American Journal of Botany

RESEARCH ARTICLE

Is selfing a reproductive assurance promoting polyploid establishment? Reduced fitness, leaky self-incompatibility and lower inbreeding depression in neotetraploids

Catarina Siopa[1,2], Maria C. Dias[1], Mariana Castro[1], João Loureiro[1], and Sílvia Castro[1]

Manuscript received 11 October 2019; revision accepted 14 January 2020.

[1] Centre for Functional Ecology, Department of Life Sciences, University of Coimbra, 3000-456 Coimbra, Portugal

[2] Author for correspondence (e-mail: catarinasiopa@gmail.com)

Citation: Siopa, C., M. C. Dias, M. Castro, J. Loureiro, and S. Castro. 2020. Is selfing a reproductive assurance promoting polyploid establishment? Reduced fitness, leaky self-incompatibility and lower inbreeding depression in neotetraploids. *American Journal of Botany* 107(3): 1–13.

doi:10.1002/ajb2.1441

PREMISE: Newly formed polyploids face significant obstacles to persistence and population establishment because of fitness costs of intercytotype mating. Selfing provides the opportunity to escape mate limitation, enabling production of new individuals and increasing the likelihood of fixation of new polyploid lineages. Still, association between self-compatibility and polyploidy is not always clear. We compared self-incompatibility and inbreeding depression in neotetraploids and their diploid progenitor to explore the direct effects of whole genome duplications on self-incompatibility and the implications of ploidy-driven changes for polyploid establishment.

METHODS: Outcross and self-pollinations were performed in diploids and synthetic neotetraploids of *Jasione maritima* var. *maritima*, and reproductive success was measured through fruit and seed production and seed germination. Self- and outcross offspring were grown under controlled conditions, and plant performance was measured through several fitness parameters.

RESULTS: Neotetraploids showed an overall lower performance than diploids. Reproductive success was negatively affected by selfing in both cytotypes. However, greater variation in the expression of self-incompatibility was observed in neotetraploids; additionally, developmental and physiological parameters were not affected by selfing on neotetraploids, with an overall similar fitness of outcrossed and selfed individuals, resulting in lower inbreeding depression indexes.

CONCLUSIONS: Neotetraploids might have benefited from selfing at initial stages after their formation. Genome duplications resulted in leaky self-incompatibility, enabling the production of offspring under minority cytotype disadvantage with similar fitness as outcrossed offspring. Our results support theoretical assumptions that selfing might be important for neopolyploid establishment, although changes in self-incompatibility might not be abrupt.

KEY WORDS Campanulaceae; inbreeding depression; *Jasione maritima*; neotetraploids; plant fitness; polyploidization; self-incompatibility; selfing.

Polyploidy is a pervasive phenomenon in the evolutionary history of flowering plants (Levin, 1983; Adams and Wendel, 2005; Soltis et al., 2009; Albert et al., 2013). However, despite its importance in past and contemporary history, the underlying processes by which polyploids emerge and succeed in nature are still poorly understood (Soltis et al., 2010; Ramsey and Ramsey, 2014). The first stages after polyploid formation are characterized by small population sizes, usually growing in sympatry with the diploid progenitors (Levin, 2002; Ramsey, 2011). Levin (1975) hypothesized that, under random mating, neopolyploids are disadvantaged in comparison with the diploids, because they occur at a lower frequency. Assuming that mating success in mixed-ploidy populations is frequency dependent, after emergence, the new cytotype will struggle to produce offspring because most of the matings will occur with diploids, producing odd ploidy offspring and reducing its reproductive success. Under this scenario, the new cytotype is subjected to minority

cytotype exclusion; it is expected to be eliminated from the population (Levin, 1975; Felber, 1991; Rodríguez, 1996; Husband, 2000).

Based on the minority cytotype exclusion theory, previous studies defined very restrictive conditions for neopolyploids establishment (Levin, 1975; Felber, 1991; Rodríguez, 1996). However, it is currently considered that the probability of polyploid establishment is much higher than previously expected, and is dependent on the acquisition of new ecological or reproductive features that may confer an advantage to the neopolyploid (e.g., Thomson and Lumaret, 1992; Rodríguez, 1996; Rausch and Morgan, 2005; Ramsey, 2007; Oswald and Nuismer, 2011a). Breeding barriers between neopolyploids and their progenitors, such as heterogeneous spatial distribution due to low dispersal (Baack, 2005) or different microhabitat preferences (Hao et al., 2013), temporal or mechanical isolation caused by different flowering phenology or morphology (Petit et al., 1997; Nuismer and Cunningham, 2005), different pollinator behavior or preferences (Segraves and Thompson, 1999; Nuismer and Cunningham, 2005; Kennedy et al., 2006), and gametic isolation (Jersáková et al., 2010; Castro et al., 2011) may all promote assortative mating and increase the fitness of neopolyploids. Alternatively, if assortative mating is not achieved, neopolyploids may have new traits that increase their fitness and fertility, allowing competition with diploids, or that increase their capacity to disperse and/or colonize new niches, thereby escaping competition with diploids (Ramsey and Schemske, 2002). Strategies such as higher rates of asexual reproduction or selfing (Baack, 2005; Fowler and Levin, 2016; Van Drunen and Husband, 2018), perenniality (Rodríguez, 1996), production of unreduced gametes (Felber, 1991; Felber and Bever, 1997; Ramsey, 2007; Suda and Herben, 2013) or higher competitive ability (Fowler and Levin, 1984; Rodríguez, 1996) may all enable the neopolyploids to cope with minority cytotype disadvantage. If one or some of these traits change after polyploidization, the probability of establishment of the neopolyploid increases (e.g., Fowler and Levin, 1984; Rodríguez, 1996; Baack, 2005; Rausch and Morgan, 2005).

One of the theories proposed for the successful establishment of neopolyploids is that polyploid plants are expected to have higher rates of self-fertilization than their diploid progenitors (Grant, 1956; Mable, 2004; Barringer, 2007; Husband et al., 2008). Rausch and Morgan (2005), using deterministic and stochastic models that included reproductive variables such as self-fertilization and inbreeding depression, showed that variations in these parameters significantly influence the conditions necessary for the establishment of neopolyploids. Shifts towards self-compatibility in polyploids are hypothesized based on key changes in the ecological and genetic context in which the sexual system is being selected, namely mate availability and genetic load. Following polyploidization, either within the progenitor's population or when colonizing new habitats, neopolyploids are subjected to limited availability of mates of the same ploidy, thus giving rise to selection for reproductive assurance and the evolution of self-fertilization (Baker, 1955; Pannell and Barrett, 1998; Brochmann et al., 2004). Selfing provides the opportunity to escape mate limitation, enabling the production of new individuals, and increasing the likelihood of fixation of new polyploid lineages (Levin, 1975; Rausch and Morgan, 2005; Buggs and Pannell, 2006). Additionally, chromosome doubling may shield neotetraploids from detrimental effects of inbreeding depression, i.e., the reduction in fitness of self-fertilized progeny relative to outcrossed progeny (Charlesworth and Charlesworth, 1987; Husband, 2016), increasing the tolerance for self-fertilization in neopolyploids (Lande and Schemske, 1985; Hedrick, 1987; Ronfort, 1999). If inbreeding depression is buffered by polyploidization, self-fertilization would be favored as a mating system, at least on the first stages of establishment (Rausch and Morgan, 2005; Ozimec and Husband, 2011).

The association between mating system and polyploidy in plants was first suggested by Grant (1956), who identified a higher incidence of polyploidy in self-compatible lineages than in outcrossing ones. Higher rates of self-fertilization in polyploids in comparison with their diploid relatives has been observed in a few flowering plants (Levan, 1936; Husband and Schemske, 1997; Cook and Soltis, 2000; Husband et al., 2008), and similar overall patterns have also been observed in larger analyses using phylogenetically independent contrasts and cross-species comparisons (Mable, 2004; Barringer, 2007; Husband et al., 2008; Robertson et al., 2011). Still, the association between self-compatibility and polyploidy is not always clear and the direct effects of genome duplications in mating systems and their role in neopolyploid establishment are still largely unexplored (Mable, 2004; Husband et al., 2008). Self-compatibility is a labile character that evolved repeatedly, according to the plant context in a given moment (Barret, 2002; Kalisz et al., 2004; Sutherland et al., 2018), and the strength of the self-incompatibility system was shown to vary within and between populations (e.g., Johnston and Schoen, 1996; Cook and Soltis, 1999, 2000; Mable, 2004; Brennan et al., 2005; Costa et al., 2017). Thus, a complete shift to self-compatibility might not be a necessary condition at initial stages of polyploid emergence; instead, partial self-compatibility could be enough to reduce the problems of reproductive assurance in newly arisen polyploid individuals (Mable, 2004; Husband et al., 2008). For example, in *Chamerion angustifolium*, self-pollinated neotetraploids had lower inbreeding depression than established tetraploids (Husband et al., 2008; Ozimec and Husband, 2011), which may allow neotetraploids to produce offspring under limited mate availability; however, this phenomenon appears to dissipate with repeated generations of self-fertilizations (Ozimec and Husband, 2011). In this context, pairwise comparison of self-incompatibility and inbreeding depression levels in neotetraploids and their diploid progenitors provide significant insights on the direct effects of genome duplications and its implications for establishment at initial stages after polyploid formation.

Jasione L. belongs to the Campanulaceae family and is found in a wide range of ecosystems, from dune systems to rocky alpine areas. *Jasione maritima* (Duby) Merino is an allogamous perennial plant found in dune systems with a disjoint distribution, growing from French Gironde to coastal areas of the northwest part of the Iberian Peninsula. The variety *maritima* is a polyploid complex harboring both diploids ($2n = 2x = 12$ chromosomes) and tetraploids ($2n = 4x = 24$ chromosomes) and, based on morphological resemblance, tetraploids are hypothesized to have originated from autopolyploidization event(s) (Castro, 2018). The two cytotypes present an allopatric distribution, with diploids occurring in the northern area of the distribution and tetraploids in southern locations of the Spanish coast (Castro, 2018). Preliminary pollination experiments suggest the presence of a strong self-incompatibility system in both diploids and tetraploids (M. Castro and S. Castro, Centre for Functional Ecology (CFE) – University of Coimbra, personal observations), likewise the closely related *J. montana* (Parnell, 1987). Recently, neotetraploids of *J. maritima* var. *maritima* have been synthesized by Castro et al. (2018), which allowed us to study the immediate effect of genome duplications in neotetraploid traits.

The main objective of this work was to understand the role of selfing in the establishment of neopolyploids using the polyploid *J. maritima* var. *maritima* as the study system. The following specific questions were addressed: (1) Do genome duplications lead to self-compatibility in neotetraploids? (2) What is the fitness cost of selfing in diploid and neotetraploid offspring? To address these questions, outcross and self-pollinations were performed in diploid and synthetic neotetraploid individuals, and reproductive success was measured through fruit and seed production and seed germination. Subsequently, self and outcross offspring of diploids and neotetraploids were grown in a greenhouse experiment, and plant performance was measured through several fitness parameters. The traits selected included: key life-cycle traits such as fruit and seed production, seed germination, production of reproductive structures, and flowering probability; key developmental traits indicative of plant fitness, such as plant biomass and height and number of leaves; and key physiological traits linked with plant performance, such as photosynthetic reactions, composition of carbohydrates and pigments, relative water content, and stomata traits. We hypothesize that fruit and seed production will be strongly reduced after self-pollination when compared with outcross pollination because of the gametic self-incompatibility system; nevertheless, genome duplications are expected to generate lower incompatibility levels in neotetraploids, enabling the production of offspring in the absence of sexual mates of the same ploidy. Also, we hypothesize that plant fitness will be strongly reduced after self-fertilization in comparison with outcrossing because of recessive alleles expression (inbreeding depression); however, inbreeding depression is expected to be lower in neotetraploids in comparison with diploids because of multiple copies of the alleles, enabling neotetraploids to cope with increasing rates of selfing at initial stages. The results will enable us to assess the role of genome duplications, which drive changes in mating patterns and their role in neotetraploid establishment.

MATERIALS AND METHODS

Plant material and general experimental design

Seeds were collected during the fruiting period (July 2013) in three *Jasione maritima* var. *maritima* diploid populations in La Coruña, Spain (Pedrosa Beach, Mourín, 43°9'29.448"N, 9°11'28.536"W; Afora Beach, Fisterra, 42°54'30.636"N, 9°16'23.808"W; and Lariño, 42°46'15.708"N, 9°7'20.172"W). Seeds of at least 30 individual plants were sampled per population. Population SC73 corresponds to the diploid locality at the contact zone with *J. maritima* var. *maritima* tetraploid populations (Castro, 2018).

Seeds collected in the field were used to (1) produce synthetic neotetraploids (see *Neotetraploid production*), and (2) to obtain diploid plants. The diploid plants were subjected to the same treatment as synthetic neotetraploids but correspond to plants that did not polyploidize; this procedure enabled us to have all the plants affected similarly by the chemical treatment. Both synthetic neotetraploid and diploid plants were used to make controlled pollinations (see *Cytotype reproductive success*). Seeds obtained after controlled pollinations were subsequently used to study the performance of the F1 offspring obtained after outcrossing and selfing (see *Plant performance*). Flow cytometry was used to identify neotetraploids and unconverted diploids, and to confirm the ploidy levels of all the F1 plants (see *Ploidy level estimation*).

Neotetraploid production

Synthetic neotetraploids were obtained in the laboratory by Castro et al. (2018), through the treatment of diploid *J. maritima* var. *maritima* seedlings with colchicine. Briefly, seeds were placed in Petri dishes (one mother family per Petri dish) with moistened filter paper, maintained at 4°C for 1 wk and transferred to a growth chamber (at 24°C with a cycle of 16 h/8 h light/dark exposure photoperiod) for seed germination. The 3–4 days-old seedlings were submerged into a solution of 0.5% colchicine for 14 h and subsequently washed several times with ddH_2O. Treated seedlings were planted in trays filled with standard soil and maintained in the greenhouse. The ploidy level of all plants that survived was screened using flow cytometry (see *Ploidy level estimation*) and both plants identified as tetraploids and unconverted diploid plants were transplanted individually to 1 L pots filled with standard soil. Plants were maintained in the greenhouse until flowering and were subsequently used for the controlled pollinations (see *Cytotype reproductive success*).

Ploidy level estimation

DNA-quantification analyses using flow cytometry were performed to assess the ploidy level of all the plants of the experiment. Briefly, ~20 mg of fresh leaves of the sampled plant and 20 mg of leaves of an internal reference standard (*Solanum lycopersicum* 'Stupické', 2C = 1.96 pg; Doležel et al., 1992) were placed in a Petri dish and chopped after adding 1 mL of Woody Plant Buffer to obtain a nuclear suspension (Loureiro et al., 2007). After removing the debris by filtration using a 50 µm nylon filter, a DNA staining fluorochrome (propidium iodide, 50 $\mu g \cdot mg^{-1}$) and RNAse (50 $\mu g \cdot mol^{-1}$) were added to the solution to stain the nuclei and to degrade the double-stranded RNA, respectively. Samples were analyzed in a CyFlow Space flow cytometer (Partec GmbH., Görlitz, Germany) and the following data were obtained using the software Partec FloMax v2.4d (Partec GmbH, Münster, Germany): a histogram with the fluorescence pulse integral in linear scale (FL); forward light scatter (FS) vs. side light scatter (SS), both in logarithmic scale; FL vs. time; and FL vs. SS in log scale. In the graphic of FL vs. SS, we define a polygon to remove the debris. As a quality standard, only histograms with coefficients of variation (CV) below 5% were accepted, otherwise the sample was repeated (Doležel et al., 2007).

Genome size was estimated from the data mentioned above with the following equation: genome size of *J. maritima* var. *maritima* (pg) = (*J. maritima* var. *maritima* FL) / (*S. lycopersicum* FL) × *S. lycopersicum* genome size, using the mean values of relative fluorescence (FL) of the obtained peaks. Based on previous genome size estimates and chromosome counts, ploidy level was assigned to each plant as follows: diploids having genome size values of 2.98 ± 0.07 pg, and neotetraploids having values of 6.06 ± 0.11 pg (Castro, 2018).

Reproductive success of each cytotype

Unconverted diploid and neotetraploid plants were grown until flowering, watered twice a week during winter and daily during spring and summer. Two controlled pollinations were performed in 45 different mother-families from each cytotype (15 plants per

population) during the flowering period (May–June 2015): (1) self-pollination, and (2) outcross pollination. Whenever possible, we selected one inflorescence per plant for each treatment (recipient inflorescences) and an additional inflorescence as pollen donor for self-pollinations. Before flowering, plants were isolated from pollinators using pollinator exclusion cages. Additionally, all the manipulated inflorescences were bagged with small mesh bags to avoid contaminations due to plant handling within the cage. The bags were kept until fruiting to prevent seed loss as the fruit is a dehiscent capsule. *Jasione maritima* has capituliform inflorescences composed of small individual flowers with secondary pollen presentation (for illustrations in the closely related *J. montana* see Yeo, 1993) that precludes emasculation, and thus we were unable to emasculate the flowers. Consequently, selfing is not completely ruled out in outcross treatments but is expected to have a negligible contribution in this polyspermic self-incompatible plant, because under mixed pollen loads outcross pollen is expected to be more successful than self-pollen. Because the flowers of the inflorescence opened gradually, 4–5 pollinations were made in alternate days, until all the flowers of the recipient inflorescence received pollen. Outcross pollinations were made using at least five pollen donor inflorescences from the same population, and the pollen of each donor was applied in sequence by gently rubbing the recipient and donor inflorescences with each other. Likewise, self-pollinations were made by gently rubbing the recipient inflorescence with the donor-bagged inflorescences of the same plant. After fruit maturation, the inflorescences were stored individually in identified paper bags. In the laboratory, the number of flowers, fruits, and morphologically viable seeds were quantified for each infructescence under a binocular microscope.

Diploid and neotetraploid seeds obtained after self and outcross pollinations were used to assess seed germination. For each cytotype, 25 seeds of 45 different mother-families from each treatment (15 plants per population) were selected and placed in Petri dishes to germinate (one mother-family per Petri dish) with moistened filter paper, as described above. Seed germination was assessed one month after transference to the growth chamber.

The following parameters were calculated: fruit production (proportion of flowers that developed into fruits), seed production (mean number of seeds per reproductive unit), and seed germination (proportion of seeds that germinate). Additionally, the index of self-incompatibility (ISI) was calculated for each diploid and neotetraploid plant as $ISI = 1 - (SP_{S\text{-}P} / SP_{OP})$ (where SP = seed production, $_{S\text{-}P}$ = self-pollination, and $_{OP}$ = outcross pollination; Lloyd, 1965). ISI can range from 0–1, where fully self-compatible plants score 0 and complete self-incompatible plants 1. Although we tried to have both self-pollination and outcross treatments per plant, not all plants had enough inflorescences to do it; thus, for those plants having only self-pollination treatment, we used the mean seed production value of outcrossing (calculated separately for each cytotype) to calculate the ISI.

Plant performance: experimental design

One 3–4 days-old seedling was randomly selected from each Petri dish of the germination trials (see *Cytotype reproductive success*) and transplanted individually to 1 L pots filled with standard soil and sand in the proportion of 1:3. A total of 180 seedlings were transplanted, i.e., 90 diploids and 90 neotetraploids, within which 45 seedlings per pollination treatment (15 plants per population). The plants were grown in the greenhouse from November 2016 to September 2017, watered 2–3 times a week during winter, and daily during spring and summer. In the first month after transplantation, seedling mortality was negligible (3%) and due to seedling manipulation. Hence, these seedlings were replaced by other seedlings from the same mother family and only after the first month, we started to record mortality; still, the mortality was also very low (4% in the end of the experiment) and, thus, mortality was not included in the study. The ploidy level of all the plants was confirmed using flow cytometry (see *Ploidy level estimation*) in July 2017.

In September 2017, we measured several developmental and physiological traits (see *Plant performance: development and physiological traits*) and harvested the plants. Photosynthesis parameters were acquired in all plants before harvesting. Stomatal measurements were assessed in 20 diploid and 20 neotetraploid plants. Eight plants per treatment and cytotype (including individuals from all the populations) were randomly selected and harvested to quantify relative water content, electrolyte leakage, photosynthetic pigments, and carbohydrate content. The remaining plants were harvested, classifying the plant as reproductive or vegetative, counting the number of leaves and reproductive branches, measuring plant height, and separating belowground and aboveground biomass to individual paper bags. Samples were then placed to dry at 68°C for 48 h. Below- and aboveground biomass were assessed by weighing the corresponding parts on a precision scale (0.01 mg accuracy).

Inbreeding depression

Reduction in fitness of self-pollinated offspring relative to outcrossed offspring was estimated for each fitness trait by calculating the coefficient of inbreeding depression as $ID = 1 - (F_{S\text{-}P} / F_{OP})$ (where F = fitness trait, $_{S\text{-}P}$ = self-pollination, and $_{OP}$ = outcross pollination; Charlesworth and Charlesworth, 1987). The following fitness traits were used to calculate inbreeding: fruit and seed production, seed germination, number of leaves and reproductive structures, plant height and biomass, and probability of flowering. A cumulative inbreeding index was also calculated as the product of selected traits, namely seed production, seed germination, number of reproductive structures, and total biomass. Fruit set and probability of flowering were not included in the formula because they were already taken into account in the calculation of seed production and number of reproductive structures parameters.

Plant performance: development and physiological traits

Photosynthesis parameters were measured in vivo with a fluorometer (FluorPen FP 100-Max; Photon Systems Instruments, Drasov, Czech Republic). Chlorophyll fluorescence parameters, minimum fluorescence (Fo), and maximum fluorescence (Fm) were measured after leaf dark adaptation for 60 min by applying a weak-intensity modulated light followed by a high saturation pulse of white light (>1500 $\mu mol \cdot m^{-2} \cdot s^{-1}$). Then, leaves were acclimated to ambient light and the steady-state fluorescence (Fs) was averaged over 2.5 s, followed by exposure to a saturating light (>7500 $\mu mol \cdot m^{-2} \cdot s^{-1}$) to determine the maximal fluorescence (FM). Maximum and effective quantum yield of photosystem II (Fv/Fm and ϕPSII, respectively) were calculated as $Fv/Fm = ((Fm-Fo)/(Fm))$ and $\phi PSII = ((FM-Fs)/(FM))$.

Relative water content, electrolyte leakage, and stomatal measurements were measured using fresh leaves. For relative water

content, two leaves per plant were collected, weighed (fresh mass, FM), and placed in 1.5 mL microtubes. The microtubes were filled with water and placed over night in the dark at 4°C to obtain the turgid mass (TM). Leaves were then dried for 7 days at 70°C to obtain the dry mass (DM). Relative water content was calculated as (FM – DM)/(TM – DM).

Membrane permeability was assessed by measuring leakage of UV-absorbing substances (UVAS; Redman et al., 1986). Two to three leaves per plant were collected, washed, and reserved on microtubes containing deionized water and incubated overnight at 25°C. The absorbance was then measured in a spectrophotometer at 280 nm (A280 nm) (Jenway 7305 Spectrophotometer, Staffordshire, UK). Afterwards, leaves were autoclaved for 20 min (120°C) and the absorbance was measure again (A'280 nm). Relative electrolyte leakage of UVAS was calculated as A280 nm/A'280 nm.

Stomatal measurements included stomatal density and length. For that, a leaf from 20 plants per cytotype was collected. After peeling the epidermis of the abaxial leaf face, the epidermis was placed in a microscopic slide and prepared following Weyers and Travis (1981). Stomatal density was measured by counting the number of stomata of five different microscope fields of view per plant (magnification = 200×), and the field-of-view area was obtained to calculate stomatal density (number of stomata/mm^2). For stomatal length, six stomata per plant were selected and photographed, and measurements obtained using the ImageJ software (National Institutes of Health, Bethesda, Maryland, USA).

Photosynthetic pigments and carbohydrate content were quantified in the eight selected individuals per treatment and cytotype. For that, leaf material was immediately frozen in liquid nitrogen and stored at –80°C in individual aluminum foil envelopes. For pigment content, macerated frozen leaf samples were homogenized with acetone/50 mM Tris buffer pH 7.8 (80:20, v:v) and centrifuged. The absorbance of the supernatant was read at 470, 537, 647, and 663 nm on a spectrophotometer (PerkinElmer 2300 EnSpire Multilabel Reader, Turku, Finland). Chlorophyll *a*, chlorophyll *b*, and carotenoid content were calculated according with Sims and Gamon (2002) using the following formulas: Chl *a* = 0.01373 A663 – 0.000897 A537 – 0.003046 A647; Chl *b* = 0.02405 A647 – 0.004305 A537 – 0.005507 A663; carotenoids = (A470 – (17.1 × (Chl *a* + Chl *b*) – 9.479 × Anthocyanins)) / 119.26; anthocyanins = 0.08173 A537 – 0.00697 A647 – 0.002228 A663.

For total soluble sugars, macerated frozen leaf samples were homogenized with 80% ethanol, and incubated for 1 h in a bath at 80°C (Irigoyen et al., 1992). After a 10 min centrifugation at low temperature, the obtained supernatant was incubated with an anthrone solution (40 mg anthrone + 20 mL sulfuric acid + 1 mL H_2O) for 10 min at 100°C and centrifuged. The supernatant was used to read the absorbance at 625 nm (PerkinElmer 2300 EnSpire Multilabel Reader, Turku, Finland). For starch content, the pellet obtained from the total soluble sugars extraction was used (Osaki et al., 1991). Perchloric acid (30%) was added to the pellet and the mixture was incubated for 1 h in a bath at 60°C, and subsequently centrifuged. The obtained supernatant was incubated with an anthrone solution (40 mg anthrone + 20 mL sulfuric acid + 1 mL H_2O) for 10 min at 100°C and centrifuged. The supernatant was used to read the absorbance at 625 nm. Total soluble sugars and starch content were calculated using a glucose standard curve, constructed with the absorbance of solutions of known glucose concentrations.

Statistical analyses

Generalized linear models (GLM) were used to explore the effect of ploidy level and pollination treatment in the reproductive, developmental, and physiologic traits. Ploidy level (diploid or neotetraploid) and pollination treatment (self- or outcross pollination) were defined as fixed factors; initially, population was defined as a random factor using generalized linear mixed models (GLMM), but was subsequently removed from the models because its variance was lower than the variance of the residuals (Bolker et al., 2009), and thus GLM were used instead. The following response variables were analyzed: fruit and seed production; seed germination; number of leaves; number of reproductive structures; plant height; probability of flowering; belowground-, aboveground-, and total biomass; stomata density; stomata length; Fv/Fm; ΦPSII; chlorophylls *a*, *b* and carotenoids content; total soluble sugars and starch content; relative water content; and relative electrolyte leakage. Fruit production, seed germination, relative water content, and relative electrolyte leakage were arcsine transformed; number of leaves, plant height, belowground-, aboveground-, and total biomass and stomatal length were log-transformed; and reproductive structures, starch content, Fv/Fm, and ΦPSII were square-root transformed. A Gaussian distribution with an identity link function was used to model all response variables except the probability of flowering where a binomial distribution with a logit link function was used. Differences between least-square means were tested pairwise through multiple comparisons. Seed production and index of self-incompatibility (ISI) did not meet the GLM assumptions, even after transformation; a Kruskal-Wallis one-way Analysis of Variance on Ranks test with ploidy and pollination treatment combined, followed by the Dunn's Test for multiple comparisons were used to analyze seed production, and a Wilcoxon signed Rank test with ploidy level as a fixed factor was used to analyze ISI. Differences between cytotypes for inbreeding indexes were explored with a t-test or Wilcoxon signed Rank test when normality was not observed. Finally, one-sample t-test or one-sample signed Rank test was used to determine if inbreeding indexes differed significantly from 0. Analyses and graphics buildup were performed using R software version 3.4.3, using the packages "car" (Fox et al., 2015) and "lme4" (Bates et al., 2014) for assumptions tests and GLM analysis, and "lsmeans" package (Lenth, 2016) for multiple comparisons tests.

RESULTS

Reproductive success under different mating strategies

Fruit production was significantly higher in unconverted diploids than in neotetraploids, regardless of the pollination treatment (Table 1, Fig. 1A). Significant differences between ploidy levels and pollination treatments were observed in seed production and seed germination (Table 1, Fig. 1B, C). Neotetraploids produced significantly fewer seeds than diploids, and self-pollination produced significantly fewer seeds than outcrossing (Fig. 1B). A similar pattern was observed for seed germination, although seed germination of self-pollinated diploids did not differ from the one of outcrossed neotetraploids (Fig. 1C). No significant differences were observed in the ISI (Table 1, Fig. 1D), although the individual values for diploid plants were all above 0.8 (threshold for self-incompatible

TABLE 1. Effect of ploidy level and pollination treatment (self- and outcross pollination) on the studied variables measured on diploids and neotetraploids of *Jasione maritima* var. *maritima*.

Variable	Ploidy level	Crossing	Crossing * Ploidy
Fruit production	$F_{1,132} = 26.37$, $\mathbf{P < 0.001}$	$F_{1,121} = 0.001$, $P = 0.983$	$F_{1,121} = 1.09$, $P = 0.299$
Seed production	$H_{1,128} = 67.60$, $\mathbf{P < 0.001}$		NA
Seed germination	$F_{1,150} = 34.48$, $\mathbf{P < 0.001}$	$F_{1,150} = 37.06$, $\mathbf{P < 0.001}$	$F_{1,150} = 0.52$, $P = 0.471$
Index of self-incompatibility	$W^2_{1,62} = 535.00$, $P = 0.435$	NA	NA
Aboveground biomass	$F_{1,130} = 5.82$, $\mathbf{P = 0.017}$	$F_{1,130} = 7.17$, $\mathbf{P = 0.008}$	$F_{1,130} = 0.99$, $P = 0.322$
Belowground biomass	$F_{1,130} = 7.88$, $\mathbf{P = 0.006}$	$F_{1,130} = 3.59$, $P = 0.060$	$F_{1,130} = 0.002$, $P = 0.968$
Total biomass	$F_{1,130} = 6.62$, $\mathbf{P = 0.011}$	$F_{1,130} = 5.76$, $\mathbf{P = 0.018}$	$F_{1,130} = 0.46$, $P = 0.497$
Number of leaves	$F_{1,162} = 68.62$, $\mathbf{P < 0.001}$	$F_{1,162} = 6.00$, $\mathbf{P = 0.015}$	$F_{1,162} = 2.17$, $P = 0.143$
Plant height	$F_{1,162} = 0.21$, $P = 0.647$	$F_{1,162} = 6.18$, $\mathbf{P = 0.014}$	$F_{1,162} = 1.96$, $P = 0.164$
Probability of flowering	$X^2_{1,161} = 17.41$, $\mathbf{P < 0.001}$	$X^2_{1,161} = 1.65$, $P = 0.199$	$X^2_{1,161} = 1.19$, $P = 0.275$
Reproductive structures	$F_{1,230} = 58.62$, $\mathbf{P < 0.001}$	$F_{1,230} = 5.680$, $\mathbf{P = 0.018}$	$F_{1,230} = 0.745$, $P = 0.389$
Total Soluble Sugars	$F_{1,28} = 12.57$, $\mathbf{P = 0.001}$	$F_{1,28} = 3.25$, $P = 0.082$	$F_{1,28} = 7.95$, $\mathbf{P = 0.009}$
Starch	$F_{1,28} = 2.69$, $P = 0.112$	$F_{1,28} = 0.08$, $P = 0.786$	$F_{1,28} = 0.49$, $P = 0.490$
Relative Water Content	$F_{1,26} = 9.18$, $\mathbf{P = 0.005}$	$F_{1,28} = 5.88$, $\mathbf{P = 0.023}$	$F_{1,28} = 3.19$, $P = 0.086$
Relative Electrolyte Leakage	$F_{1,28} = 1.74$, $P = 0.198$	$F_{1,28} = 0.02$, $P = 0.881$	$F_{1,28} = 6.70$, $\mathbf{P = 0.015}$
Maximum quantum yield of photosystem II (F_v/F_m)	$F_{1,167} = 7.22$, $\mathbf{P = 0.008}$	$F_{1,167} = 0.26$, $P = 0.611$	$F_{1,167} = 0.34$, $P = 0.562$
Effective quantum yield of photosystem II (ϕ_{PSII})	$F_{1,167} = 9.00$, $\mathbf{P = 0.003}$	$F_{1,167} = 1.54$, $P = 0.216$	$F_{1,167} = 0.01$, $P = 0.920$
Chlorophyll *a*	$F_{1,28} = 0.69$, $P = 0.413$	$F_{1,28} = 0.68$, $P = 0.418$	$F_{1,28} = 2.52$, $P = 0.124$
Chlorophyll *b*	$F_{1,28} = 0.96$, $P = 0.335$	$F_{1,28} = 0.31$, $P = 0.582$	$F_{1,28} = 2.86$, $P = 0.102$
Carotenoids	$F_{1,28} = 1.50$, $P = 0.230$	$F_{1,28} = 0.34$, $P = 0.565$	$F_{1,28} = 1.64$, $P = 0.210$
Stomata Density	$F_{1,77} = 37.75$, $\mathbf{P < 0.001}$	$F_{1,77} = 1.48$, $P = 0.228$	$F_{1,77} = 0.27$, $P = 0.602$
Stomata Length	$F_{1,78} = 172.77$, $\mathbf{P < 0.001}$	$F_{1,78} = 0.81$, $P = 0.372$	$F_{1,78} = 0.02$, $P = 0.904$

Notes: significant *P* values are highlighted in bold.

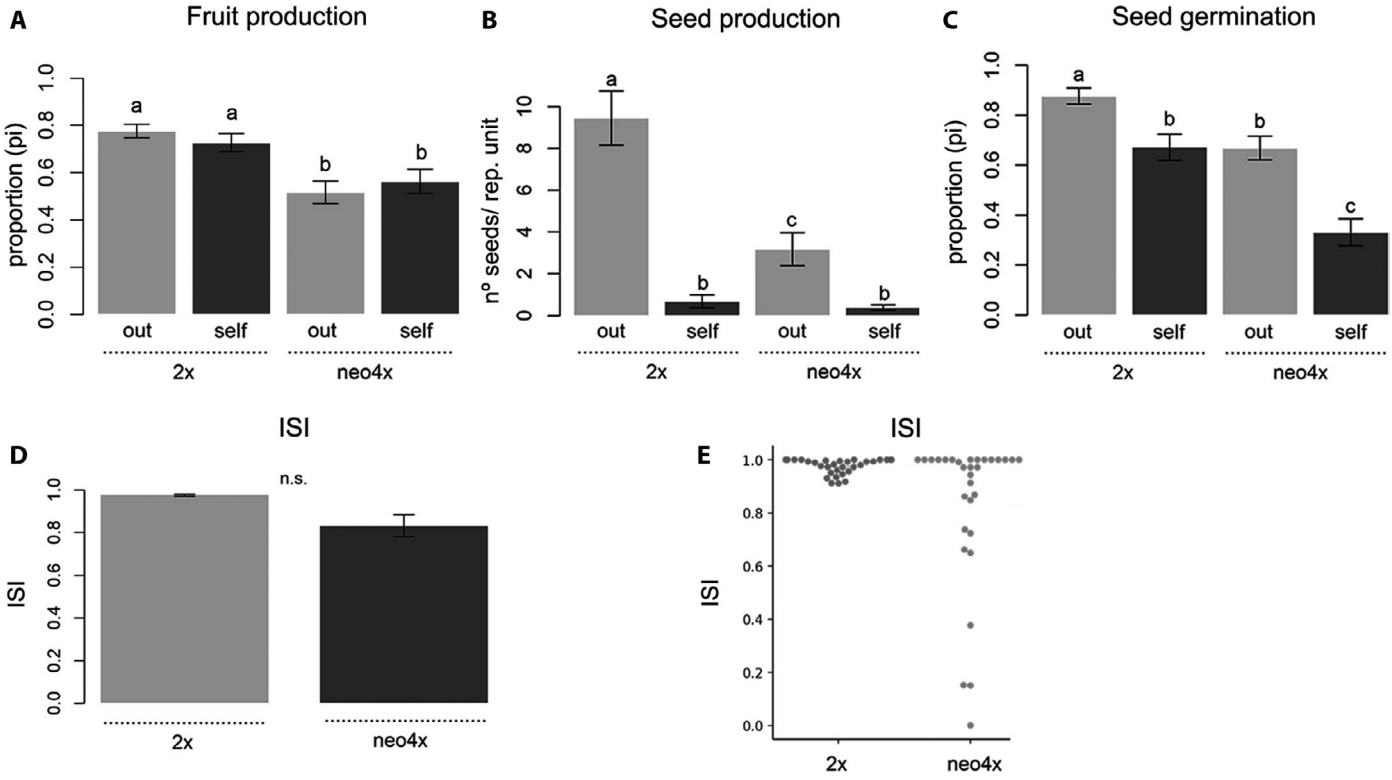

FIGURE 1. Reproductive success under self- and outcross pollination treatments (self and out, respectively) for diploids (2*x*) and neotetraploids (neo4*x*) of *Jasione maritima* var. *maritima*: (A) Fruit production (proportion of flowers that developed into fruit). (B) Seed production (mean number of seeds per reproductive unit). (C) Seed germination (proportion of seeds that germinate). (D) Index of Self-Incompatibility (ISI; ranges from 0, indicating self-incompatibility, to 1, indicating full self-compatibility). (E) Individual ISI values with self-incompatibility thresholds highlighted (ISI < 0.2 self-incompatible plants; 0.2 < ISI < 0.8 partially self-compatible plants; ISI > 0.8 self-compatible plants; Lloyd, 1965). Values are presented as mean and standard error of the mean; different letters represent statistical differences at $P < 0.05$; n.s. denotes nonsignificant differences at $P > 0.05$.

plants; Lloyd, 1965), while neotetraploids presented higher variability with a few individuals showing ISI values below 0.2 (threshold for self-compatible plants; Lloyd, 1965), and several between 0.8 and 0.2 (Fig. 1E).

Plant developmental performance under different mating strategies

Ploidy level and pollination treatment affected total and aboveground biomass, while belowground biomass was only affected by ploidy level (Table 1, Fig. 2A, Appendix S1). Overall, biomass was lower in neotetraploids than in diploids, and in self-pollinated plants in comparison with outcrossed ones (Fig. 2A; Appendix S1). Number of leaves was also significantly affected by ploidy level and pollination treatment (Table 1). Outcrossed diploids produced more leaves than self-pollinated diploids, which in turn produced more leaves than neotetraploids regardless of the treatment (Fig. 2B). Plant height was affected only by treatment (Table 1), although significant differences were only found between outcrossed and self-pollinated diploids (Fig. 2C). Reproductive structures were significantly affected by ploidy level and pollination treatment, while probability of flowering was affected only by ploidy (Table 1). Diploids produced more reproductive structures and had a higher probability of flowering than neotetraploids; no significant differences between outcrossed and self-pollinated treatments on both ploidies were observed, although both variables were lower after selfing in the diploids (Fig. 2D, E).

Inbreeding depression

In unconverted diploids, inbreeding values were significantly different from zero for all traits, except for fruit production; in neotetraploids, fruit production, plant height, reproductive structures, and probability of flowering inbreeding values did not differ from zero (Table 2). Overall, neotetraploids presented lower inbreeding depression indexes than diploids for most variables, except seed germination and belowground biomass (Table 2). However, significant differences between cytotypes were obtained only for seed germination, with diploids having lower inbreeding index than neotetraploids, and for plant height with neotetraploids having lower inbreeding index than diploids (Table 2). Neotetraploids presented a lower cumulative inbreeding depression in comparison with diploids, although no significant differences were observed (Table 2).

Plant physiological performance under different mating strategies

Significant differences were observed in the interaction factor for total soluble sugars content (Table 1), with outcrossed diploid plants presenting higher total soluble sugars than the remaining groups (Fig. 2F), but no differences were observed in starch content (Table 1, Appendix S1).

Ploidy level and pollination treatment significantly affected relative water content (Table 1), with higher values in neotetraploids than in diploids, although this was only significant for self-pollinated

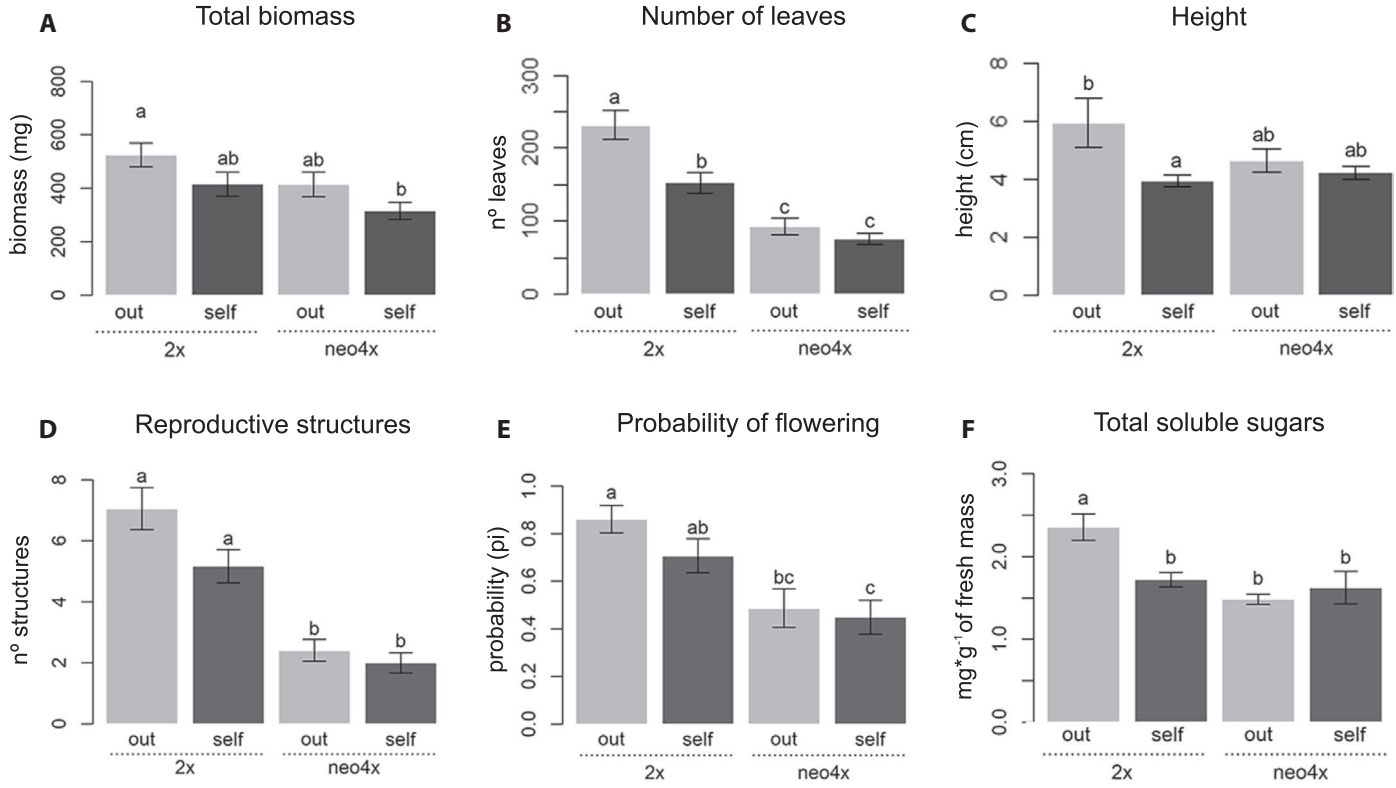

FIGURE 2. Plant developmental performance under self- and outcross pollination treatments (self and out, respectively) for diploids (2*x*) and neotetraploids (neo4*x*) of *Jasione maritima* var. *maritima*: (A) Total biomass (mg). (B) Number of leaves. (C) Plant height (cm). (D) Number of reproductive structures. (E) Probability of flowering (pi). (F) Total soluble sugars (mg·g^{-1} fresh mass). Values are presented as mean and standard error of the mean; different letters represent statistical differences at $P < 0.05$.

TABLE 2. Inbreeding depression indexes for reproductive and developmental traits for diploids (2*x*) and neotetraploids (neo4*x*).

Variable	Inbreeding depression (mean ± SE)		Statistical test (cytotype comparisons)
	2*x*	Neo4*x*	
Fruit production	0.064 ± 0.049 n.s.	**0.024 ± 0.105 n.s.**	$W_{1,66} = 640.00$, $P = 0.447$
Seed production	0.975 ± 0.005***	**0.830 ± 0.052*****	$W_{1,62} = 535.00$, $P = 0.435$
Seed germination	**0.233 ± 0.061****	0.505 ± 0.082***	$W_{1,72} = 428.50$, **$P = 0.006$**
Number of leaves	0.341 ± 0.043***	**0.170 ± 0.042****	$F_{1,78} = 2.88$, $P = 0.094$
Plant height	0.338 ± 0.034***	**0.088 ± 0.052 n.s.**	$F_{1,78} = 17.10$, **$P \leq 0.001$**
Probability of flowering	0.179 ± 0.062**	**0.078 ± 0.118 n.s.**	$W_{1,114} = 1684.00$, $P = 0.973$
Reproductive structures	0.267 ± 0.077***	**0.170 ± 0.140 n.s.**	$W_{1,114} = 1608.00$, $P = 0.697$
Aboveground biomass	0.339 ± 0.072***	**0.225 ± 0.083****	$F_{1,62} = 1.10$, $P = 0.299$
Belowground biomass	**0.197 ± 0.093***	0.296 ± 0.075***	$F_{1,62} = 0.67$, $P = 0.416$
Total biomass	0.287 ± 0.076***	**0.238 ± 0.077*****	$F_{1,62} = 0.20$, $P = 0.658$
Cumulative inbreeding	0.942 ± 0.016***	**0.922 ± 0.019*****	$F_{1,114} = 0.61$, $P = 0.438$

Notes: lower inbreeding depression values are highlighted in bold; statistical comparisons between cytotypes are provided for each trait; the occurrence of significant differences from 0 are also indicated for each inbreeding index after running a one-sample t-test or one-sample signed Rank test; n.s. denote nonsignificant differences between cytotypes at $P > 0.05$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

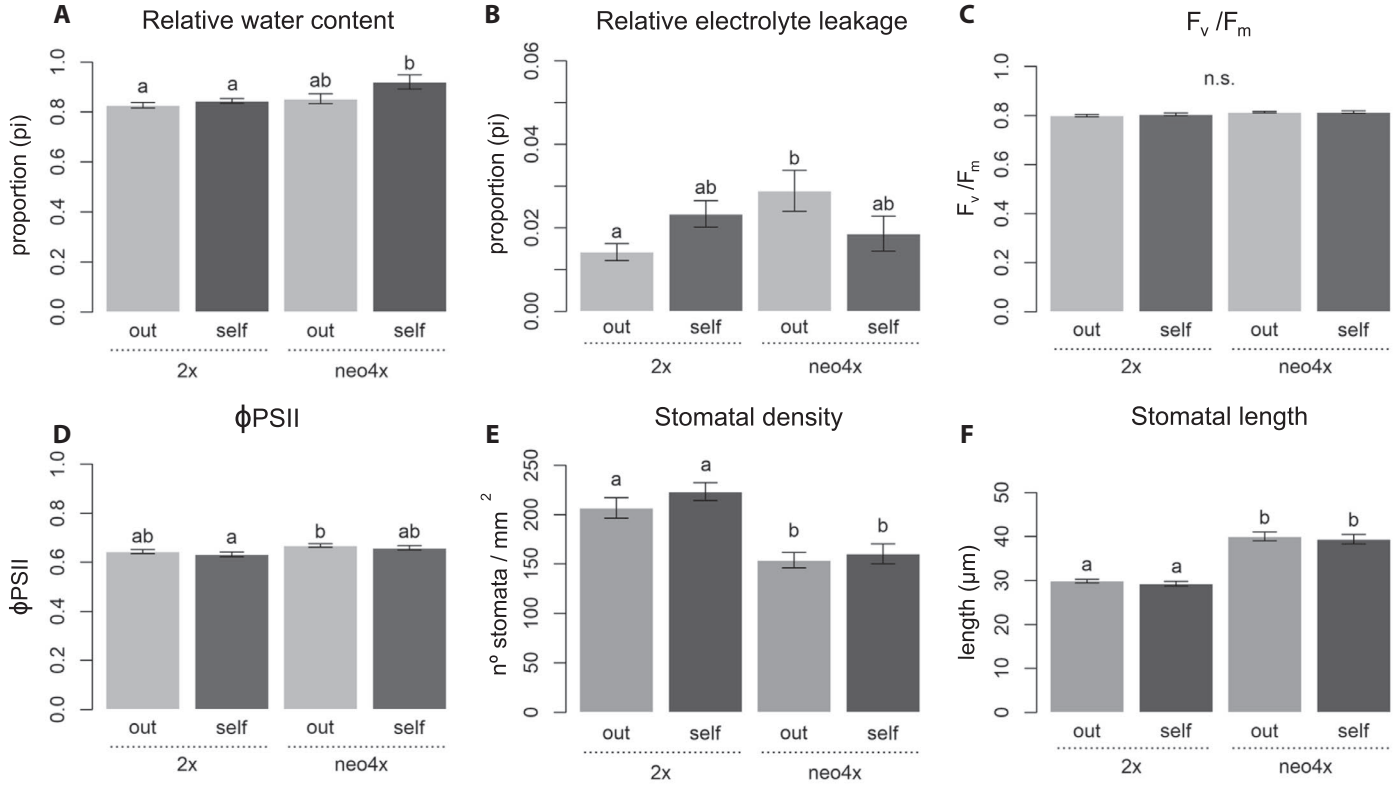

FIGURE 3. Plant physiological performance under self- and outcross pollination treatments (self and out, respectively) for diploids (2*x*) and neotetraploids (neo4*x*) of *Jasione maritima* var. *maritima*: (A) Relative water content (pi). (B) Relative electrolyte leakage (pi). (C) Maximum quantum yield of photosystem II (Fv/Fm). (D) Effective quantum yield of photosystem II (ΦPSII). (E) Stomata density (number of stomata/mm^2). (F) Stomata length (µm). Values are presented as mean and standard error of the mean; different letters represent statistical differences at $P < 0.05$; n.s. denotes nonsignificant differences at $P > 0.05$.

neotetraploid plants (Fig. 3A). Significant differences were observed in the interaction factor for relative electrolyte leakage (Table 1), with outcrossed neotetraploids presenting significantly higher values than outcrossed diploids and with both self-pollinated cytotypes having intermediate values (Fig. 3B).

Maximum and effective quantum yield of photosystem II (Fv/Fm and ΦPSII, respectively) were both affected by ploidy level, only (Table 1), although post hoc tests failed to detect differences for Fv/Fm (Fig. 3C). The ΦPSII was significantly higher in the neotetraploids than in diploids, but significant differences were only detected between self-pollinated diploids and outcrossed neotetraploids (Fig. 3D). No differences were observed between ploidy levels, and pollination treatments for pigment content (Table 1, Appendix S1).

Ploidy levels significantly affected stomata density and length, while treatment had no effect (Table 1). Stomata density was significantly higher in diploids in comparison with the neotetraploids (Fig. 3E); whereas stomata length of neotetraploids was significantly higher than that of diploids (Fig. 3F).

DISCUSSION

In *Jasione maritima* var. *maritima*, neotetraploids showed an overall lower performance than unconverted diploids, and reproductive success was negatively affected by selfing in both cytotypes. However, a high variation in the expression of self-incompatibility was observed in neotetraploids; additionally, developmental and physiological parameters were not affected by selfing on neotetraploids, with an overall similar performance of outcrossed and selfed individuals, which was reflected in tendentially lower indexes of inbreeding depression in neotetraploids when compared with diploids. These results provide insights of the potential role of selfing in the establishment of neopolyploids, as discussed further in the following sections.

Effect of whole genome duplications

In our experiment, neotetraploids showed an overall lower performance than diploids that most probably results from a combination of processes due to polyploidization. Whole genome duplications impose major changes in cellular architecture and gene expression, and cause epigenetic instability and difficulties in mitosis and meiosis (e.g., Mittelsten et al., 1996; Comai, 2005; Rastogi et al., 2005; Risso-Pascotto et al., 2005; Hollister, 2015; Doyle and Coate, 2019). These changes are potentially disadvantageous for polyploid development, survival, and establishment (Comai, 2005), and the lower performance of neotetraploids observed here may reflect some of these changes. Additionally, as in here, in the great majority of the studies, the direct effects of whole genome duplications rely on synthetized neopolyploids (e.g., Maheraldi et al., 2009; Oswald and Nuismer, 2011b; Husband et al., 2016; Castro et al., 2018), using c-mitotic agents. These treatments themselves may affect plant fitness and self-incompatibility (e.g., Pandey, 1968; Münzbergová, 2017), limiting the interpretation of results. In our comparisons, we tried to minimize the colchicine effect by using unconverted plants to represent diploids, thus, such plants were also subjected to colchicine treatment. Although not ideal, this is the general protocol to study the direct effect of genome duplications (e.g., Husband et al., 2008; Ramsey and Ramsey, 2014; Castro et al., 2018) in polyploid complexes where neotetraploids were not found in nature.

Changes in self-incompatibility

Our pollination experiments showed that *Jasione maritima* var. *maritima* is self-incompatible, and likely has a gametophytic self-incompatibility (GSI) system as described in the Campanulaceae family (Stepheson et al., 2000; Ægisdóttir et al., 2007; Good-Avila et al., 2008). Reproductive success was significantly reduced after self-pollination for both diploids and neotetraploids, except for fruit set. Consequently, both cytotypes presented a high ISI (>0.8; Lloyd, 1965). However, the strength of self-incompatibility was not uniform. Neotetraploids presented higher variability and slightly lower ISI than diploids, with some partially self-compatible individuals ($0.2 < ISI < 0.8$) and even a few self-compatible plants ($ISI < 0.2$; Lloyd, 1965). The variation observed in neotetraploids contrast with a strong self-incompatibility detected in diploids ($ISI > 0.9$), suggesting changes in the GSI system following genome duplication.

Breakdown of gametophytic self-incompatibility has been described as a direct result of genome duplications (e.g., Solanaceae, Rosaceae and other families; Lewis, 1947; Pandey et al., 1968; Entani et al., 1999; Adachi et al., 2009; Sutherland et al., 2018), in which pollen tubes containing two copies of the same pollen S-allele (homoallelic) are arrested if the cognate S-RNase is present in the pistil, while pollen grains containing two different pollen S-alleles (heteroallelic) are compatible and thus are allowed to fertilize (de Nettancourt, 1977; Entani et al., 1999; Luu et al., 2000), although accumulation of non-functional S haplotypes has also been shown (e.g., sour cherry; Hauck et al., 2006; Tao and Lezzoni, 2010). In diploids, the haploid gametophyte expresses a single protein and is arrested if recognized by S-RNases in the pistil; however, tetraploids will produce a mixture of homoallelic and heteroallelic gametophytes and self-fertilization will be reduced but not eliminated. This mechanism is consistent with incompatibility breakdown in other Campanulaceae (Sutherland et al., 2018) and with leaky self-incompatibility observed in *J. maritima* neotetraploids.

Variation in the strength of self-incompatibility with genome duplications may have increased the likelihood of successful establishment of *J. maritima* neotetraploid, when the population of neotetraploids is small and the number of available mates is limited. Newly formed neotetraploids, in sympatry with their diploid counterparts, are subjected to frequency-dependent selection, having difficulties in persisting and in establishing viable populations (Levin, 1975; Thompson and Lumaret, 1992; Husband, 2000). Under this scenario, some levels of self-compatibility will enable the production of viable offspring. Selfing may thus ameliorate the negative effects associated with minority cytotype disadvantage (Levin, 1975; Rodriguez, 1996; Rausch and Morgan, 2005; Fowler and Levin, 2016), has shown before in natural populations (e.g., Baack, 2005; Buggs and Pannel, 2006; Husband et al., 2008). By reducing the fitness costs of intercytotype mating due to neotetraploid minority status, self-compatibility opens the possibility for selfing, increasing the probability of neotetraploid establishment within diploid populations.

Fitness of self and outcross offspring

The primary force governing selfing is inbreeding depression (Lande and Schemske, 1985; Charlesworth and Charlesworth, 1987; Lloyd, 1992; Husband and Schemske, 1996, 1997). Here we observed that, contrarily to diploids that presented a significant reduction in the fitness of selfed progeny relative to outcrossed one, neotetraploid selfed and outcrossed offspring presented similar fitness performances for the studied traits. Consequently, neotetraploids presented tendentially lower levels of inbreeding depression than diploids. The few available studies report lower inbreeding levels in polyploids compared with diploid relatives (e.g., Husband and Schemske, 1997; Rosquist, 2001; Galloway et al., 2003; Husband et al., 2008; Ozimec and Husband, 2011). Polyploidization may decrease the magnitude of inbreeding depression due to immediate masking of deleterious genes (Lande and Schemske, 1985), although this may vary with the magnitude of mutational effects and dominance relations (Lande and Schemske, 1985; Ronfort, 1999). This shield against the effects of inbreeding depression may favor offspring production through self-reproduction, increasing the number of neotetraploid individuals.

However, repeated selfing will have medium- to long-term consequences for inbreeding levels and plant fitness, reaching a point where selfing might no longer be favorable (Lloyd, 1992; Ozimec and Husband, 2011). Continuous self-reproduction increases the level of homozygosity, exposing deleterious recessive mutations and, consequently, causing reduced fitness on inbred progeny (Charlesworth

and Charlesworth, 1987; Lynch and Walsh, 1998; Stone, 2002). In *J. maritima*, the high proportion of self-incompatible plants and presence of some partially self-compatible ones suggest that, while selfing may represent a reproductive assurance being strongly favored in initial generations (e.g., after polyploid formation or long-distance dispersal; Ozimec and Husband, 2011; Costa et al., 2017; Sutherland et al., 2018), it could be a transient state (Schemske and Lande, 1985), with outcross being favored in future scenarios in case inbreeding depression levels increase (Ozimec and Husband, 2011). Thus, an intermediate breakdown of the SI system in some *J. maritima* individuals may benefit neopolyploid establishment, enabling the formation of some viable offspring, while maintaining the capacity for outcrossing (Lloyd, 1992).

Direct effects of whole genome duplications

In this study, by comparing diploids with neotetraploids, it was possible to disentangle the direct effects of whole genome duplications. Besides the effects described above, genome duplications clearly affected stomata size and density. Bigger cells and organ size have been described as a direct effect of increased DNA content in polyploid organisms (the so-called "gigas effect"; Stebbins, 1971; Ramsey and Ramsey, 2014). Here, neotetraploids of *J. maritima* have bigger stomata at lower densities when compared with diploids. This pattern has long been documented in polyploid plants (e.g., Sax and Sax, 1937; Masterson, 1994; Mishra, 1997; Maherali et al., 2009) and may significantly affect physiologic performance and ecological tolerances of polyploid plants (Levin, 2002). For example, a trade-off between stomata size and number may facilitate the maintenance of photosynthesis rates under water stressful conditions after polyploidization (Li et al., 1996). Several studies pointed that polyploids tend to have higher drought tolerances in stress environments, such as dune systems (Li et al., 1996; Li et al., 2009; Pustovoitova et al., 1996; Allario et al., 2013). Here, *J. maritima* neotetraploids presented higher values for water status (relative water content), an indicator of plant sensitivity to dehydration (Sánchez-Rodríguez et al., 2010). Higher relative water content may act as a buffer in scenarios of water deficit, delaying water potential threshold and increasing plant resistance to drought.

Chlorophyll fluorescence parameters, an overall indicator of photosynthesis, were also higher in neotetraploids. However, this higher efficiency was not reflected in increased carbohydrates (total soluble sugars) and biomass production. Likely, neotetraploids invest more resources in defense mechanisms (e.g., increase of antioxidant battery) compromising growth, while diploids allocate most of their resources in growth. Polyploids are referred to have enhanced accumulation of secondary metabolites and antioxidant enzymes, conferring increased tolerance and enabling them to better cope with stressful conditions (Zhang et al., 2010; Tan et al., 2015; Kong et al., 2017). Further studies exploring physiologic traits and ecological tolerances of *J. maritima* cytotypes under stress gradients are needed to confirm these hypotheses.

CONCLUSIONS

Neotetraploids of *J. maritima* var. *maritima* might have benefited from selfing at initial stages after their formation. Genome duplications resulted in leaky self-incompatibility enabling the production of offspring under minority cytotype disadvantage, with selfed offspring having similar fitness as outcrossed ones. This work supports theoretical assumptions that selfing might be important in neopolyploid establishment phase, although changes in self-incompatibility might not be abrupt and variability in the expression of self-incompatibility is expected.

ACKNOWLEDGMENTS

The authors are thankful to Lucie Mota and Marta Gonçalves for all their assistance in the pollination experiment. This research was supported by POPH/FSE funds by the Portuguese Foundation for Science and Technology (FCT) with a doctoral grant to MC (SFRH/BD/89910/2012), a starting grant and exploratory project to SC (IF/01267/2013), a junior research contract to MCD (SFRH/BPD/100865/2014), and by Project RENATURE financed by the "Programa Operacional Regional do Centro 2014-2020 (Centro2020) - CENTRO-01-0145-FEDER-000007". The authors also thank two anonymous reviewers and the editor for their helpful suggestions to improve the manuscript.

AUTHOR CONTRIBUTIONS

S.C., M.C., and J.L. designed the experiment and conducted field collections; C.S., M.C., S.C., and M.C.D. conducted the common garden experiment; C.S. and M.C.D. conducted laboratory analyses; C.S. analyzed the data with the other authors participating in the discussion of the results; C.S. and S.C., with contribution of all the authors, wrote the manuscript.

DATA AVAILABILITY

Data set results of the measured parameters are available at FigShare: https://doi.org/10.6084/m9.figshare.11592642.v1.

SUPPORTING INFORMATION

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

APPENDIX S1. Plant developmental and physiological performance under self- and outcross pollination treatments (self and out, respectively) for diploids (2*x*) and neotetraploids (neo4*x*) of *Jasione maritima* var. *maritima*: (A) Aboveground biomass (mg). (B) Belowground biomass (mg). (C) Starch content ($mg \cdot g^{-1}$ fresh mass). (D) Chlorophyll *a* content ($mg \cdot g^{-1}$ fresh mass). (E) Chlorophyll *b* content ($mg \cdot g^{-1}$ fresh mass). (F) Carotenoid content ($mg \cdot g^{-1}$ fresh mass). Values are presented as mean and standard error of the mean; different letters represent statistical differences at $P < 0.05$; n.s. denotes nonsignificant differences at $P > 0.05$.

LITERATURE CITED

Adachi, Y., S. Komori, Y. Hoshikawa, N. Tanaka, K. Abe, H. Bessho, M. Watanabe, and A. Suzuki. 2009. Characteristics of fruiting and pollen tube growth of apple autotetraploid cultivars showing self-compatibility. *Journal of the Japanese Society for Horticultural Science* 78: 402–409.

Adams, K. L., and J. F. Wendel. 2005. Polyploidy and genome evolution in plants. *Current opinion in plant biology* 8: 135–141.

Ægisdóttir, H. H., D. Jespersen, P. Kuss, and J. Stöcklin. 2007. No inbreeding depression in an outcrossing alpine species: the breeding system of *Campanula thyrsoides*. *Flora-Morphology, Distribution, Functional Ecology of Plants* 202: 218–225.

Albert, V. A., W. B. Barbazuk, J. P. Der, J. Leebens-Mack, H. Ma, J. D. Palmer, S. Rounsley, et al. 2013. The *Amborella* genome and the evolution of flowering plants. *Science* 342: 1241089.

Allario, T., J. Brumos, J. M. Colmenero-Flores, D. J. Iglesias, J. A. Pina, L. Navarro, M. Talon, et al. 2013. Tetraploid Rangpur lime rootstock increases drought tolerance via enhanced constitutive root abscisic acid production. *Plant, cell & environment* 36: 856–868.

Baack, E. J. 2005. To succeed globally, disperse locally: effects of local pollen and seed dispersal on tetraploid establishment. *Heredity* 94: 538.

Baker, H. G. 1955. Self-compatibility and establishment after 'long distance' dispersal. *Evolution* 9: 347–348.

Barrett, S. C. 2002. Evolution of sex: the evolution of plant sexual diversity. *Nature reviews genetics* 3: 274.

Barringer, B. C. 2007. Polyploidy and self-fertilization in flowering plants. *American Journal of Botany* 94: 1527–1533.

Bates, D., M. Maechler, B. Bolker, and S. Walker. 2014. lme4: linear mixed-effects models using Eigen and S4. Website: http://CRAN.R-project.org/package=lme4 [accessed 5 October 2019].

Bolker, B. M., M. E. Brooks, C. J. Clark, S. W. Geange, J. R. Poulsen, M. H. H. Stevens, and J. S. S. White. 2009. Generalized linear mixed models: a practical guide for ecology and evolution. *Trends in Ecology & Evolution* 24: 127–135.

Brennan, A. C., S. A. Harris, and S. J. Hiscock. 2005. Modes and rates of selfing and associated inbreeding depression in the self-incompatible plant Senecio squalidus (Asteraceae): a successful colonizing species in the British Isles. *New Phytologist* 168: 475–486.

Brochmann, C., A. K. Brysting, I. G. Alsos, L. Borgen, H. H. Grundt, A. C. Scheen, and R. Elven. 2004. Polyploidy in arctic plants. *Biological journal of the Linnean society* 82: 521–536.

Buggs, R. J., and J. R. Pannell. 2006. Rapid displacement of a monoecious plant lineage is due to pollen swamping by a dioecious relative. *Current Biology* 16: 996–1000.

Castro, S., Z. Münzbergová, J. Raabová, and J. Loureiro. 2011. Breeding barriers at a diploid–hexaploid contact zone in *Aster amellus*. *Evolutionary Ecology* 25: 795–814.

Castro, M., S. Castro, and J. Loureiro. 2018. Production of synthetic tetraploids as a tool for polyploid research. *Web Ecology* 18: 129–141.

Charlesworth, D., and B. Charlesworth. 1987. Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology, Evolution, and Systematics*. 18: 237–268.

Comai, L. 2005. The advantages and disadvantages of being polyploid. *Nature Reviews Genetics* 6: 836.

Cook, L. M., and P. S. Soltis. 1999. Mating systems of diploid and allotetraploid populations of *Tragopogon* (Asteraceae). I. Natural populations. *Heredity* 82: 237.

Cook, L. M., and P. S. Soltis. 2000. Mating systems of diploid and allotetraploid populations of *Tragopogon* (Asteraceae). II. Artificial populations. *Heredity* 84: 410.

Costa, J., V. Ferrero, M. Castro, J. Loureiro, L. Navarro, and S. Castro. 2017. Variation in the incompatibility reactions in tristylous *Oxalis pes-caprae*: large-scale screening in South African native and Mediterranean basin invasive populations. *Perspectives in Plant Ecology, Evolution and Systematics* 24: 25–36.

de Nettancourt, D. 1977. The genetic basis of self-incompatibility. *In* Incompatibility in angiosperms, 28–57. Springer, Berlin, Germany.

Doležel, J., J. Greilhuber, and J. Suda. 2007. Flow cytometry with plant cells: analysis of genes, chromosomes and genomes. Wiley-VCH, Weinheim, Germany.

Doležel, J., S. Sgorbati, and S. Lucretti. 1992. Comparison of three DNA fluorochromes for flow cytometric estimation of nuclear DNA content in plants. *Physiologia Plantarum* 85: 625–631.

Doyle, J. J., and J. E. Coate. 2019. Polyploidy, the nucleotype, and novelty: the impact of genome doubling on the biology of the cell. *International Journal of Plant Sciences* 180: 1–52.

Entani, T., S. Takayama, M. Iwano, H. Shiba, F. S. Che, and A. Isogai. 1999. Relationship between polyploidy and pollen self-incompatibility phenotype in *Petunia hybrida* Vilm. *Bioscience, Biotechnology, and Biochemistry* 63: 1882–1888.

Felber, F. 1991. Establishment of a tetraploid cytotype in a diploid population: effect of relative fitness of the cytotypes. *Journal of Evolutionary Biology* 4: 195–207.

Felber, F., and J. D. Bever. 1997. Effect of triploid fitness on the coexistence of diploids and tetraploids. *Biological Journal of the Linnean Society* 60: 95–106.

Fowler, N. L., and D. A. Levin. 1984. Ecological constraints on the establishment of a novel polyploid in competition with its diploid progenitor. *The American Naturalist* 124: 703–711.

Fowler, N. L., and D. A. Levin. 2016. Critical factors in the establishment of allopolyploids. *American Journal of Botany* 103: 1236–1251.

Fox, J., S. Weisberg, D. Adler, D. Bates, G. Baud-Bovy, and S. Ellison. 2015. car: Companion to applied regression. Website: http://CRAN.R-project.org/package=car [accessed 5 October 2019].

Galloway, L. F., J. R. Etterson, and J. L. Hamrick. 2003. Outcrossing rate and inbreeding depression in the herbaceous autotetraploid, *Campanula americana*. *Heredity* 90: 308–315.

Good-Avila, S. V., D. Majumder, H. Amos, and A. G. Stephenson. 2008. Characterization of self-incompatibility in *Campanula rapunculoides* (Campanulaceae) through genetic analyses and microscopy. *Botany* 86: 1–13.

Grant, V. 1956. The influence of breeding habit on the outcome of natural hybridization in plants. *The American Naturalist* 90: 319–322.

Hao, G. Y., M. E. Lucero, S. C. Sanderson, E. H. Zacharias, and N. M. Holbrook. 2013. Polyploidy enhances the occupation of heterogeneous environments through hydraulic related trade-offs in *Atriplex canescens* (Chenopodiaceae). *New Phytologist* 197: 970–978.

Hauck, N. R., K. Ikeda, R. Tao, and A. F. Iezzoni. 2006. The mutated S1-haplotype in sour cherry has an altered S-haplotype–specific F-box protein gene. *Journal of Heredity* 97: 514–520.

Hedrick, P. W. 1987. Genetic load and the mating system in homosporous ferns. *Evolution* 41: 1282–1289.

Hollister, J. D. 2015. Polyploidy: adaptation to the genomic environment. *New Phytologist* 205: 1034–1039.

Husband, B. C. 2000. Constraints on polyploid evolution: a test of the minority cytotype exclusion principle. *Proceedings of the Royal Society of London, B, Biological Sciences* 267: 217–223.

Husband, B. C. 2016. Effect of inbreeding on pollen tube growth in diploid and tetraploid *Chamerion angustifolium*: Do polyploids mask mutational load in pollen? *American Journal of Botany* 103: 532–540.

Husband, B. C., and D. W. Schemske. 1996. Evolution of the magnitude and timing if inbreeding depression in plants. *Evolution* 50: 54–70.

Husband, B. C., and D. W. Schemske. 1997. The effect of inbreeding depression in diploid and tetraploid populations of *Epilobium angustifolium* (Onagraceae): implications for the genetic basis of inbreeding depression. *Evolution* 51: 737–746.

Husband, B. C., B. Ozimec, S. L. Martin, and L. Pollock. 2008. Mating consequences of polyploid evolution in flowering plants: current trends and insights from synthetic polyploids. *International Journal of Plant Sciences* 169: 195–206.

Husband, B. C., S. J. Baldwin, and H. A. Sabara. 2016. Direct vs. indirect effects of whole-genome duplication on prezygotic isolation in *Chamerion angustifolium*: Implications for rapid speciation. *American Journal of Botany* 103: 1259–1271.

Irigoyen, J. J., D. W. Einerich, and M. Sánchez-Díaz. 1992. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfalfa (*Medicago sativa*) plants. *Physiologia Plantarum* 84: 55–60.

Jersáková, J., S. Castro, N. Sonk, K. Milchreit, I. Schödelbauerová, T. Tolasch, and S. Dötterl. 2010. Absence of pollinator-mediated premating barriers in mixed-ploidy populations of *Gymnadenia conopsea* sl (Orchidaceae). *Evolutionary Ecology* 24: 1199–1218.

Johnston, M. O., and D. J. Schoen. 1996. Correlated evolution of self-fertilization and inbreeding depression: an experimental study of nine populations of Amsinckia (Boraginaceae). *Evolution* 50: 1478–1491.

Kalisz, S., D. W. Vogler, and K. M. Hanley. 2004. Context-dependent autonomous self-fertilization yields reproductive assurance and mixed mating. *Nature* 430: 884.

Kennedy, B. F., H. A. Sabara, D. Haydon, and B. C. Husband. 2006. Pollinator-mediated assortative mating in mixed ploidy populations of *Chamerion angustifolium* (Onagraceae). *Oecologia* 150: 398–408.

Kong, D., Y. Li, M. Bai, Y. Deng, G. Liang, and H. Wu. 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 Physiology and Biochemistry* 112: 87–96.

Lande, R., and D. W. Schemske. 1985. The evolution of self-fertilization and inbreeding depression in plants. I. Genetic models. *Evolution* 39: 24–40.

Lenth, R.V. 2016. Least-squares means: the R package lsmeans. *Journal of Statistical Software* 69: 1–33. Website: https://cran.r-project.org/package=lsmeans [accessed 5 October 2019].

Levan, A. 1936. Polyploidy and self-fertility in Allium. *Hereditas* 22: 278–280.

Levin, D. A. 1975. Minority cytotype exclusion in local plant populations. *Taxon* 24: 35–43.

Levin, D. A. 1983. Polyploidy and novelty in flowering plants. *The American Naturalist* 122: 1–25.

Levin, D. A. 2002. The role of chromosomal change in plant evolution. Oxford University Press.

Lewis, D. 1947. Competition and dominance of incompatibility alleles in diploid pollen. *Heredity* 1: 85.

Li, W. L., G. P. Berlyn, and P. M. S. Ashton. 1996. Polyploids and their structural and physiological characteristics relative to water deficit in *Betula papyrifera* (Betulaceae). *American Journal of Botany* 83: 15–20.

Li, W. D., D. K. Biswas, H. Xu, C. Q. Xu, X. Z. Wang, J. K. Liu, and G. M. Jiang. 2009. Photosynthetic responses to chromosome doubling in relation to leaf anatomy in *Lonicera japonica* subjected to water stress. *Functional Plant Biology* 36: 783–792.

Lloyd, D. G. 1965. Evolution of self-compatibility and racial differentiation in *Leavenworthia* (Cruciferae). *Contributions from the Gray Herbarium of Harvard University* 195: 3–134.

Lloyd, D. G. 1992. Self-and cross-fertilization in plants. II. The selection of self-fertilization. *International Journal of Plant Sciences* 153: 370–380.

Loureiro, J., E. Rodriguez, J. Doležel, and C. Santos. 2007. Two new nuclear isolation buffers for plant DNA flow cytometry: a test with 37 species. *Annals of Botany* 100: 875–888.

Luu, D. T., X. Qin, D. Morse, and M. Cappadocia. 2000. S-RNase uptake by compatible pollen tubes in gametophytic self-incompatibility. *Nature* 407: 649.

Lynch, M., and B. Walsh. 1998. Genetics and analysis of quantitative traits, vol. 1, 535–557. Sinauer, Sunderland, MA.

Mable, B. K. 2004. Polyploidy and self-compatibility: is there an association? *New Phytologist* 162: 803–811.

Maherali, H., A. E. Walden, and B. C. Husband. 2009. Genome duplication and the evolution of physiological responses to water stress. *New Phytologist* 184: 721–731.

Masterson, J. 1994. Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. *Science* 264: 421–424.

Mishra, M. K. 1997. Stomatal characteristics at different ploidy levels in *Coffea* L. *Annals of Botany* 80: 689–692.

Mittelsten Scheid, O., L. Jakovleva, K. Afsar, J. Maluszynska, and J. Paszkowski. 1996. A change of ploidy can modify epigenetic silencing. *Proceedings of the National Academy of Sciences, USA* 93: 7114–7119.

Münzbergová, Z. 2017. Colchicine application significantly affects plant performance in the second generation of synthetic polyploids and its effects vary between populations. *Annals of Botany* 120: 329–339.

Nuismer, S. L., and B. M. Cunningham. 2005. Selection for phenotypic divergence between diploid and autotetraploid *Heuchera grossulariifolia*. *Evolution* 59: 1928–1935.

Osaki, M., T. Shinano, and T. Tadano. 1991. Redistribution of carbon and nitrogen compounds from the shoot to the harvesting organs during maturation in field crops. *Soil Science and Plant Nutrition* 37: 117–128.

Oswald, B. P., and S. L. Nuismer. 2011a. A unified model of autopolyploid establishment and evolution. *The American Naturalist* 178: 687–700.

Oswald, B. P., and S. L. Nuismer. 2011b. Neopolyploidy and diversification in *Heuchera grossulariifolia*. *Evolution* 65: 1667–1679.

Ozimec, B., and B. C. Husband. 2011. Effect of recurrent selfing on inbreeding depression and mating system evolution in an autopolyploid plant. *Evolution* 65: 2038–2049.

Pandey, K. K. 1968. Colchicine-induced changes in the self-incompatibility behaviour of *Nicotiana*. *Genetica* 39: 257–271.

Pannell, J. R., and S. C. Barrett. 1998. Baker's law revisited: reproductive assurance in a metapopulation. *Evolution* 52: 657–668.

Parnell, J. 1987. Variation in *Jasione montana* L. (Campanulaceae) and related species in Europe and North Africa. *Watsonia* 16: 249–267.

Petit, C., P. Lesbros, X. Ge, and J. D. Thompson. 1997. Variation in flowering phenology and selfing rate across a contact zone between diploid and tetraploid *Arrhenatherum elatius* (Poaceae). *Heredity* 79: 31.

Pustovoitova, T. N., N. E. Zhdanova, V. N. Zholkevich, G. V. Eremin, and E. G. Rassvetaeva. 1996. Drought resistance, recovery capacity, and phytohormone content in polyploid plum leaves. *Russian Journal of Plant Physiology* 43: 232–235.

Ramsey, J. 2007. Unreduced gametes and neopolyploids in natural populations of *Achillea borealis* (Asteraceae). *Heredity* 98: 143.

Ramsey, J. 2011. Polyploidy and ecological adaptation in wild yarrow. *Proceedings of the National Academy of Sciences* 108: 7096–7101.

Ramsey, J., and T. S. Ramsey. 2014. Ecological studies of polyploidy in the 100 years following its discovery. *Philosophical Transactions of the Royal Society, B, Biological Sciences* 369: 20130352.

Ramsey, J., and D. W. Schemske. 2002. Neopolyploidy in flowering plants. *Annual Review of Ecology, Evolution, and Systematics* 33: 589–639.

Rastogi, S., and D. A. Liberles. 2005. Subfunctionalization of duplicated genes as a transition state to neofunctionalization. *BMC Evolutionary Biology* 5: 28.

Rausch, J. H., and M. T. Morgan. 2005. The effect of self-fertilization, inbreeding depression, and population size on autopolyploid establishment. *Evolution* 59: 1867–1875.

Redmann, R. E., J. Haraldson, and L. V. Gusta. 1986. Leakage of UV-absorbing substances as a measure of salt injury in leaf tissue of woody species. *Physiologia Plantarum* 67: 87–91.

Risso-Pascotto, C., M. S. Pagliarini, and C. B. do Valle. 2005. Multiple spindles and cellularization during microsporogenesis in an artificially induced tetraploid accession of *Brachiaria ruziziensis* (Gramineae). *Plant Cell Reports* 23: 522–527.

Robertson, K., E. E. Goldberg, and B. Igić. 2011. Comparative evidence for the correlated evolution of polyploidy and self-compatibility in Solanaceae. *Evolution: International Journal of Organic Evolution* 65: 139–155.

Rodriguez, D. J. 1996. A model for the establishment of polyploidy in plants. *The American Naturalist* 147: 33–46.

Ronfort, J. 1999. The mutation load under tetrasomic inheritance and its consequences for the evolution of the selfing rate in autotetraploid species. *Genetics Research* 74: 31–42.

Rosquist, G. 2001. Reproductive biology in diploid *Anthericum ramosum* and tetraploid *A. liliago* (Anthericaceae). *Oikos* 92: 143–152.

Sánchez-Rodríguez, E., M. Rubio-Wilhelmi, L. M. Cervilla, B. Blasco, J. J. Rios, M. Rosales, L. Romero, and J. M. Ruiz. 2010. Genotypic differences in some physiological parameters symptomatic for oxidative stress under moderate drought in tomato plants. *Plant Science* 178: 30–40.

Sax, K., and H. J. Sax. 1937. Stomata size and distribution in diploid and polyploid plants. *Journal of the Arnold Arboretum* 18: 164–172.

Schemske, D. W., and R. Lande. 1985. The evolution of self-fertilization and inbreeding depression in plants. II. Empirical observations. *Evolution* 39: 41–52.

Segraves, K. A., and J. N. Thompson. 1999. Plant polyploidy and pollination: floral traits and insect visits to diploid and tetraploid *Heuchera grossulariifolia*. *Evolution* 53: 1114–1127.

Sims, D. A., and J. A. Gamon. 2002. Relationships between leaf pigment content and spectral reflectance across a wide range of species, leaf structures and developmental stages. *Remote Sensing of Environment* 81: 337–354.

Soltis, D. E., V. A. Albert, J. Leebens-Mack, C. D. Bell, A. H. Paterson, C. Zheng, D. Sankoff, et al. 2009. Polyploidy and angiosperm diversification. *American Journal of Botany* 96: 336–348.

Soltis, D. E., R. J. Buggs, J. J. Doyle, and P. S. Soltis. 2010. What we still don't know about polyploidy. *Taxon* 59: 1387–1403.

Stebbins, G. L. 1971. Chromosomal Evolution in Higher Plants. Edward Arnold Ltd., London.

Stephenson, A. G., S. V. Good, and D. W. Vogler. 2000. Interrelationships among inbreeding depression, plasticity in the self-incompatibility system, and the breeding system of *Campanula rapunculoides* L. (Campanulaceae). *Annals of Botany* 85: 211–219.

Stone, J. L. 2002. Molecular mechanisms underlying the breakdown of gametophytic self-incompatibility. *The Quarterly Review of Biology* 77: 17–32.

Suda, J., and T. Herben. 2013. Ploidy frequencies in plants with ploidy heterogeneity: fitting a general gametic model to empirical population data. *Proceedings of the Royal Society, B, Biological Sciences* 280: 20122387.

Sutherland, B. L., B. M. Quarles, and L. F. Galloway. 2018. Intercontinental dispersal and whole-genome duplication contribute to loss of self-incompatibility in a polyploid complex. *American Journal of Botany* 105: 249–256.

Tan, F. Q., H. Tu, W. J. Liang, J. M. Long, X. M. Wu, H. Y. Zhang, and W. W. Guo. 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 Biology* 15: 89.

Tao, R., and A. F. Iezzoni. 2010. The S-RNase-based gametophytic self-incompatibility system in *Prunus* exhibits distinct genetic and molecular features. *Scientia Horticulturae* 124: 423–433.

Thompson, J. D., and R. Lumaret. 1992. The evolutionary dynamics of polyploid plants: Origins, establishment and persistence. *Trends in Ecology and Evolution* 7: 302–307.

Van Drunen, W. E., and B. C. Husband. 2018. Immediate vs. evolutionary consequences of polyploidy on clonal reproduction in an autopolyploid plant. *Annals of Botany* 122: 195–205.

Weyers, J. D., and A. J. Travis. 1981. Selection and preparation of leaf epidermis for experiments on stomatal physiology. *Journal of Experimental Botany* 32: 837–850.

Yeo, P. F. 1993. Secondary pollen presentation: form, function and evolution. Springer Science & Business Media, Berlin, Germany.

Zhang, X. Y., C. G. Hu, and J. L. Yao. 2010. Tetraploidization of diploid *Dioscorea* results in activation of the antioxidant defence system and increased heat tolerance. *Journal of Plant Physiology* 167: 88–94.