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RESEARCH PAPER

Polyploidy affects the seed, dormancy and seedling characteristics of a perennial grass, conferring an advantage in stressful climates

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ABSTRACT

- Polyploidy (the state of having more than two genome copies) is widely distributed in flowering plants and can vary within species, with polyploid races often associated with broad ecological tolerances. Polyploidy may influence within-species variation in seed development, germination and establishment. We hypothesized that interactions between polyploidy and the seed developmental environment would affect subsequent dormancy, germination and early growth traits, particularly in stressful environments.
- Using seeds developed in a common garden under ambient and warmed conditions, we conducted germination trials under drought and temperature stress, and monitored the subsequent growth of seedlings. The study species, *Themeda triandra*, is a widespread, keystone, Australian native grass and a known polyploid complex.
- Tetraploid plants produced heavier, more viable seeds than diploids. Tetraploids were significantly more dormant than diploids, regardless of seed developmental environment. Non-dormant tetraploids were more sensitive to germination stress compared to non-dormant diploids. Finally, tetraploid seedlings were larger and grew faster than diploids, usually when maternal plants were exposed to developmental temperatures atypical to the source environment.
- Seed and seedling traits suggest tetraploids are generally better adapted to stressful environments than diploids. Because tetraploid seeds of *T. triandra* are more dormant they are less likely to germinate under stress, and when they do germinate, seedling growth is rapid and independent of seed developmental environment. These novel results demonstrate that polyploidy, sometimes in interaction with developmental environment and possibly also asexuality, can have within-species variation in seed and seedling traits that increase fitness in stressful environments.

INTRODUCTION

Physical seed traits, dormancy, germination and subsequent seedling establishment have direct relevance for plant fitness and persistence (Cochrane *et al.* 2015b) and are essential elements in the ecology and evolution of plant life histories (Simons & Johnston 2000; Jiménez-Alfaro *et al.* 2016; Larson & Funk 2016). Existing research on plant fitness and adaptation to novel environments has often overlooked the early developmental stages of plant life (however, see Moyers & Kane 2010; Dwyer & Erickson 2016) despite these early developmental stages of plants having been identified as a major bottleneck to plant recruitment (Lloret *et al.* 2004; Fay & Schultz 2009). Critical seed traits, including germination and dormancy, commonly show significant intraspecific and inter-population variation, which may confer both a broad geographic range under current climate conditions, and an increased ability to withstand a changing climate (Walck *et al.* 2011). However, patterns of within-species variation in seed traits, and the mechanisms that underpin that variation, remain poorly understood and are often overlooked when considering adaptation to novel or stressful environments (Cochrane *et al.* 2015a).

Seed dormancy enables plants to avoid germination under stressful conditions, and is thought to have evolved to ensure that germination occurs at a time when the likelihood of seedling survival is highest (Hilhorst 1995; Vleeshouwers *et al.* 1995). Dormancy can be governed by environmental and genetic factors, and seeds can exhibit differences in the status and degree of dormancy (Cross *et al.* 2018). The temporal delay of germination imposed by dormancy can lead to larger dispersal distances from the mother plant and stagger germination, which can increase the chance of germination success and decrease competition between siblings (Vleeshouwers *et al.* 1995; Baskin & Baskin 2014). The timing of dormancy alleviation can govern the spatial and temporal distribution of seeds and seedlings, factors which greatly influence the microenvironment to which seedlings are exposed at later life stages (de Casas *et al.* 2015). Seed dormancy is advantageous in variable and unpredictable climates (Ooi 2012), since seeds with lower

dormancy status may be more likely to germinate in suboptimal conditions.

Germination also plays a key role in species' persistence because it sets the circumstances for subsequent plant development and selection (Donohue *et al.* 2010). The environmental cues that trigger germination (*e.g.* water availability and temperature) are often highly specific and therefore germination can be sensitive to environmental change and variability (Probert 2000; Fay & Schultz 2009; Cochrane *et al.* 2014; Edwards *et al.* 2016). The range of temperatures under which a seed will germinate, its thermal germination niche, is one way in which seeds can vary in environmental response (Cochrane *et al.* 2014). Within-species variation in germination characteristics can assist plant populations to persist in variable and changing climates.

Seedling establishment and early growth following germination are also critical stages of plant development and are also highly sensitive to environmental factors and variability therein (Fay & Schultz 2009). Across species, seedling size often correlates with seed size (Bretagnolle *et al.* 1995; Kidson & Westoby 2000). Larger seedlings are better able to compete for critical resources such as nutrients and light, often due to faster growth, resulting in increased adult fitness (Moles & Westoby 2004). Fast growth rates are beneficial to seedling survival, particularly when environmental conditions may be unpredictable, as an ability to acquire resources is necessary when such resources might be limited. Seedling size and biomass allocation are important indicators of plant fitness, in particular, a plant's ability to access and compete for resources in novel environments (Funk *et al.* 2008). Early growth responses will affect which plants survive in a stressful or changing climate. Seedling establishment and early growth have been used to monitor the effects of climate change on plant communities (Wookey *et al.* 1995; Sternberg *et al.* 1999; Classen *et al.* 2010) as these traits are particularly suitable for detecting future alterations in community composition as a consequence of climate change (Lloret *et al.* 2004; Hoyle *et al.* 2013).

The environmental conditions to which seeds are exposed during development have significant consequences for variation in seed and early life traits (Nicotra *et al.* 2010; Walck *et al.* 2011; Zhang *et al.* 2012). Seed mass, seed longevity, seed dormancy, germination percentage and rate, seedling establishment and growth are all influenced by seed developmental environment (Finch-Savage & Leubner-Metzger 2006; Walck *et al.* 2011). Both warmer developmental temperatures and maternal drought stress can decrease seed dormancy (Steadman *et al.* 2004; Carta *et al.* 2016), and seeds with reduced dormancy status may be more likely to germinate in unsuitable conditions (Ooi 2012). Higher germination proportions and faster germination rates have also been observed in seeds developed in a warmer maternal environment (Hoyle *et al.* 2008; Zhang *et al.* 2012; Dwyer & Erickson 2016). Thus, seed developmental environment can impact the conditions that plants are exposed to throughout their life, and have implications for plant fitness.

As well as the effects of developmental environment, the conditions to which a seed is exposed after dispersal also affect germination rate and proportion. In many cases, relatively warm conditions during germination accelerate germination rate and increase final germination proportion until an optimum temperature is reached; beyond which the rate and proportion decline rapidly (Fenner 1991; Blödner *et al.* 2007; Walck *et al.* 2011; Mondoni *et al.* 2012). Given that warmer environments tend to reduce dormancy and increase the rate and proportion of germination, a warming climate may significantly alter reproductive success due to seedlings being exposed to conditions non-optimal for survival. These cumulative effects can lead to reductions in the number of seeds persisting in the soil seed bank (Dwyer & Erickson 2016), and ultimately drastically change population stability and persistence. Elucidating the interactions between environmental cues and variation in seed and seedling traits is therefore a vital step in understanding future plant population dynamics in response to global change.

Polyploidy (often resulting from whole genome duplication), the heritable condition of possessing more than two complete sets of chromosomes (Comai 2005; Ramsey 2011), has been a key evolutionary mechanism in the diversification of flowering plants (Madlung 2013; Fialová *et al.* 2014). Ploidy level can vary within a species, and polyploid cytotypes often occur in climatically more variable or extreme habitats than their diploid progenitors (te Beest *et al.* 2012; Ramsey & Ramsey 2014). One hypothesis is that polyploidy conveys evolutionary or competitive advantages in novel or stressful environments (Fawcetta *et al.* 2009; Eliášová & Münzbergová 2014). However, despite abundant evidence for ploidy-linked variation in physiological and morphological traits in adult plants (*e.g.* Masterson 1994; Li *et al.* 1996, 2009; Hodgson *et al.* 2010), the ecological consequences of polyploidy for early life traits and reproductive success are relatively little studied (Eliášová & Münzbergová 2014; Godfree *et al.* 2017). The few studies that do exist indicate that polyploidy can increase seed weight (Bretagnolle *et al.* 1995; Eliášová & Münzbergová 2014; Godfree *et al.* 2017), perhaps seed dormancy (Hacker 1988), and both germination percentage and rate (Bretagnolle *et al.* 1995; Hahn *et al.* 2013). At least some of these traits can alter seedling competition and other elements of life history (Moles & Westoby 2004; Zhang *et al.* 2012). Whether such differences may be advantageous under climate change, and what ecological, physiological and evolutionary roles (Walck *et al.* 2011; Madlung 2013) polyploidy may play in determining early life cycle traits, remains poorly understood.

This research aimed to test the long-standing hypothesis in evolutionary biology that polyploid genotypes have a fitness advantage over diploids in climatically variable or extreme habitats. We investigated this by focusing on the generally overlooked early life history (physical seed traits, seed dormancy, germination and seedling characteristics) and intergenerational effects among diploid and tetraploid populations of the keystone C_4 grass species, *Themeda triandra*. Previous work (Godfree *et al.* 2017) has shown that tetraploid *T. triandra* plants maintain higher reproductive output under increased abiotic stress than diploid plants, and also display fixed differences in seed development (larger seed) that are likely beneficial in such environments. However, it is not known whether the seeds produced by tetraploids also display polyploid advantage in other important traits, such as dormancy and germination. Here, we aim to provide some of the first experimental research results addressing polyploid advantage in seed and seedling traits, and whether these might provide an intergenerational reproductive advantage under climate change (Walck *et al.* 2011). We

conducted four investigations using diploid and tetraploid (polyploid) seeds and seedlings of *T. triandra* to determine whether polyploidy and seed developmental environment interact to affect (i) seed physical traits, (ii) seed dormancy status, (iii) seed germination of non-dormant seeds in stressful conditions, and (iv) early seedling growth.

MATERIAL AND METHODS

Study species and system

Themeda triandra Forssk (*Poaceae*, kangaroo grass) is a perennial C_4 grass species found throughout Australia, and also in parts of Asia and Africa. In Australia, it is native and common in a diverse range of habitats, including coastal, alpine and arid areas (Sindel *et al.* 1993). *Themeda triandra* is a dominant ground-cover species in a number of threatened ecological communities, such as Box-Gum Grassy Woodlands (Australian Government Department of the Environment & Energy (DoEE) 1999), and is a key species used in restoration of grasslands and woodlands, including degraded agricultural land and mine sites (Morgan & Lunt 1999; Cole & Lunt 2005). *Themeda triandra* is a polyploid complex, with chromosome numbers ranging from $2n = 20$ to $2n = 80$ (Hayman 1960). Diploid ($2n = 20$) and tetraploid ($2n = 40$) cytotypes display a strong pattern of cytogeographic divergence, with diploids found in mesic environments of the cooler, wetter southeast and tetraploids in the warmer, drier, centre and west (Hayman 1960). Some interface populations containing both cytotypes do exist and generally occur along the contact zone of the two ploidy levels (Hayman 1960; Godfree *et al.* 2017). Hayman (1960) concluded that the origin of tetraploid Australian races of *T. triandra* was either through autopolyploidy or hybridization between different diploid ecotypes, leading to segmental allopolyploidy. Further studies in South Africa and India concluded that the majority of polyploids in *T. triandra* are segmental allopolyploids derived from divergent diploid biotypes (Birari 1980; Liebenberg 1986) with closely related genomes. Whole genome duplication in Australian races of *T. triandra* probably involved a similar process. The species is believed to reproduce primarily sexually when diploid and to be a facultative apomict when tetraploid, with the mode of reproduction somewhat responsive to environmental cues (Evans & Knox 1969). However, *T. triandra* displays considerable variability in reproductive pathways both within and between cytotypes (Evans & Knox 1969), and the presence or frequency of apomictic reproduction under different levels of stress have not been quantified. There are very few examples of triploid individuals, and these have been detected in populations where diploids and tetraploids coexist, suggesting that hybridization between the cytotypes is occasionally possible (Hayman 1960) although infrequent.

Germination characteristics of *T. triandra* are relatively well described and show considerable variation among and within populations (Groves *et al.* 1982; Saleem *et al.* 2009). Similar to many other native grasses, *T. triandra* seeds are often not ripe at dispersal and require 'dry after-ripening', a period (usually several months) of dry storage on the plant at ambient temperatures; *ex situ*, the same can be achieved by storage at low humidity and ambient temperatures, and can be accelerated with higher temperatures. An after-ripening requirement generally indicates physiological dormancy (Groves *et al.* 1982; Saleem *et al.* 2009). Following dormancy alleviation, germination of *T. triandra* is highest at temperatures of approximately 30 °C (Clarke *et al.* 2000; Cole & Lunt 2005).

Seeds for this study were collected from plants grown in a common garden at the CSIRO Black Mountain site in Canberra, ACT (S 35.27°, E 149.11°; full details in Godfree *et al.* 2017). Individual ramets (hereafter referred to as plants) of the study species *T. triandra* were collected from three regions in south-eastern New South Wales between October and November 2013. The three source regions, Albury (ALB), Batemans Bay (BBAY) and Sydney (SYD), represent three climatically different regions where both diploid (DI; $2n = 20$) and tetraploid (TE; $2n = 40$) cytotypes of this species are known to co-occur (Hayman 1960). The three regions, each at least 150 km apart, experience different climate regimes, ranging from moderate temperatures and relatively high rainfall on the coast (BBAY), to hot summers, cool winters and much lower rainfall in the seasonally dry, warm temperate region (ALB). Diploid and tetraploid plants were collected from each source region to avoid confounding regional and ploidy level effects. Collections from 12 populations were included in this study: from two sites comprised of diploid plants and two sites comprised of tetraploid plants within each of the three regions.

Plants were translocated to a common garden where they were grown in plots of 12 individuals (one from each collection population). There were 12 plots of 12 plants, resulting in 144 plants, which formed the maternal lines in this study. For this study, seeds were collected from plots experimentally subjected to either ambient conditions or passive warming that aimed to raise average maximum air temperatures by 2 °C to simulate projected warming by 2050. An average increase in air temperature of 1.4 °C above ambient levels was achieved using polycarbonate hexagonal open-top chambers (Godfree *et al.* 2011). These treatments commenced on 1 November 2014 and were maintained through to harvest. Treatments are referred to as seed developmental environments in this study. For further details on source plants, climate information, common garden design and realized climate treatments, see Godfree *et al.* (2017). Plants in the common garden were grown for 20 months, seed harvested in April 2015 (Godfree *et al.* 2017), with an additional harvest in December 2015.

Harvests were made when seed had matured and was beginning to be shed, to coincide with natural seed dispersal. Paper bags containing harvested material were slowly dried in ambient conditions and then placed in a dry room at 15% relative humidity (RH) and 15 °C until used for experiments. Maternal lines were collected and stored separately. Seed was cleaned by hand, using tweezers and a winnowing system to remove stems, florets, spikelets, chaff and awns. The removal of awns does not damage the caryopsis or enclosing structure of the seeds and does not affect seed dormancy or germination (Sindel *et al.* 1993). Seeds from the April 2015 harvest were stored for 12 months before use, approximately 4 months in ambient (ca. 23 °C) and 8 months at 15 °C/15% RH. Seeds from the December 2015 harvest were stored for 14 days in ambient conditions (ca. 30 °C max. and 11 °C min.) and 103 days at 15 °C/15% RH before the first experiment commenced.

Confirmation of seed cytotypes

The seeds used in this study were collected from open-pollinated diploid and tetraploid plants that were grown adjacent to each other in a series of outdoor plots as described in Godfree *et al.* (2017). To determine whether the cytotype of seed/seedlings matched that of the 48 maternal lines used in our experiments, we used DNA flow cytometry. DNA flow cytometry indicates the relative amount of DNA in the nuclei of a sample *versus* a standard and is now widely accepted as the most convenient and rapid method for ploidy screening in plants (Loureiro *et al.* 2006; Doležel *et al.* 2007). As ploidy differences, if present, may affect germination speed and germination success, we sampled early germinating seedlings (from the first 2 weeks of germination), late germinating seedlings (from the second 2 weeks of germination) and non-germinated seeds. At least four seedlings from each of the 48 maternal lines (24 diploid mothers and 24 tetraploid mothers) and two non-germinated seeds from 20 maternal lines (ten from diploid mothers and ten from tetraploid mothers) were separately analysed, with a total sample size of 200 seedlings and 40 non-germinated seeds. For each sample, fresh seedling shoot material was chopped with a sharp razor blade in 1 ml ice-cold Galbraith's buffer (Galbraith *et al.* 1983) together with fresh material of the plant reference standard, *Pisum sativum* cv. Ctirad (garden pea; 2C = 9.09 pg), to release nuclei from cells and form an aqueous suspension. This was filtered through a 4-μm filter to remove large particles that may block the flow stream. A total of 50 $\mu g \cdot ml^{-1}$ of the DNA-binding flurochrome, propidium iodide, was added together with 50 $\mu g \cdot ml^{-1}$ RNase to ensure that only the DNA was stained. For non-germinated seeds, the seed coats were removed, and all other seed material was analysed. The same protocol was followed as in the seedling samples, except that a LB01 (Doležel *et al.* 1989) buffer was used in place of Galbraith's in some samples. Samples were analysed using a Beckman Coulter Quanta SC cytometer (Beckman Coulter, Lane Cove, NSW, Australia), with a 488 nm laser to excite the propidium iodide. Outputs were viewed and analysed using Quanta software.

Physical seed traits

To determine whether cytotype and seed developmental environment affected physical seed traits, we measured seed viability and mass. Seed viability was assessed *via* cut tests, which were conducted on all experimental seed at the conclusion of each experiment. Empty seeds (no visible endosperm or embryo) were classified as nonviable. Seed mass was determined for a sample of 100 seeds from 48 maternal lines using a Sartorius balance (type: BP210S, Germany).

Seed dormancy

To test the hypothesis that polyploidy and seed developmental environment affect seed dormancy at dispersal, and investigate the effects of dormancy alleviation treatments, temporarily dry-stored (117 days after harvest) seed (December collection) was subjected to periods of artificial dry after-ripening (DAR) and subsequently moved to a germination assay. Artificial DAR conditions were maintained at constant 40 °C and 40% RH over the experimental period. Seed received one of four artificial DAR durations: 0 months (no treatment), 1 month, 2 months or 3 months. Artificial DAR conditions were created in a laboratory oven (Thermoline Scientific, Australia), using four airtight electrical boxes (Fibox Electrical enclosure box (EK0E) 280 × 280 × 130 mm, NHP Electrical Engineering Products) containing a solution of anhydrous lithium chloride (LiCl; Bacto Laboratories, Australia) to control RH at 40%. The LiCl solution was prepared following the Royal Botanic Garden, Kew protocol (Newton *et al.* 2009). Open glass vials containing seed from each maternal line were placed on a rack suspended above the liquid. Each vial contained 25 seeds, each electrical box contained three vials from 12 maternal lines, one vial per maternal line for each DAR duration. As seeds were removed from each of the DAR treatments, the germination assay was replicated four times, each replicate containing one of four maternal lines from each of three regions, two cytotypes and two seed developmental environment combinations, making up the 48 maternal lines used in this experiment (192 dishes in total).

To determine the proportion of dormant seed, seed from each vial was plated onto 90-mm plastic Petri dishes containing 1% water agar, sealed with Parafilm and placed in an incubator (Thermoline Scientific) at 30 °C/20 °C, 12 h/12 h day/night cycle. The agar solution was sterilized in an autoclave before use, and plating conducted in a laminar flow cabinet (Gelaire Laminar Air Flow, HLAF Series, Australia) to minimize non-seed borne contamination and fungal/bacterial growth. Each incubator shelf contained plates from all maternal line, organized by region × ploidy × seed developmental environment combinations (12 plates per shelf). Germination was defined as radical emergence of ≥2 mm beyond the seed coat and was scored twice weekly. At 2 weeks, non-germinated seed was transferred onto new plates to continue the germination monitoring, and germinated seedlings were potted for use in a seedling experiment. After 4 weeks, once germination had ceased, non-germinated seed was cut in half and examined. Seeds were classified as empty (nothing inside), dead (full, but soft and black/brown inside) or viable and dormant (full, firm and white).

Seed germination in stress

To determine whether and how polyploidy and seed developmental environment affect germination of seed under warmed and/or drought germination conditions, a four-way factorial experiment was designed using seed from the coastal region. The four factors were ploidy (2 levels), seed developmental environment (2 levels), germination temperature (2 levels) and germination drought (3 levels). The experiment was replicated five times, with five maternal lines from the same ploidy level and seed developmental environment, giving a total of 20 maternal lines used in this experiment and a total of 120 germination plates. Seed for this experiment was harvested in April 2015 and stored at 15% RH/15 °C for 12 months. It was considered likely, based on other authors' investigations, that dormancy had been alleviated in most of the seed by this time (Sindel *et al.* 1993; Saleem *et al.* 2009). Cut tests of seed germinated in benign/optimal conditions confirmed that the likely dormant portion was low and approximately equivalent among cytotypes and seed developmental environments (data not shown).

Two incubators were used to impose the temperature treatment. The high temperature incubator was set at 30 °C/20 °C, 12 h/12 h day/night cycle, and low temperature at 25 °C/15 °C, 12 h/12 h day/night cycle. The seeds were germinated in solutions of synthesis grade polyethylene glycol 8000 (PEG; Bacto Laboratories) to alter the osmotic potential of the water and therefore assess the effect of drought on germination. Three osmotic potentials were used: no drought (deionized water), medium drought (−0.5 MPa) and high drought (−1.0 MPa). Due to osmotic potential changing with temperature, four solutions of PEG were prepared to achieve the desired osmotic potentials at the two experimental temperatures. Solutions of PEG were prepared following Michel (1983).

Seeds were germinated in 90-mm plastic Petri dishes on two layers of filter paper (Whatman, qualitative, grade 1), irrigated with 6 ml of solution and sealed with Parafilm and plastic wrap to prevent evaporation. Non-germinated seeds were transferred onto fresh plates after 3 weeks to ensure the initial drought conditions were maintained over the entire 6-week germination period, as any water evaporation could have changed the osmotic potentials initially created. Most plates contained 25 seeds, however, due to limited amounts of seed from the April harvest, particularly from diploid maternal lines, some plates contained between 13 and 25 seeds each. Germination was recorded twice weekly. Germination was defined as extension of the radicle ≥2 mm beyond the seed coat. After 6 weeks, when germination had ceased, seeds that had not germinated were cut in half and examined. Seeds were classified as empty (nothing inside), dead (full, but soft and black/brown inside) or non-germinated (full, firm, white, healthy) rather than dormant, as in the previous experiment, as conditions for germination may not have been met by the germination treatments.

To analyse relative sensitivity to germination stress and compare cytotypes and seed developmental environments, we calculated the difference in germination of each maternal line between the least and most stressful environments. To create a proxy for germination sensitivity, we used the following equation:

$$\text{germination sensitivity} = \frac{(\text{maxgerm} - \text{mingerm})}{\text{maxgerm}}$$

Maximum germination was the highest germination proportion from the low or no stress environment and was taken to be the optimal germination. Minimum germination was the germination proportion at −1.0 MPa and 25 °C/15 °C, which had the lowest percentage of germination for all cytotypes and seed developmental environments and was taken to be the most stressful germination environment.

Early growth

To examine the effects of polyploidy and seed developmental environment on early growth, we grew seedlings from the 48 maternal lines germinated in the dormancy experiment described above. Three traits were measured: seedling mass (whole seedling biomass), seedling height (length of longest, living blade from ground to blade tip) and root mass ratio (RMR; the proportion of whole seedling biomass allocated to root mass) at 8 weeks.

After 14 days of the germination test, seedlings were transplanted into forestry tubes (50 mm × 50 mm × 125-mm deep) containing a mix of 60:40 crushed quartz and Perlite (500 coarse). Seedlings were grown in a propagation house at the Australian National Botanic Gardens, Canberra, ACT, Australia, under common conditions for 8 weeks. We aimed to have five seedlings from each of the 48 maternal lines and, where possible, potted up to eight seedlings from each germination plate to compensate for potential seedling death. Seven maternal lines did not have enough germination or surviving seedlings, to allow five seedlings to be measured. For these maternal lines, a further 30 seedlings were potted 1 month after the original seedlings, to provide a balanced design for analysis. The second set of seedlings was subjected to the same growing conditions and growth duration as the first set of seedlings. Where there were more than five established seedlings per maternal line, excess seedlings were randomly selected and removed.

Seedlings were hand-watered daily, and after 6 weeks, seedlings were fertilized with 5 ml liquid fertiliser (half-strength; Peter's Professional water-soluble fertilizer potash special) twice each week. At 8 weeks, the longest blade of each seedling was measured from the soil surface to leaf tip, and seedlings were harvested for measurement of biomass allocation. Seedling roots were washed to remove substrate, and the above- and belowground material of the seedling were separated and placed in paper bags. Bags were placed in an oven at 60 °C for 72 h to dry completely, before weighing using a Mettler-Toledo AB304-S analytical balance (Mettler-Toledo, Switzerland).

Statistical analyses

All statistical analyses were carried out using the statistical package Genstat 18th edition (VSN International 2016). All data were checked prior to conducting the statistical analyses to ensure that assumptions of normality were met and that no transformations were required. No outliers were removed. Due to the balanced design of all three experiments and the normal distribution of residuals, ANOVA was carried out to compare means of the seed and seedling traits measured in all experiments. To adjust for variation in seed viability, empty seeds (determined by cut test) were subtracted from samples in the dormancy and germination experiments before calculating dormancy and germination proportions.

To determine which factors influence physical seed traits, we used an ANOVA with the effects of cytotype (two levels), seed developmental environment (two levels) and region (three levels) and all interactions. The model was applied for each response variable, seed viability and seed mass. To analyse which factors influence seed dormancy, a model with the following fixed factors was created: cytotype (2 levels; diploid, tetraploid) and seed developmental environment (2 levels; warmed, ambient), region (3 levels; ALB, BBAY or SYD) and DAR duration (4 levels; 0, 1, 2 and 3 months). Incubator shelf (4 levels) was treated as a blocking factor/random effect. Seed developmental environment had no effect on dormancy and was removed to simplify the model. The final model was applied to analyse the proportions of dormant, dead and empty seed. For the drought and temperature stress experiment, viability adjusted germination was analysed with ANOVA to determine main effects and two-, three- and four-way interactions. Factors considered in the model were: cytotype (2 levels), seed

developmental environment (2 levels), germination temperature (2 levels; high 30 °C/20 °C, low 25 °C/15 °C) and germination drought (3 levels; no 0 MPa, medium −0.5 MPa, high −1.0 MPa). Incubator shelf (5 levels) was applied as a blocking factor. Stepwise removal of non-significant higher-order interactions did not affect the results, but simplified the model for a clearer presentation of the analysis. The final model presents main effects and two-way interactions, with all non-significant three- and four-way interactions removed. The final model was run for each of the following response variables: germination proportion, germination rate, proportion of non-germinated healthy seed and proportion of dead seed. Finally, to determine which factors influence early growth traits, we used an ANOVA with the fixed effects of cytotype (2 levels), seed developmental environment (2 levels) and region (3 levels). The full model with all interactions is presented, since a number of interactions between the fixed factors were found to influence seedling traits. Seedling weight, seedling height and root mass ratio were all analysed using the same model.

RESULTS

Confirmation of seed cytotypes

Flow cytometry confirmed that 99% of sampled seedlings had the same cytotype as the mother plant. Of the 200 seedling samples analysed, only two were not of the same cytotype as the mother plant. These two plants may be the result of somatic mutation, fusion of unreduced gametes or accidental contamination during seed processing. A total of 100% of the sampled non-germinated seeds had the same cytotype as the mother plant. Given the high consistency between maternal and progeny ploidy (across early germinating, late germinating and non-germinated seeds), we henceforth refer to offspring by the cytotype of the parent throughout the paper. Our flow cytometry results also showed the monoploid genome size (Cx value) was the same for both cytotypes, consistent with the findings reported in Godfree *et al.* (2017).

Physical seed traits

Assessment of seed viability *via* cut tests revealed that tetraploid seed had significantly higher viability than diploid seed (Table 1a). Diploid and tetraploid cytotypes were affected differently by seed developmental environment depending on the region of origin (Fig. 1). Specifically, viability of tetraploid seed from BBAY and SYD regions was not affected by developmental warming, nor was diploid seed from BBAY (Fig. 1a and b). However, developmental warming decreased seed viability in diploids from SYD and increased viability in diploid seed from ALB. Warming decreased viability in ALB tetraploid seed.

Tetraploid seed was significantly heavier, on average 35%, than diploid seed, regardless of region. Seeds from ALB mothers were heaviest and seed from SYD mothers had the smallest seed mass (Fig. 1). Seed mass was not affected by seed developmental environment (Table 1a).

Seed dormancy

Seed dormancy varied strongly with the main effects of cytotype (TE *versus* DI), artificial DAR duration (40 °C and 40% RH for 0, 1, 2 or 3 months) and region (SYD, BBAY and ALB; $P < 0.001$; Table 2), although significant cytotype × DAR duration and region × DAR duration interactions were also present (Table 2). At the time of initial testing (0 months DAR), seed dormancy was significantly higher in tetraploids (55%) than in diploids (35%; Fig. 2a). Regional differences in the proportion of dormant seed were also apparent, with SYD maternal plants producing the fewest dormant seeds and ALB plants producing the most (Fig. 2c).

Artificial DAR effectively alleviated seed dormancy in both cytotypes of *T. triandra*. Tetraploid seed was initially more deeply dormant than diploid seed, and dormancy declined at a similar rate over the first month. However, after 2 and 3 months in the DAR conditions, dormancy in both cytotypes was similar (≈10%; Fig. 2a). Region also weakly affected dormancy alleviation over time, with seed from SYD plants undergoing a more rapid loss of dormancy than ALB seed, especially after the first and second months (Fig. 2c–d).

In contrast, seed death was not directly related to cytotype ($P > 0.05$; Table 2) and was only weakly affected by DAR duration ($P = 0.015$; Table 2, Fig. 2b). There was, however, a weakly significant trend ($P = 0.038$; Table 2) for seed death to differ over time between diploid and tetraploid seed, with the majority of tetraploid and diploid seed death occurring in the first and second months, respectively (Fig. 2d).

Seed germination under stress

Germination of non-dormant seed was significantly affected by the main model effects of cytotype ($P < 0.001$), germination drought status (no, medium and high drought; $P < 0.001$) and marginally by germination temperature (30 °C/20 °C *versus* 25 °C/15 °C; $P = 0.074$; Table 3). Seed development environment (warm *versus* ambient) had no impact on germination and there were no significant higher effects (*i.e.* two-way interactions; Table 3). Overall, there was a consistent trend for a higher proportion of diploid seed to germinate in both temperature treatments (Fig. 3a) and in all experimental drought stress environments (Fig. 3b).

Higher germination temperature had a significant effect of reducing the proportion of healthy, non-germinated seed at the end of the experiment (Fig. 3a), especially in tetraploids, with the cytotype × temperature interaction term being significant ($P = 0.035$; Table 3). Tetraploid seeds retained a higher proportion of non-germinated seed overall (~50% *versus* ~30%; Fig. 3a).

The application of PEG in germination treatments, to reduce osmotic potential and impose water stress, resulted in reduced germination in both cytotypes, especially in the highest drought stress treatment (−1.0 MPa). Drought stress also significantly slowed germination rate (the average time taken to reach 50% of total germination (T_{50})), which increased from 6 days at 0 MPa to 20 days at −1.0 MPa. However, T_{50} did not differ significantly between cytotypes, seed developmental environment or experimental germination temperatures (Table 3).

A germination index was created to compare the decline in germination in the −1.0 MPa stressful germination treatment to the larger of the other two (0 and −0.5 MPa) benign treatments from either temperature. The index of germination sensitivity confirmed that tetraploid seed was less tolerant of germination stress than diploid seed (Fig. 4, Table 4), and

Table 1. ANOVA of the effects of cytotype, seed developmental environment (SDE) and region on (a) physical seed traits – seed mass and viability, and (b) seedling growth traits – seedling mass, height and root mass ratio. Variance ratio (*v.r.*) represents *F*-values and F probability (*F pr.*) represent *P*-values. Statistically significant results are highlighted in bold.

		(a)				(b)					
		seed mass		seed viability		seedling mass		seedling height		root mass ratio	
	df	*v.r.*	*F pr.*	*v.r.*	*F pr.*	*v.r.*	*F pr.*	*v.r.*	*F pr.*	*v.r.*	*F pr.*
block stratum	3	2.99		4.13		0.09		0.89		2.4	
cytotype	1	31.51	**<0.001**	39.34	**<0.001**	17.22	**<0.001**	8.22	**0.005**	2.23	0.137
SDE	1	0	0.948	0.28	0.599	3.66	0.057	10.38	**0.001**	0.17	0.676
region	2	13.8	**<0.001**	3.39	**0.021**	16.03	**<0.001**	5.45	**0.005**	1.66	0.193
cytotype × SDE	1	0.06	0.81	0.41	0.523	0.93	0.335	6.13	**0.014**	1.82	0.178
cytotype × region	2	1.39	0.263	0.05	0.952	0.33	0.717	0.36	0.698	0.1	0.902
SDE × region	2	0.69	0.509	3.09	**0.048**	0.15	0.857	1.02	0.362	1.84	0.161
cytotype × SDE × region	2	2.83	0.073	6.12	**0.003**	5.91	**0.003**	3.55	**0.03**	5.56	**0.004**

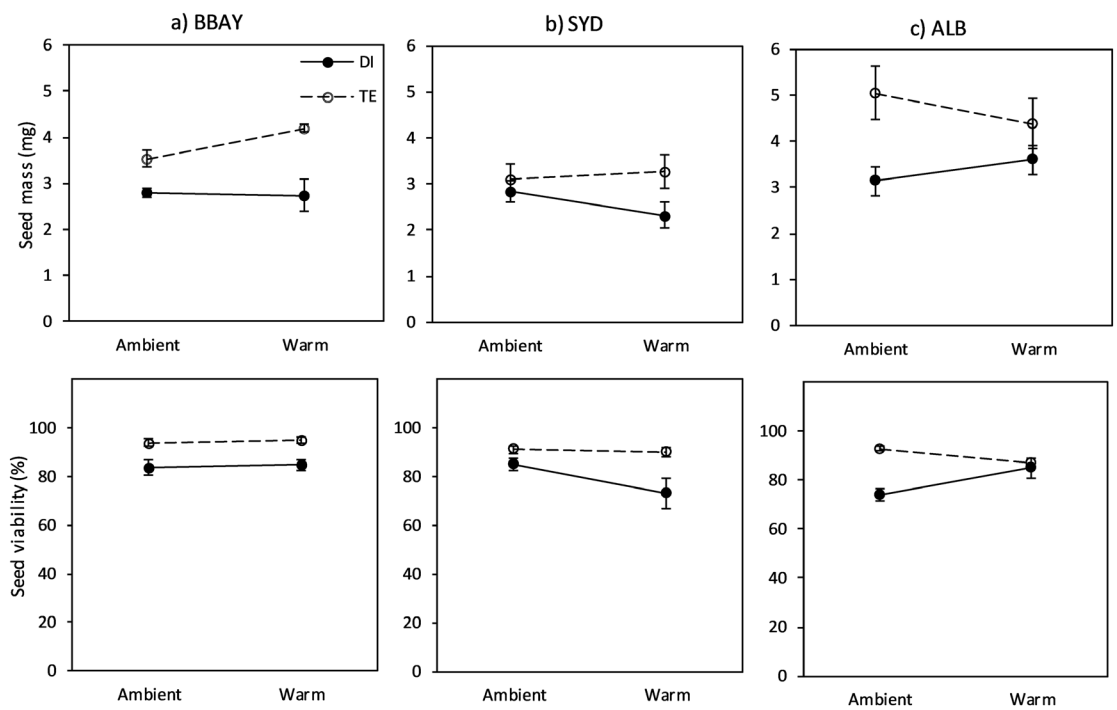

Fig. 1. Effects of cytotype (diploid (DI) and tetraploid (TE)) and seed developmental environment (SDE; ambient and warm) on mean seed mass and mean seed viability across (a) Batemans Bay (BBAY), (b) Sydney, (SYD) and (c) Albury (ALB) regions. Error bars ± SE.

tetraploid seed had a narrower set of conditions favourable for germination (germination niche) compared with diploid seed.

Finally, the frequency of seed death differed across cytotypes (19% of diploid *versus* 13% of tetraploid) and also with seed developmental environment (Table 3). Seed from a warm seed developmental environment had a lower death rate (14%) compared to seed from the ambient seed developmental environment (18%).

Collectively, a higher percentage of diploid seed germinated, and the diploid seeds that did not germinate were more likely to be dead (50% germ, 31% healthy non-germ, 19% dead) than tetraploid seed (37% germ, 51% healthy non-germ, 13% dead), a pattern suggesting more specific germination requirements in the tetraploids.

Early growth

Assessment of seedling mass, seedling height and RMR revealed that cytotype, region and seed developmental environment all significantly affected early growth traits in *T. triandra* (Table 1b). Cytotype differences in seedling mass depended on both seed developmental environment and region (three-way interaction $P = 0.003$; Table 1b), with tetraploid seedlings from the warm developmental environment being heavier in BBAY and SYD seedlings, but lighter in seedlings from ALB (Fig. 5a). Across treatments, however, consistent with seed size differences, seedlings from ALB tended to be heavier than those from BBAY and SYD (region effect $P < 0.001$), and tetraploids tended to be heavier than diploids (cytotype effect $P < 0.001$;

Table 2. ANOVA of the effects of cytotype, dry after-ripening (DAR) duration and region on seed dormancy status and seed death. Variance ratio (*v.r.*) represents *F*-values and *F* probability (*F pr.*) represent *P*-values. Statistically significant results are highlighted in bold

		dormant		dead	
	df	*v.r.*	*F pr.*	*v.r.*	*F pr.*
block stratum	3	1.44		3.32	
cytotype	1	47.98	**<0.001**	3.65	0.058
DAR duration	3	114.62	**<0.001**	3.59	**0.015**
region	2	22.51	**<0.001**	5.84	**0.004**
cytotyoe × DAR duration	3	8.71	**<0.001**	2.87	0.038
cytotype × region	2	0.17	0.844	0.8	0.453
DAR duration × region	6	3.08	**0.007**	1.23	0.295

Table 1b). Seedling height was also influenced by interactive effects of cytotype, region and developmental environment ($P = 0.03$; Table 1b). A consistent pattern was for tetraploid seedlings to be taller than diploid seedlings when sourced from the warm developmental environment; this pattern was absent in seedlings derived from the cool environment (Fig. 5b). Tetraploid seedlings also tended to be taller overall (mean across treatments = 76 mm *versus* 69 mm) and ALB seedlings were, on average, taller than seedlings from other regions (Fig. 2b). Collectively, these data indicate that diploid seedlings from the cooler coastal regions (BBAY and SYD) performed relatively poorly compared to tetraploids when exposed to warmer maternal growing conditions, while tetraploid seedlings from a hot and dry region (ALB) tended to be larger when maternal plants were exposed to cool (ambient) growing conditions. In other words, when the difference in temperature between the region of origin and the experimental plots was large (>10 °C), the experimental warming of plots had a larger effect on seedling growth, and maternal plants produced seeds with a higher tetraploid advantage.

Analysis of biomass allocation also revealed that the effects of cytotype and seed developmental environment vary significantly depending on region (three-way interaction $P = 0.004$; Table 1b, Fig. 5c). Few patterns were evident, although the RMR of seedlings from SYD was strikingly lower for diploids from the warm developmental environment but higher from the cool development environment. In contrast, the RMR of tetraploids sourced from the warm environment was higher than that of diploids. Apart from this, RMR was similar across regions, cytotypes and developmental environments (main effect $P > 0.05$ for all).

DISCUSSION

The results of our study show that polyploidy in *T. triandra* is significantly associated with variation in seed and seedling traits that are known to confer a fitness advantage in extreme or stressful environments. Tetraploid plants generally produced larger, more viable seed than diploids, and tetraploid seeds had higher dormancy and lower germination when under drought stress. Tetraploid seedlings also tended to have higher biomass and height than diploid seedlings. Overall, these patterns indicate that seed sourced from tetraploid plants has a narrower germination niche than diploid seed, but that once germinated they grow faster under more optimal conditions than those sourced from diploid plants. The ability of plants to establish reproducing populations in new environments, and to persist in fluctuating, extreme or changing climates (te Beest *et al.* 2012; Hahn & Müller-Schärer 2013), are known to be associated with dispersal ability, a high relative growth rate, wide environmental tolerance,

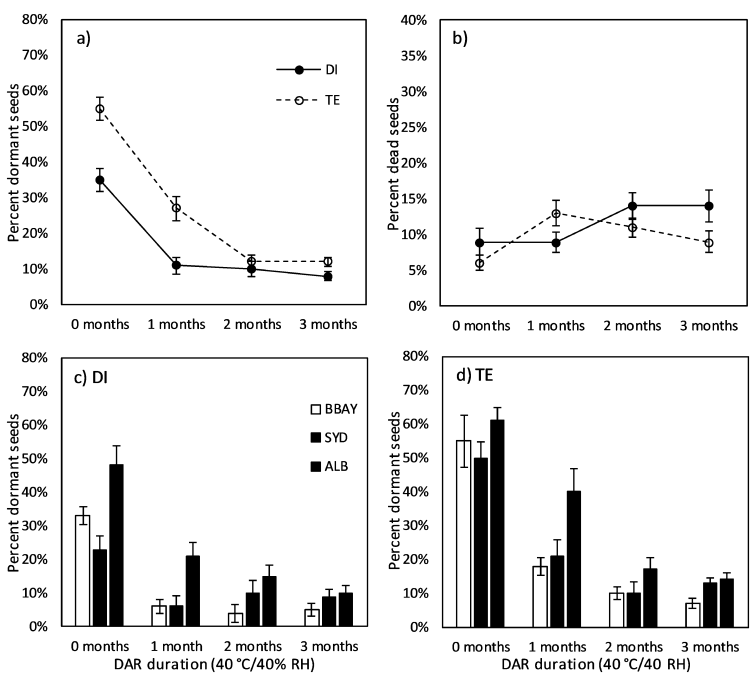

Fig. 2. The effect of four dry after-ripening (DAR) durations on (a) mean seed dormancy and (b) mean seed death of diploid (DI) and tetraploid (TE) seed. Panels (c) and (d) show differences among the Batemans Bay (BBAY), Sydney (SYD) and Albury (ALB) regions for each cytotype, (c) diploid and (d) tetraploid, respectively. 0 months DAR is equivalent to dormancy status at the time the experiment (artificial DAR) started. Error bars ± SE.

Table 3. ANOVA of the effects of cytotype, seed developmental environment (SDE) and germination environment (drought and temperature) on the number of non-germinated healthy and dead seeds and the proportion and rate of germination. Variance ratio (*v.r.*) represents *F*-values and *F* probability (*F pr.*) represent *P*-values. Statistically significant results highlighted in bold

		not germinated				germinated			
		healthy		dead		proportion		rate (T50)	
	df	*v.r.*	*F pr.*	*v.r.*	*F pr.*	*v.r.*	*F pr.*	*v.r.*	*F pr.*
block stratum	4	2.53		1.64		2.79		0.41	
cytotype	1	86.62	**<0.001**	13.8	**<0.001**	24.11	**<0.001**	0.02	0.882
SDE	1	3.99	**0.048**	6.47	**0.012**	0.00	0.944	0.36	0.552
germination drought	2	50.7	**<0.001**	1.05	0.355	39.44	**<0.001**	80.59	**<0.001**
germination temp	1	5.88	**0.017**	0.01	0.926	3.27	0.074	0.11	0.738
cytotype × SDE	1	0.08	0.779	0.57	0.45	2.13	0.148	1.31	0.272
cytotype × germination drought	2	0.11	0.9	0.9	0.41	0.37	0.69	0.15	0.859
cytotype × germination temp	1	6.94	**0.01**	1.09	0.3	1.92	0.169	2.12	0.149
SDE × germination drought	2	0.85	0.43	0.48	0.619	1.04	0.359	0.09	0.911
SDE × germination temp	1	6.45	**0.013**	1.16	0.284	1.49	0.226	1.91	0.17
germination drought × germination temp	2	2.96	0.056	2.34	0.101	0.48	0.618	2.12	0.125

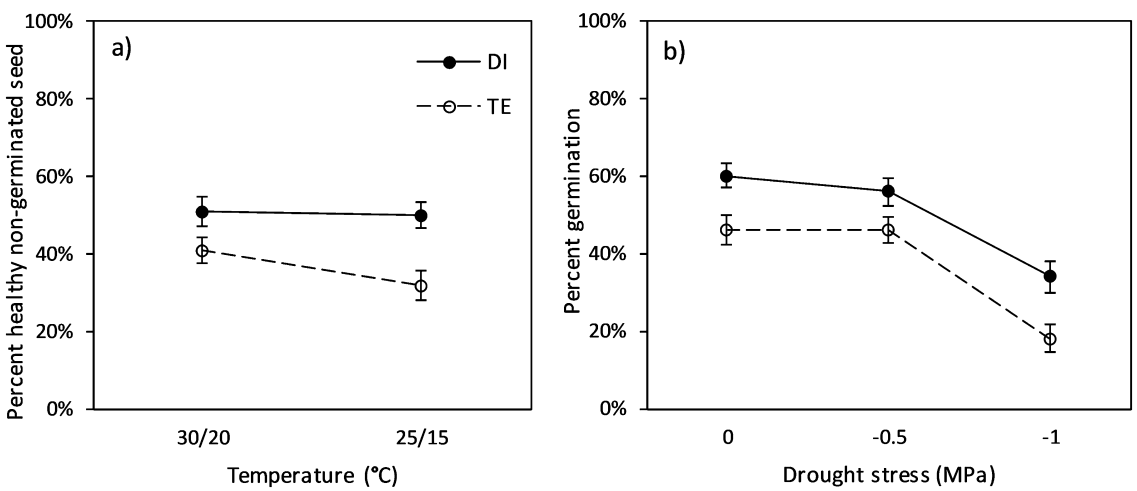

Fig. 3. Effects of (a) germination temperature on the proportion of healthy, non-germinated seed remaining after 40 days in germination treatments on two cytotypes: diploid (DI) and tetraploid (TE). (b) Effects of drought stress on the mean germination proportion of DI and TE seeds. Values are averaged across seed developmental environments or germination temperatures, respectively, as they did not significantly alter the effect of temperature or drought on germination proportion (the interaction term was not significant). Error bars ± SE.

high competitive ability and avoidance of genetic bottlenecks, among other factors (Levin 2000). Our investigation into the early life stages of *T. triandra* demonstrate that seed and seedling traits associated with polyploidy, and not simply variation in seed production and whole-plant resource allocation (*e.g.* Godfree *et al.* 2017), are likely to be highly advantageous in such environments. Below we discuss the effects of ploidy level and seed development environment on seed dormancy, germination and establishment under stress, and implications for restoration and conservation.

Reproductive barriers between cytotypes

Within the diploid–tetraploid complex of *T. triandra* we found evidence of reproductive barriers between cytotypes preventing inter-cytotypic hybridization that might result in inferior triploid offspring. Flow cytometry revealed that tetraploids did not produce any triploid offspring, despite open-pollination and growing in very close proximity to diploids, which suggests that gene flow from diploids to tetraploids is low. This lack of inter-cytotypic hybridization may be due to genetic incompatibilities between cytotypes, or, as has been observed previously, that tetraploids may reproduce largely through apomixis (Evans & Knox 1969). The ability of apomicts to reproduce successfully in the absence of suitable mates is thought to aid in the colonization of novel environments (Madlung 2013), and persistence in a wider range of habitats and climates, where chances of successful pollination might be low. Apomixis is also thought to negate the issue of minority cytotype. When polyploids arise and are the minority cytotype they have a breeding disadvantage, as it is more likely they will be pollinated by haploid pollen from the majority cytotype (diploid) resulting in non-viable or less fit triploid offspring, leading to the exclusion of that cytotype (Levin 1975). Apomicts overcome this evolutionary obstacle, often by avoiding the need for fertilization to produce viable seed, and produce clonal seed that can disperse through the landscape. If apomixis is common,

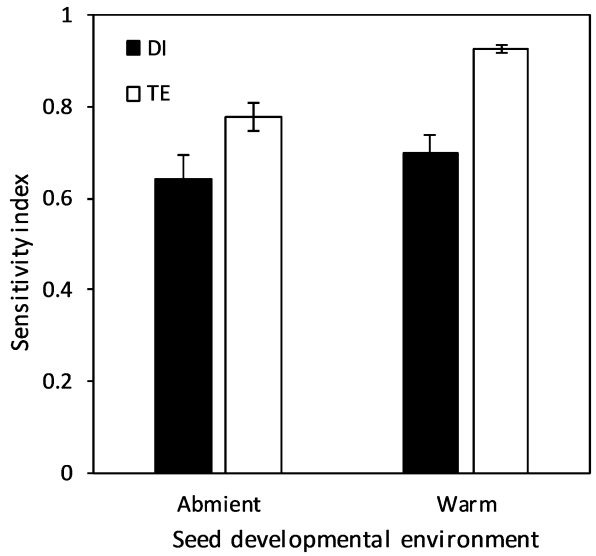

Fig. 4. Sensitivity to germination stress of diploids (DI) and tetraploids (TE) from ambient and warm seed developmental environments. The germination sensitivity index compared the decline in germination in the −1.0 MPa stressful germination treatment to the larger of the other two 0 and −0.5 MPa benign treatments. Error bars ± SE.

Table 4. ANOVA of the effects of cytotype and seed developmental environment (SDE) on sensitivity to germination stress. Variance ratio (*v.r.*) represents *F*-values and *F* probability (*F pr.*) represent *P*-values. Statistically significant result highlighted in bold

	relative sensitivity to germination stress		
	df	*v.r.*	*F pr.*
block stratum	4	1.43	
cytotype	1	5.59	**0.036**
SDE	1	1.77	0.208
cytotype × SDE	1	0.34	0.571

and predominantly present in polyploid races, this may be one mechanism enabling tetraploid success in novel or more stressful environments. Apomixis, a well-known correlate of polyploidy (Evans & Knox 1969; Carman 1997; Koltunow & Grossniklaus 2003), could be an additional influencing factor driving the observed differences in early life traits revealed in this research. Future research to uncover the origin and mechanism of maintenance of *T. triandra* polyploidy and elucidate the effects of genome duplication and apomixis would provide important insights to the ploidy effects uncovered in this study.

Diploids of *T. triandra* are believed to reproduce sexually (Hayman 1960; Evans & Knox 1969) and, interestingly, we also found that diploids did not produce triploid seed. Diploid mothers did, however, produce more empty seeds. Norrmann *et al.* (1994) found post-zygotic abortion to occur in several *Paspalum* species (*Poaceae*) following diploid–tetraploid cross-pollination, which impeded the production of triploid hybrids. Post-zygotic abortion of triploids could therefore explain both the lack of triploid offspring and the higher proportion of empty diploid seed. To test this would require further experimental work to specifically investigate cross-pollination between cytotypes and subsequent seed viability.

Differences in seed dormancy

Perhaps the most notable result of our research was the marked difference in seed dormancy between cytotypes. We found polyploid seed had higher dormancy status and a greater degree of dormancy (the proportion of a population of seeds that did not germinate in conditions otherwise favourable for germination) than diploid seed. Together, these traits delay germination and increase the likelihood that germination occurs at a time when conditions favour successful seedling establishment and early growth, thus ensuring higher seedling survival and adult plant fitness. In highly variable and extreme climates, regulating the timing and location of germination is crucial for reproductive success. The temporal delay in germination introduced by dormancy can contribute to the soil seed bank and aid further seed dispersal, both beneficial mechanisms for persisting in novel or variable climates.

Given these patterns, the lack of studies relating seed dormancy to reproductive success in polyploid plants is surprising. In one of the few studies that explicitly explored this relationship, Hacker (1988) found that seed dormancy increased with ploidy level in the tropical African grass *Digitaria milanjiana*. However, this relationship was confounded by the tendency for higher ploidy cytotypes to occur in regions with low rainfall. Indeed, polyploids often occur in climates that are also associated with increased seed dormancy, which makes it difficult to elucidate the specific causal role played by polyploidy in these systems. Our seed, however, originated from a common garden experiment in which diploid and tetraploid plants from three distinct regions grew under controlled conditions, thus allowing us to assess relationships between seed dormancy and ploidy levels without the confounding effects of source or developmental climate conditions. Thus, while previous studies have speculated that differences in germination strategy observed between diploids and tetraploids could be, in part, due to increased dormancy (Eliášová & Münzbergová 2014), our study of *T. triandra* has provided the first definitive evidence of this relationship. Understanding variation in seed dormancy between cytotypes could greatly improve our understanding of the cytogeographic distributions seen in other species, and enhance both our ability to predict how plant populations might respond to a changing climate, and also understand longer-term evolutionary processes such as speciation (Willis *et al.* 2014).

The regional differences in dormancy observed in this study, despite maternal plants being grown under common conditions, are in keeping with previous findings that link seed dormancy variation to the long-term maternal environment (Hacker 1988; Carta *et al.* 2016). We found that seed from the region (ALB) with the lowest rainfall, hottest summers and coldest winters was most dormant, while seed from the more mesic coastal region (BBAY) produced seed with the least dormancy, which is consistent with the findings of Carta *et al.* (2015). It is important to note, however, that regional differences in dormancy cannot always be explained by climate conditions (Schütz & Milberg 1997). Our findings suggest that long-term climate effects do act on seed dormancy and are perhaps retained in maternal genomes and passed to the next generation independent of, or in concert with, effects of the seed developmental environment. While local climate may modulate seed dormancy to some degree, in this case genetic factors also seem to play a part, but whether these are evolved or epigenetic remains to be determined.

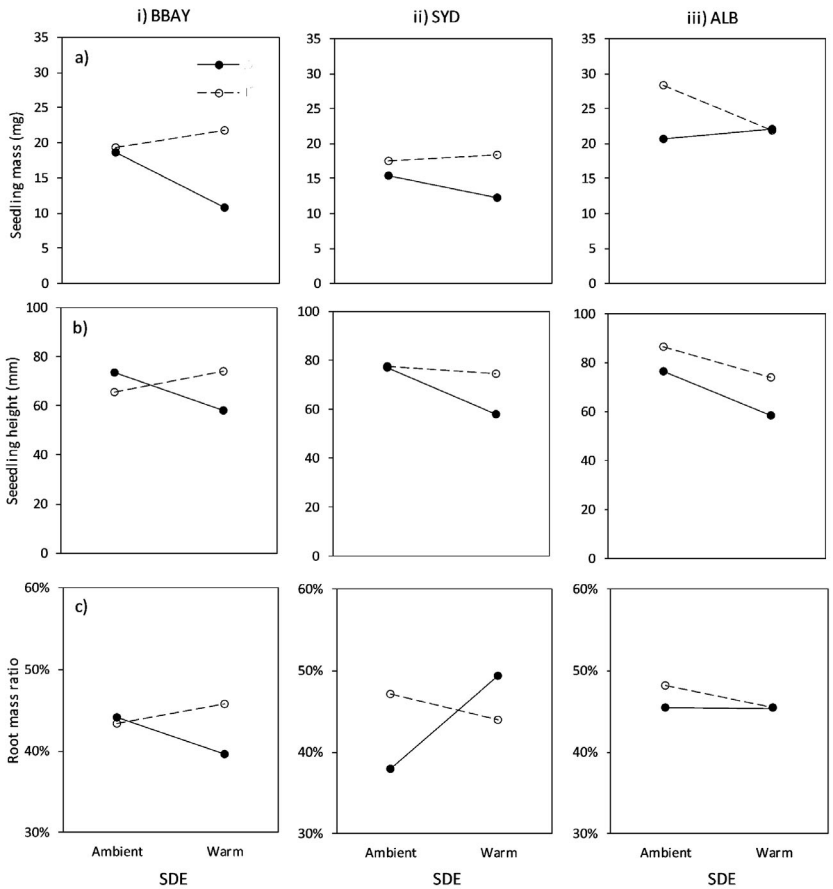

Fig. 5. The effect of seed developmental environment (ambient or warmed) on (a) seedling mass, (b) seedling height and (c) root mass ratio (RMR) of two cytotypes, diploid (DI) and tetraploid (TE) from the three collection regions, (i) Batemans Bay (BBAY), (ii) Sydney (SYD) and (iii) Albury (ALB).

Effect of polyploidy on germination and establishment in stressful germination environments

Responses to germination cues in *T. triandra* also displayed cytotypic differences: a higher proportion of non-dormant diploid seed germinated than tetraploid seed in all germination environments. This may indicate that diploids are more resilient to climate stress during germination, but it could also indicate that they are more likely to express the 'wrong phenotype' and germinate in response to unreliable environmental cues in drought-prone habitats.

The differences in germination that we found between environments could indicate that diploids have a broader thermal germination niche than tetraploids. However, due to the limited range of experimental temperatures used, it could also indicate that diploids and tetraploids simply occupy different germination niches. Our data suggest that the potentially warmer and/or narrower thermal germination niche of tetraploids might be favoured in a warming climate. On the other hand, populations with narrow germination niches can be more vulnerable to a changing climate, since a shift in environmental conditions is more likely to result in germination requirements no longer being met or occurring at different times of the year. In this sense, the wider germination tolerances observed in diploids of *T. triandra* might be advantageous in a changing climate. However, high germination proportions do not necessarily translate to high fitness, because it must occur when environmental conditions are favourable for seedling establishment and survival. As climate extremes become more common, specific germination niches may in fact protect seedlings from harmful climate conditions. While many studies have used temperature gradient plates to identify germination niche breadth and thresholds in native Australian species (Kebreab & Murdoch 1999; Cochrane *et al.* 2014; Cochrane *et al.* 2015a), further experimental work is required to understand the impact of ploidy on germination niche in this and other relevant plant species.

In addition to failing to show the expected decrease in seed dormancy due to developmental warming that is reported in the literature (Steadman *et al.* 2004; Hoyle *et al.* 2008; Ooi 2012; Carta *et al.* 2015), we also found no evidence that warming substantially increased seed germination (cf. Fenner 1991; Blödner *et al.* 2007; Walck *et al.* 2011; Mondoni *et al.* 2012). Notably, the temperature differences investigated in the existing literature (at least 10 °C in most cases) were much larger than those produced in our common garden (approximately 1–3 °C, average 1.4 °C), which represent a realistic level of warming for the next few decades. Differences in diploid and tetraploid seedling growth are, therefore, perhaps more indicative of effects of shorter-term predicted temperature increases. We found that seedlings derived from diploid seed produced by maternal plants in the warmer environment were smaller than those produced under

ambient conditions, while tetraploids appeared unaffected by the warmer conditions. This may indicate that tetraploids will be more resilient to the potentially disadvantageous effects of a warmer climate. It is also possible that observed regional differences in the performance of diploid and tetraploid cytotypes under warm *versus* ambient experimental conditions might partly reflect underlying differences between the optimal climate niche of the source populations and the climate conditions in the common garden study. Specifically, the difference between climate conditions of the source location and common garden was larger for coastal (BBAY and SYD) populations than warmer drier (ALB) populations. Further investigations performed under a broader range of climate conditions would help to resolve this.

The observed variation in traits between cytotypes could be interpreted as advantageous or not depending on the environment in which they are growing. In their paper, Tyler *et al.* (1978) studied the altitudinal distribution of two cytotypes within the perennial grass species *Festuca pratensis* and concluded that the cytotype which dominated the more densely populated lower slopes, where competition was high but conditions for growth more favourable, displayed seed and seedling traits that complemented survival in such conditions. Diploid seed of *T. triandra* are less dormant and will germinate under a wider set of conditions compared to tetraploids. Since diploids dominate in mesic regions of Australia where earlier germination incurs minimal risk of extreme climates, it is likely that early seedlings would be better able to out-compete other plants in these more densely populated environments. In contrast, our results suggest that polyploids have a fitness advantage in environments where conditions are more challenging to seedling establishment and survival. In particular, higher seed dormancy and narrow germination niches equip seed to survive long periods of unfavourable conditions (Koornneef *et al.* 2002), traits both present in tetraploid *T. triandra* seed. Furthermore, once germination does occur, faster growing polyploid seedlings can establish quickly and compete for potentially limited resources. In the long term, as rapid climate change shifts mesic regions of Australia into increasingly warmer and drier climate regimes, we might expect to see tetraploid gradually replace diploid populations, particularly in extant contact zones.

Implications for seed sourcing for restoration and conservation

Selection of appropriate seed is fundamental to improve planting success of restoration projects. Sourcing suitable and quality seed ensures that new populations become functional, self-sustaining and resilient to environmental challenges (Broadhurst *et al.* 2008). Informed approaches for sourcing seed that capitalize on inherent trait diversity and adaptive capacity offer the potential for significantly improving the success of landscape restoration (Prober *et al.* 2016), particularly in environments that are significantly degraded or under threat of serious changes to climate, such as prolonged drought or rapid warming. For decades, locally sourcing seed for restoration was believed to be the best approach, as plants already adapted to that environment would be more successful and it would reduce the chance of deleterious out-breeding (Mckay *et al.* 2005; Mijnsbrugge *et al.* 2010). However, many studies have highlighted the shortfalls of this approach to seed sourcing, which include limited and poor-quality seed supply and lack of adaptive capacity (Broadhurst *et al.* 2008; Gelliea *et al.* 2016). With the realization that environmental restoration must consider the effects of future climate change, restoring environments that will persist in future conditions, rather than those that once or currently exist, is becoming more widely accepted (Havens *et al.* 2015), and seed sourcing practices and approaches must follow suit.

Given this, we suggest that when seed sourcing *T. triandra* for restoration, consideration of cytotype could have significant implications for the success and resilience of the restored populations and related ecosystems. While polyploid seed has many advantageous traits for success in changing and unpredictable climates, such as increased seed dormancy and more specific germination requirements, diploid seed may germinate more readily in a wider range of environments. We therefore suggest trialling the use of mixed diploid and tetraploid seed together. Selecting and utilising both diploid and tetraploid seed for restoration is one way to increase trait diversity and take advantage of the short-term and long-term adaptive capacity of the different cytotypes. However, Broadhurst *et al.* (2012) highlight some of the potential consequences of sourcing polyploid seed without properly understanding the complexities that come with cytotype disparity and suggest a cautionary approach when utilizing polyploids in restoration. Our recommendations take this into consideration and are based on the evidence for reproductive barriers between diploid and tetraploid cytotypes in *T. triandra*. Therefore, the potential cross-breeding outcomes, such as non-viable or less fit offspring as a result of unstable triploid production which could lead to population decline, may be of minimal concern, given the infrequency with which they occur *in situ* or in common garden trials.

Grassland ecosystems are amongst Australia's most degraded and endangered environments and their restoration and management is a top conservation priority (Yates *et al.* 2008). The reintroduction of native grasses is a key step in restoring these landscapes, and as a dominant grass species, *T. triandra* is widely used in restoration (Stol & Prober 2015). We believe our research proves that exploring and ensuring the use of both diploid and polyploid sources of *T. triandra* seed is a feasible and worthwhile option for improving restoration success in a changing climate. Furthermore, polyploid complexes are common in many grasses (Visser & Molofsky 2015), therefore, research investigating polyploid complexes and early growth could be significant in improving seed sourcing for many species used in grassland conservation.

CONCLUSION

We have demonstrated that polyploid seed is more dormant than diploid seed and that this is not confounded with climate or region. Additionally, tetraploid seed has more specific germination requirements, and seedlings are larger, grow faster and are more resilient to the effects of developmental warming compared to diploids. Such traits are important in plant regeneration, adaptation and persistence. The challenge of establishing and sustaining resilient and adaptable plant communities is becoming ever more important in a rapidly changing climate. Considering the prevalence of polyploidy in flowering plants and, as evidenced in *T. triandra*, their potential to persist and succeed in extreme and variable climates, polyploidy could be

an important consideration in sourcing resilient seed and plants for future-proofing restoration.

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