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Research Article

Phenology and polyploidy in annual *Brachypodium* species (Poaceae) along the aridity gradient in Israel

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Abstract Local adaptation of plants along environmental gradients provides strong evidence for clinal evolution mediated by natural selection. Plants have developed diverse strategies to mitigate stress, for example, drought escape is a phenological strategy to avoid drought stress, while polyploidy was proposed as a genomic adaptation to stress. Polyploidy as an adaptation to aridity (an environmental parameter integrating temperature and precipitation) was previously documented in annual *Brachypodium* spp. (Poaceae) in the Western Mediterranean. Here, we examined whether polyploidy or phenology are associated with aridity in annual *Brachypodium* spp. along the aridity gradient in the Eastern Mediterranean. Using flow cytometry, we determined ploidy levels of plants from natural populations along the Israeli gradient, spanning ~424 km from mesic Mediterranean to extreme desert climates. In a common garden we recorded time of seedling emergence, flowering and senescence. We tested whether the proportion of allotetraploids in the populations and phenological traits were associated with aridity. Contrary to a previous study in the Western Mediterranean, we found no effect of aridity on the proportion of allotetraploids and diploids within populations. Interestingly, phenology was associated with aridity: time of emergence was later, while flowering and senescence were earlier in desert plants. Our results indicate that in the Eastern Mediterranean, adaptation of *Brachypodium* to aridity is mediated mainly by phenology, rather than ploidy level. Therefore, we suggest that genome duplication is not the main driver of adaptation to environmental stress; rather, phenological change as a drought escape mechanism may be the major adaptation.

Key words: abiotic stress, adaptation, *Brachypodium*, climatic gradient, polyploidy.

1 Introduction

Over the past 100 years, Earth's climate has become warmer and precipitation regimes have changed (Araújo & Rahbek, 2006). For example, the Middle East region is predicted to experience a major reduction in annual rainfall (up to 30% less) by the next century (IPCC, 2007; Sowers et al., 2011). As a consequence of the forecasted changes, climate change is expected to alter natural selection on native plant populations (Hoffmann & Sgrò, 2011). Insights on how selection will change in the future and how populations will respond can be obtained by comparing selection regimes in current environments to selection regimes in environments similar to those predicted for the future (Etterson, 2004). Climatic gradients provide a useful tool to study adaptations of plants to environmental conditions, because they can be a surrogate for experimental climate change and replace space for time (Davis et al., 2005; Holzapfel et al., 2006; Schneider & Mazer, 2016). Of particular interest are plant species with distributions that span major gradients, where a steep change in environmental conditions stretches over a relatively short distance. The climatic gradient in Israel spans from mesic Mediterranean to extreme desert, within 424 km, therefore providing a useful "natural laboratory" for studying adaptation to aridity (Petrů & Tielbörger, 2008; Lampei & Tielbörger, 2010; Kigel et al., 2011; Golodets et al., 2015). Temperature and rainfall are usually correlated factors in a Mediterranean climate, where high temperatures are expected during drought periods and vice versa (Noy-Meir, 1973). By considering the effect of these two factors combined on phenology (i.e., life history traits) of plants, we can better understand the evolution of adaptation to aridity in plants.

Three major strategies are used by plants adapted to aridity: (i) Dehydration tolerance, in which plants are able to survive

under decreased precipitation. (ii) Dehydration avoidance, in which plants increase water uptake or decrease water loss to prevent tissue dehydration. (iii) Drought escape, achieved by modifying phenology and completing all life cycles in the comfortable season, when soil humidity is high (Ludlow, 1989). While dehydration tolerance and avoidance are achieved through physiological and biochemical traits, such as stomatal closure and increased water use efficiency (Peleg et al., 2005; Sherrard et al., 2006; Xu et al., 2009), drought escape is achieved through modifications of phenological traits (Kigel et al., 2011; Westberg et al., 2013). Of the above strategies, the escape strategy is shown to be the major strategy to mitigate abiotic stresses, including drought, in annual plants (Mulroy & Rundel, 1977; Venable & Lawlor, 1980; Shmida & Burgess, 1988; Franks et al., 2007; Rosenthal et al., 2010; Franks, 2011). Because aridity selects for early flowering and short life-cycles (Volis, 2007; Franks et al., 2014), differences in flowering and senescence times across precipitation gradients are considered evidence for adaptation to variation in rainfall (Aronson et al., 1992; Kigel et al., 2011).

The evolution of adaptation to aridity may involve genetic and genomic changes, or both.Polyploidy is a phenomenon resulting in multiplications of the basic chromosomes numbers in the genome (Goldblatt, 1980). It has been proposed that polyploidy is a key process in the evolution of vascular plants (Adams & Wendel, 2005; Mayrose et al., 2011), possibly by providing increased tolerance to abiotic stress due to larger genetic diversity and higher potential for plastic responses through the evolution of duplicate genetic pathways to produce alternative phenotypes (Bohnert et al., 1995; Levin, 2002; Madlung, 2013; Gerstein et al., 2015). Thus, the proportion of allotetraploids is expected to increase with decreased precipitation and increased aridity. Indeed, a study along the aridity climate gradient in the Iberian Peninsula found geographical structure in the distribution of annual *Brachypodium* spp., with more allotetraploids in more arid environments (Manzaneda et al., 2012). On the other hand, Maherali et al. (2009) showed that physiological tolerances in *Chamerion angustifolium* had most likely evolved post polyploidization. Furthermore, research on North American angiosperms showed that the species ranges and ecological niches of polyploid and diploid species along an environmental gradient were influenced by phylogenetic history, rather than genomic duplication (Martin & Husband, 2009). Correspondingly, a recent study along the climatic gradient in Israel failed to associate rainfall with the proportion of allotetraploid abundance in annual *Brachypodium* (Bareither et al., 2017). In a detailed study, focusing on allotetraploid species, *Brachypodium hybridum*, Kurze et al. (2017) found that earlier flowering time, higher reproductive allocation and reduced root investment are associated with ecotypic within-species differentiation towards drier regions.

Given the contrasting results of previous studies, a thorough study is needed to test for the role of polyploidy in shaping adaptation along aridity gradient. Here we tested for both genome duplication (i.e., polyploidy) and phenology as possible indicators of adaptation to aridity in a large number of populations of annual *Brachypodium* spp. along the steep aridity gradient in Israel.

Annual *Brachypodium* spp. are self–fertile grasses (Vogel et al., 2009) comprised of three different species; *B. distachyon, B. stacei* and *B. hybridum*. Each of these three species is a different karyotype (López-Alvarez et al., 2012; López-Alvarez et al., 2015): the two species, *B. distachyon* ($2n = 10$, 0.631 pg/2C) and *B. stacei* ($2n = 20$, 0.564 pg/2C) are diploid species with relatively small genomes, while *B. hybridum* ($2n = 30$, 1.265 pg/2C) is a derived allotetraploid with a larger genome size (Catalán et al., 2012). These species share similar DNA content, differing only in the number of chromosomes due to multiple centric fusion/fission events (Catalán et al., 2012). In the dry Mediterranean climate of the Iberian Peninsula, allotetraploid *Brachypodium* are more abundant (Manzaneda et al., 2012) and are better adapted to aridity (Manzaneda et al., 2015) than diploid *Brachypodium*. We expected to reveal a similar pattern among *Brachypodium* spp. along the steeper aridity gradient in Israel, such that the proportion of allotetraploids would increase with aridity. We hypothesized that phenological traits associated with drought escape would be differentially expressed along the aridity gradient and that this association would be different between ploidy levels, hence allotetraploids that were hypothesized to be more abundant in desert areas would also flower earlier than diploids that are hypothesized to be more frequent in Mediterranean regions and to flower later.

2 Material and Methods

2.1 Study area and plant material

The flora of Israel is highly diverse, mainly due to a steep North-South climatic gradient including different climatic conditions, from mesic Mediterranean to extreme desert areas. The gradient spans from >1200 to ~25 mm mean annual rainfall along a relatively short distance of ~424 km.

Seeds of annual *Brachypodium* spp. were collected from 20 populations in 2013 and mature plants from 9 additional populations were collected in 2016. Sites were located along the aridity gradient in Israel, from the Hermon Mountain in the North (>1200 mm mean annual precipitation) to Zenifim Wadi in the South (~50 mm mean annual precipitation; Table 1; see map in Fig. 1A). Annual *Brachypodium* spp. are abundant throughout the Mediterranean climate region in Israel. In order to evenly represent this distribution, we sampled from at least one population in each interval of 100 mm rain (Fig. 1A). For sites in the Mediterranean climate region, we collected all seeds from rocky south-facing slopes to reduce effects of micro-scale spatial heterogeneity. In semi-arid and desert habitats (<300 mm), populations are scattered and not continuous as in the Mediterranean region; thus, seeds were collected from plants where found, regardless of the exact micro-habitat.

In 2013, we randomly collected the seeds of one spikelet from a minimum of 20 plants per site. Seeds were germinated in the nursery of Tel Aviv University Botanical Garden (TAUBG) for ploidy estimations using flow cytometry. In 2016, live plants were collected from another 9 sites, brought to the greenhouse in the same day, and planted immediately in pots with standard potting soil mixture. Their leaves were collected

Table 1 Frequency of diploid occurrence of *Brachypodium* spp. populations collected for this study

Population number	Site	GPS coordinates	Altitude (m, above sea level)	Mean annual precipitation (mm)	Mean annual temperature (℃)	Aridity Index (R:T ratio)	Collection year	*n*	Percentage of diploid plants
1	Hermon – Mount Kahal	33°16′53″N; 35°44′2″E	1331	1097	13.36	82.16	2013	17	70.59
2	Hermon – Majdal Shams	33°16′41″N; 35°46′26″E	1296	1213	13.62	89.15	2013	32	37.50
3	Hermon – Neve Ativ	33°15′56″N; 35°44′31″E	969	1060	14.99	70.77	2013	32	25
4	Hermon – Nimrod Fortress	33°15′10″N; 35°43′4″E	760	897	16.21	55.33	2013	36	100
5	Banias	33°14′40″N; 35°41′49″E	373	793	18.16	43.66	2013	32	87.50
6	Tel Hai	33°14′11″N; 35°34′31″E	303	718	18.02	39.85	2013	32	0
7	Shtula	33° 05′4″N; 35°18′26″E	631	807	16.70	48.21	2013	32	25
8	Mount Meron	32°59′39″N; 35°24′57″E	1132	909	14.25	63.79	2013	26	76.92
9	Karmiel	32°55′40″N; 35°18′2″E	280	782	17.55	44.56	2013	32	65.63
10	Carmel – Rakafot	32°44′38″N; 35° 2′33″E	510	701	16.67	42.05	2013	32	56.25
11	Carmel – Kedumim quarry	32°43′40″N; 35° 0′52″E	297	709	17.77	39.90	2013	32	93.75
12	Carmel – Oren gorge	32°42′52″N; 34°58′23″E	54	612	18.81	32.54	2013	32	68.75
13*	Atlit	32°42′25″N; 34°56′30″E	5	538	18.81	28.12	2016	15	86.67
14	Modi'in	31°53′45″N; 34°57′30″E	140	493	19.54	25.23	2013	32	56.25
15	Eshtaol forest	31°48′43″N; 35° 0′45″E	290	586	19.01	30.88	2013	28	0
16	Kiryat Anavim	31°48′46″N; 35° 6′47″E	699	696	16.82	41.39	2013	32	0
17	Bet-Shemesh heights	31°42′58″N; 34°58′36″E	318	488	18.91	25.81	2013	32	56.25
18	Avishur Plato	31°38′49″N; 34°55′06″E	367	418	18.52	22.62	2013	42	97.62
19	Amatzya	31°31′30″N; 34°53′58″E	367	370	18.70	19.79	2013	32	18.75
20	Lahav	31°23′31″N; 34°51′42″E	421	294	18.62	15.79	2013	33	96.97
21*	Laqia	31°20′55″N; 34°51′27″E	459	258	13.97	13.97	2016	25	76
22*	Meitar	31°19′57″N; 34°56′16″E	410	248	13.12	13.12	2016	8	100
23*	Ha'Negev monument	31°16′01″N; 34°49′10″E	353	202	10.68	10.68	2016	14	85.71
24*	Molada	31°15′41″N; 35° 1′05″E	521	206	11.06	11.06	2016	12	75
25*	Arad	31°15′42″N; 35°14′18″E	581	112	5.90	5.90	2016	15	100
26*	Abu Qureinat	31°08′42″N; 34°59′5″E	432	146	7.64	7.64	2016	10	100
27*	Sde Boker	30°52′02″N; 34°46′7″E	521	100	18.47	5.41	2016	21	61.90

Continued

Table 1 Continued

Population number	Site	GPS coordinates	Altitude (m, above sea level)	Mean annual precipitation (mm)	Mean annual temperature (°C)	Aridity Index (R:T ratio)	Collection year	*n*	Percentage of diploid plants
28*	Nahal Ramon	30°36′39″N; 34°51′25″E	490	66	19.36	3.41	2016	62	1.61
29	Zenifim wadi	30° 4′21″N; 34°49′41″E	470	51	20.18	2.53	2013	22	13.64

See map in Fig. 1A for the distribution of sites along the aridity gradient in Israel. Populations marked with * were not phenologically characterized. Aridity Index (R:T ratio) was calculated by dividing the mean annual precipitation by the mean annual temperature.

the next day and used to estimate ploidy levels also using flow cytometry. Ploidy level was quantified using plants from both collections, but phenological traits were measured only from plants grown from seeds collected in 2013. A total of 620 plants from 29 populations were collected and analyzed for ploidy level.

We characterized the climate for all collecting sites by retrieving mean annual precipitation and mean annual temperature data from the Israeli Meteorological Services website (http://ims.gov.il; Table 1). In order to integrate precipitation and temperature data, we calculated an aridity index as the ratio between mean annual precipitation and mean annual temperature (hereafter R:T ratio, Manzaneda et al., 2012; Table 1). Precipitation and temperature along the Israeli aridity gradient are negatively correlated ($r = -0.88$, $P < 0.001$), thus the aridity index calculated, accounts also for their co-variation.

2.2 Ploidy level

To test our hypothesis that the proportion of allotetraploids should increase with aridity, we estimated the genome size of mature plants and plants grown from seeds that were collected along the gradient. Genome size is an indicator of ploidy level in *Brachypodium* spp., where a small genome size indicates a diploid plant and a large genome size indicates an allotetraploid (Manzaneda et al., 2012). Because both of the small genome size species are diploids and because our question focuses on the role of polyploidy in adaptation to aridity, in our analyses of genome size we did not differentiate between *B. distachyon* and *B. stacei*.

Ploidy level was estimated for 8 to 62 plants in each population (median: 32 plants per population) using BDbiosciences FACSort (flow cytometer) (Dolezel & Bartos, 2005). From each plant two or three leaves equal to 20 mg were chopped with a razor blade in a Petri dish and 0.5 mL Galbraith buffer (Galbraith, 1983) was added together with 1.5 mL containing 5 mg/mL Propidium Iodide (Sigma-Aldrich, USA) and 100 mg/mL RNaseA (Roche Diagnostics, Germany). The sample was then transferred on ice to the BDbiosciences FACSort. Plants of the sequenced *B. distachyon* line Bd21 (The International *Brachypodium* Initiative, 2010) were used in all runs of the FACS as an external standard reference. To validate our estimates, we measured ploidy levels also within families (i.e., among sibling plants) and among generations (parent and its offspring) for a sub-sample of 312 plants. In all the 312 families ploidy level was identical between parent and offspring and among sibling plants. The FACS data were analyzed using Flowing Software 2.5.1 (Turku Centre for Biotechnology, University of Turku, Finland). As an additional validation of our ploidy level results, we counted the number of chromosomes visually in 64 plants of the two ploidy levels (Doc. S1).

2.3 Phenological measurements

We hypothesized an association of earlier phenology with increased aridity along the gradient, as an indication of the drought escape strategy. In order to test differences in phenology between plants from different populations along the aridity gradient, at each of the 20 populations collected in 2013, we grew plants of 9 to 21 (mean = 16) plant families in the nursery in TAUBG. Plants were grown for two generations to reduce maternal effects on phenotypic traits (Bischoff & Müller-Schärer, 2010).

During the first growing season, from fall of 2013 to the summer of 2014, we germinated plants in a commercial germination soil mix (Melzer Nursery, Inc.) and watered for 20 min twice a day by upper mist, an amount of water equivalent to approximately 3.5 liters per day. When most plants (>80%) had at least six leaves, we transplanted them directly in the soil, into 6 m^2 experimental plots in TAUBG. The plants were planted 20 cm apart from one another to avoid competition. To reduce interspecific competition the area in and around the plots was regularly weeded. The plots had open-sided sheds covered with transparent plastic panels (Fig. S1) to provide natural environmental conditions while controlling water. The plants were watered for 3 h twice a week by drippers of 2 L/h, spread 30 cm apart from each other. In both growing seasons, all sheds received an equal amount of water.

Between mid-April and early July 2014, plants were harvested according to their individual senescence times. The seeds of these plants were stored in paper bags at 4 °C and six seeds from each family were randomly selected for germination in the next year (2014/2015). The second growing season began with the sowing of these six randomly selected seeds from each family on November 30th 2014. Of those six seeds, two seedlings were randomly chosen and transplanted in the experimental plots on mid-February 2015. Each seed was tagged individually and tracked throughout the whole growing period for phenological data (see below) and genomic data. The second growing season ended with the senescence of the last plant on July 6th 2015. The growing protocol and watering conditions were identical to the previous year.

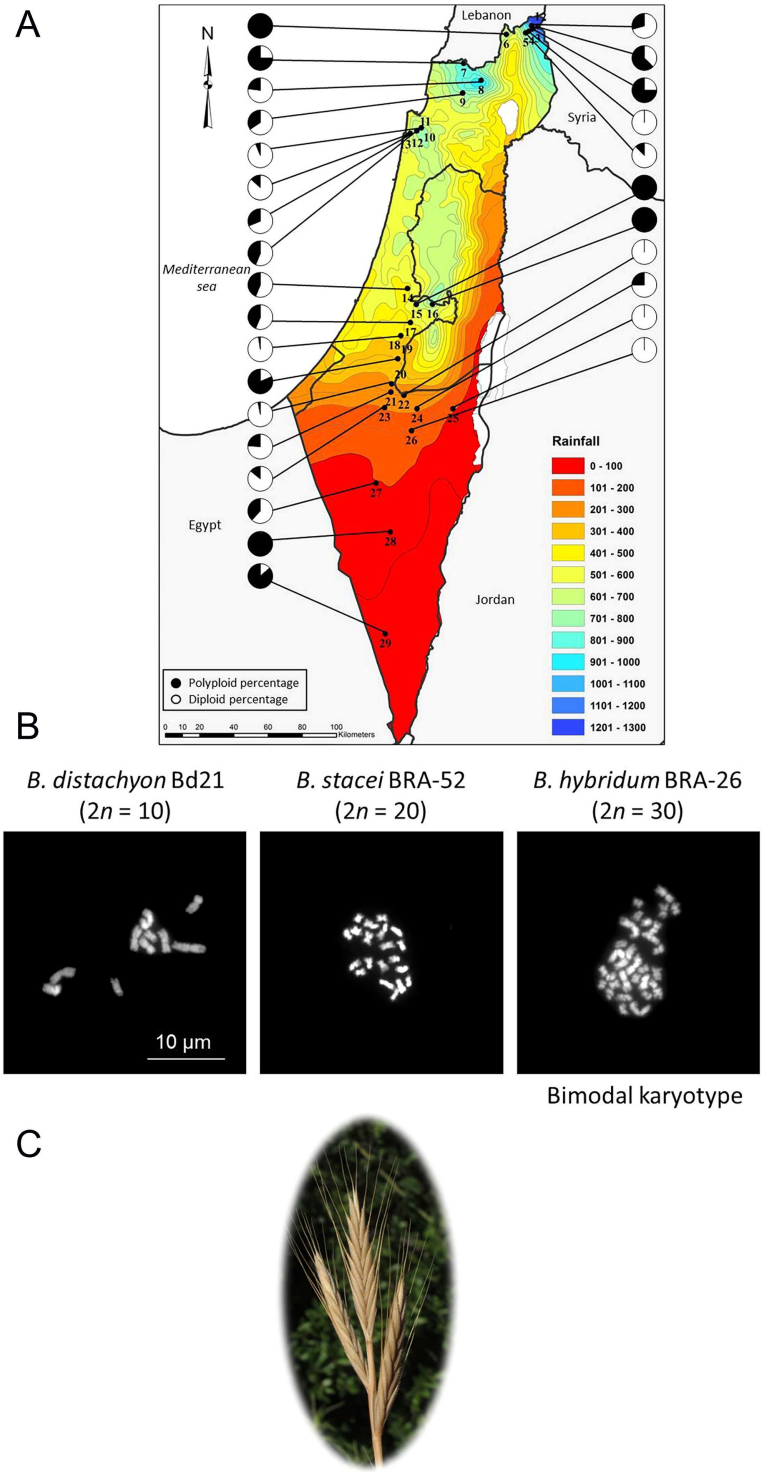

Fig. 1. A, Geographical origin of *Brachypodium* spp. accessions used in the study, on the background of the annual mean precipitation. Pie charts display the frequencies of allotetraploids and diploids in each population. Precipitation data are mean for the years 1981-2010 (Israel Meteorological Service; http://ims.gov.il). **B,** Three cytotypes of *Brachypodium* spp. stained with DAPI and imaged using a Zeiss Z2 epifluorescence microscope and CoolCube CCD camera (Hasterok et al., 2006). Image courtsey of Dr. Terezie Mandakova. **C,** *Brachypodium* spp. plant in its natural habitat in Nimrod Fortress (897 mm).

Phenological measurements of this second generation included life history traits that represent either adaptation to aridity, as described in Kigel et al. (2011), or putative differences between cytotypes, as was tested by Manzaneda et al. (2012). Three phenological traits were recorded: (i) Emergence time was recorded as the number of days from sowing until shoot emergence. (ii) Flowering time was recorded as the number of days from sowing to first appearance of awns in the first spikelet. (iii) Senescence time was measured as days from sowing to the day when all vegetative parts of the plant dried out.

In addition to phenological traits, we measured morphological traits in order to phenotypically define the differences between cytotypes (following Catalán et al., 2012). Because no morphological differences were found between diploids and allotetraploids (Fig. S2), we focused our analyses on phenological traits.

2.4 Statistical analyses

Statistical analyses were performed using R (R Development Core Team, 2014). Generalized linear model (GLM) with a defined binomial distribution was used for testing the effect of the aridity index on the proportion of diploid and allotetraploid plants in a given population.

In order to evaluate the changes of phenological traits along the aridity gradient we did analysis of variance (ANOVA), using GLM with aridity index for each site as continuous (random) variable, ploidy level as a factorial variable, and tested for interaction between ploidy level and aridity index in their effect on the phenology. Seedling emergence, flowering and senescence times are count data, measured as days, thus we used GLMs with Poisson distributions for these phenological traits. For all the phenological trait models, type II analyses were calculated to test for significant effects of all variables.

Correlations between the different phenological traits and ploidy levels were tested, using Pearson's correlation coefficient, in order to understand phenological patterns that maybe shaping life cycle variation. To account for multiple tests, Bonferroni correction was used for *P*-values obtained for all traits and ploidy levels tested.

3 Results

3.1 Distribution of polyploids along the aridity gradient

Flow cytometry revealed two distinct genome sizes, a small size of 200 fluorescence intensity units, and a large size of 400 fluorescence intensity units. The smaller size was identical to the size of the reference plant Bd21. Following Catalán et al. (2012), these plants were considered as diploids, representing either $2n = 10$ or $2n = 20$ chromosomes (*Brachypodium distachyon* and *B. stacei*, respectively; Fig. 1B). Plants with a large genome size were considered as allotetraploids, representing $2n = 30$ chromosomes (*B. hybridum*; Fig. 1B). We did not detect intermediate genome sizes.

Overall, 59.8% of the plants measured in the study were diploids and 40.2% were allotetraploids (Table 1). The proportion of allotetraploid plants within populations ranged between 0 and 100%. In three populations (Tel Hai, Kiryat Anavim, and Eshtaol forest), no diploids were detected, while in four other populations (Nimrod Fortress, Meitar, Arad and Abu Qurinat), all sampled individuals were diploids. All other populations included both ploidy levels, with diploids proportion ranging from 1.61% to 97.62% (Table 1).

Mean frequency of allotetraploid across populations was 0.53. There was no correlation between the proportion of allotetraploids and aridity along the aridity gradient (GLM with correction to binomial distribution: regression slope ≈ 0, $P = 0.512$; Fig. 2).

3.2 Ploidy level and phenology along the aridity gradient

In order to understand life history strategies and patterns of life cycle variation among *Brachypodium* plants along the aridity gradient we tested for correlations among traits and ploidy levels using Pearson correlation. For diploids, emergence time was negatively correlated with flowering time ($r = -0.027$, $P = 0.918$), emergence time was positively correlated with senescence time ($r = 0.028$, $P = 0.916$), and flowering time was positively correlated with senescence time ($r = 0.448$, $P = 0.071$). For allotetraploids, emergence time was negatively correlated with flowering time ($r = -0.317$, $P = 0.185$), emergence time was negatively correlated with senescence time ($r = -0.658$, $P = 0.002$), and flowering time was positively correlated with senescence time ($r = 0.315$, $P = 0.189$). After Bonferroni correction for multiple tests all the correlations' *P*-values were not significant, except for the correlation between emergence time and senescence time in the allotetraploids ($P = 0.002$).

Furthermore, we tested for an association between phenological traits and ploidy level, aridity, and their interaction. All three phenological traits (i.e., emergence, flowering and senescence time) were significantly affected by aridity (Table 2; see mean values for populations and ploidy levels in Table S1). In more details, emergence time in arid populations (<400 mm annual rainfall) was in average 4.8 days (±0.05), later than in Mediterranean populations (1200–400 mm annual rainfall) emerging after 4.7 days (±0.08). Arid populations started flowering after 103.5 days in average (± 0.33), earlier than Mediterranean populations that started flowering after 109.8 days (±0.43). Finally, senescence time in plants from arid populations was in

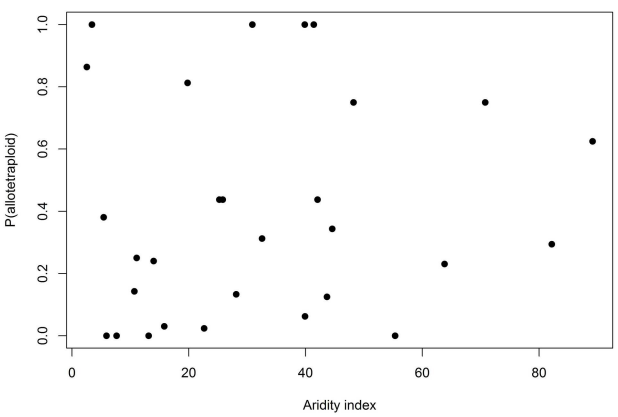

Fig. 2. Frequency of allotetraploid plants in populations of *Brachypodium* spp. in Israel as a function of aridity index.

Table 2 Effect of aridity index, ploidy level and their interactions on population mean values of phenological traits in *Brachypodium* spp. from populations along the aridity gradient in Israel

Source Phenological traits	Aridity index Significance	Ploidy level Significance	Slope diploids	Slope allotetraploids	Interaction Significance
Emergence time	***P* = 0.013**	***P* = 0.035**	0 ($P = 0.99$)	−0.019 ($P = 0.051$)	$P = 0.073$
Flowering time	***P* = 0.0009**	$P = 0.862$	**0.188** ($P = 0.003$)	**0.185** ($P = 0.001$)	$P = 0.972$
Senescence time	***P* = 0.038**	$P = 0.704$	0.065 ($P = 0.054$)	0.085 ($P = 0.078$)	$P = 0.725$

Slopes that are significantly different from zero are in bold.

average 154.9 days (±0.54), earlier than in the Mediterranean populations senescing after 180.45 days (±0.71).

Emergence time was the only trait that was significantly affected by ploidy level, but the difference was relatively small — only 0.26 days (diploids: 4.3 ± 0.04 days; allotetraploids: 4.56 ± 0.11 days; see mean values for populations and ploidy levels in Table S1). None of the tested traits revealed a significant interaction between ploidy level and aridity (Table 2; Fig. 3).

4 Discussion

Phenology and ploidy level are considered as mechanisms related to adaptation along aridity gradients (Levin, 2002; Petrů et al., 2006; Kigel et al., 2011). Our study tested this hypothetical association in annual *Brachypodium* spp. along the steep aridity gradient in Israel. Contradict to this well-established hypothesis, and despite using relatively large sample of populations, we found no evidence that allotetraploids confer an advantage to *Brachypodium* plants in arid climates. Nonetheless, life history phenological traits that are, seedling emergence, flowering time and senescence time, were significantly associated with aridity (Fig. 3; Table 2). The phenological shift towards delayed germination, and shorter life cycle, expressed in earlier flowering and senescence, may indicate an adaptation of plants from arid habitats to mitigate arid conditions. Thus, our results support the hypothesis of phenotypic adaptation of life history traits to aridity, but do not support the genomic mechanism of genome doubling as a driver of adaptation to aridity.

A positive correlation between ploidy level and aridity was previously described in annual *Brachypodium* spp. along the aridity gradient in the Iberian Peninsula, from 250–1773 mm of annual rainfall (Manzaneda et al., 2012). Here, we tested whether this hypothesized relationship is also found along the drier (50–1200 mm of annual rainfall) and geographically shorter aridity gradient in Israel. Although we found inter-population variation in the fraction of allotetraploids, the distribution of diploid and allotetraploid plants was not associated with the aridity gradient in Israel. These results are similar to those of Glennon et al. (2014) that found similar climatic niche of allotetraploids and their diploid relatives in North American and European plants. Our results are also similar to those of Bareither et al. (2017), who studied *B. distachyon* along the rain gradient in Israel and found no apparent distribution pattern of diploid and allotetraploids along the rain gradient. Nonetheless, that work tested 15 populations only, from semi-arid to mesic Mediterranean climates (114 mm to 954 mm annual rainfall) and used rainfall

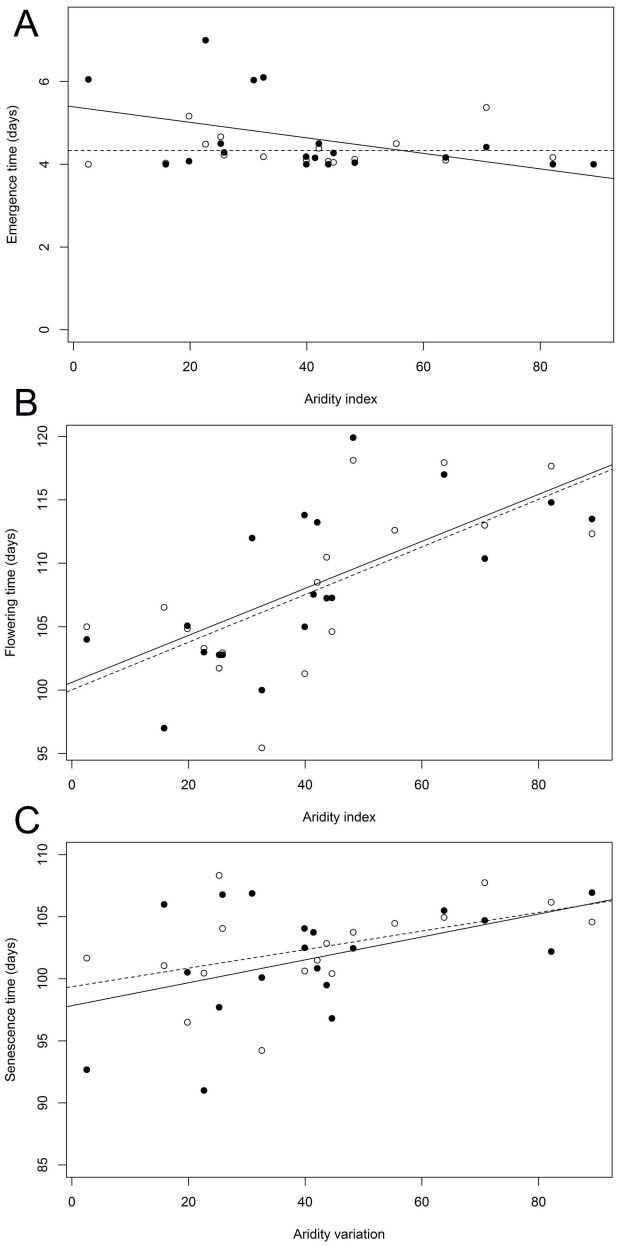

Fig. 3. Effect of ploidy level and aridity index on phenological traits of *Brachypodium* spp. from populations along the aridity gradient in Israel. **A,** Emergence time. **B,** Flowering time. **C,** Senescence time. Points are means for diploid plants (empty circles) and allotetraploid plants (full circles) within each population; lines denote partial regression slope for diploid plants (dashed line) or allotetraploid plants (solid line).

as the environmental parameter. In our study the sample size included 29 populations from extreme desert to mesic Mediterranean climates (~50 mm to >1200 mm). We also used the aridity index that accounts for both the thermal and rainfall gradients, thus providing a stronger support to the hypothesis that the climatic gradient is not affecting cytotype distribution of *Brachypodium* spp. in Israel.

Phenological variation along climatic gradient was shown before in *Brachypodium* spp. in Israel (Kigel et al., 2011; Kurze et al., 2017). These two previous studies indeed showed phenological change in *Brachypodium* populations along the gradient, but both used only rainfall as an environmental parameter, without considering the effect of temperature on aridity. Moreover, Kurze et al. (2017) studied only *B. hybridum*, and did not test the possible role of ploidy level in adaptation by comparing the allotetraploid *B. hybridum* to the diploid *B. distachyon* and *B. stacei*, as was done in this research.

4.1 Distribution of ploidy levels along aridity gradients

Many studies claimed that polyploid plants often differ from their diploid progenitors in ecophysiological tolerance to abiotic stresses (e.g., Levin, 2002; Maherali et al., 2009; Mráz et al., 2014). Allotetraploid *Brachypodium* plants are more efficient in water use under water-restricted growing conditions, compared to diploids (Manzaneda et al., 2015). Thus, it is surprising that *Brachypodium* plants along the Israeli aridity gradient deviate from this hypothesized pattern. The striking difference between the two aridity gradients suggests that different evolutionary pathways may govern adaptation to aridity in these two regions. The origin of annual *Brachypodium* spp. is proposed to be in the Middle East, from where it dispersed westward throughout the Mediterranean basin (Opanowicz et al., 2008). It is likely that during this expansion, *Brachypodium* spp. acquired different genetic mechanisms to mitigate climatic stresses. Thus, while in the Western Mediterranean the effect of genome doubling on adaptation to aridity was more pronounced, in the original region of the species complex these adaptations did not require genomic change.

In addition, differences between the two gradients may explain the different distribution of diploids and allotetraploids. Along the Israeli aridity gradient that ranges up to extreme desert (<50 mm mean annual rain and ~3 aridity index units, reflects also high average temperature) we found no pattern of ploidy level distribution. On the other hand, the gradient in the Iberian Peninsula is less extreme, with minimum precipitation of only 250 mm and about 18 aridity index units, still showing a significant association with diploid/allotetraploids distribution (Manzaneda et al., 2012). Therefore, our findings suggest that the association of ploidy level and adaptation to aridity stress is not ubiquitous across the distribution of the *Brachypodium* complex, implying that adaptation to environmental aridity in *Brachypodium* spp. is possibly locally evolved, regardless of ploidy level.

The origin of the allotetraploids in Israel is still unknown; it is unclear whether they were formed once and then spread, or whether hybridization occurred several times independently in different places along the gradient (Soltis & Soltis, 1999). Given that we found no evidence that allotetraploids confer an advantage to *Brachypodium* plants in arid climates, it is likely that the evolution of allotetraploids in Israel is neutral to climate and may have evolved multiple times in independent populations. A population genetic study is underway to uncover the genetic relationships between diploids and allotetraploids within and among populations, to shed light on this question.

4.2 Ploidy level and phenology along the aridity gradient

Drought escape is a known strategy employed by plants to mitigate water stress. Drought escape is advantageous because it allows plants to complete the entire life cycle within the growing season, when soil humidity is still high and temperature is relatively low (Ludlow, 1989; Mckay et al., 2003; Sherrard et al., 2006). Phenological traits, including emergence, flowering and senescence timing, play a central role in drought escape (Mckay et al., 2003). Among these, early flowering time is considered a key adaptive trait for drought escape in annual plants (Kigel et al., 2011). Similar to our findings, shifts in flowering phenology were revealed as an adaptation to stress in an invasive knapweed, regardless of the ploidy level (Mráz et al., 2014). Furthemore, Aronson et al. (1992) demonstrated that senescence shifted earlier in annual plants under water stress, and that this response was greater in desert populations than in Mediterranean ones. We show here that flowering and senescence times are significantly associated with aridity (Figs. 3B, 3C and Table 2), which may indicate an adaptation of plants from arid habitats to flower and senesce earlier than Mediterranean plants.

Emergence time was also affected by aridity (Fig. 3A; Table 2), but this association showed that plants originating from dry regions emerged later than those originating from mesic cool regions, which may indicate a risk-spreading strategy. This strategy may have an advantage under arid and unpredictable conditions, allowing the plants to spread the germination along the season with lower germination fractions each time (Venable et al., 1980; Metz et al., 2018).

In our study we found that, allotetraploids emerged later than diploids (Table 2; Fig. 3A). This could be explained if different cell sizes of diploids and polyploids (smaller and larger cell sizes, respectively) affect the timing of basic processes such as cell division. Thus, emergence may be delayed in polyploids in contrast to diploids (Comai, 2005). Nevertheless, the change in emergence time as a function of aridity was not significantly different between diploids and allotetraploids, thus preventing definitive conclusions about the role of ploidy level in adaptation to aridity.

5 Conclusions

The natural distribution of *Brachypodium* in Israel spans a range of different climatic conditions, from mesic Mediterranean to extreme desert, within a short geographical range along the aridity gradient (Davis et al., 2005; Holzapfel et al., 2006; Petrů & Tielbörger, 2008; Lampei & Tielbörger, 2010; Golodets et al., 2015). By replacing time with space, the gradient approach allows us to make some inferences regarding potential adaptation of plants to climate change (Petrů & Tielbörger, 2008; Lampei & Tielbörger, 2010; Talmon et al., 2011). Substituting space for time, our results suggest that the traits of plants that are successful in dry, desert habitats today may be adaptive for plants in mesic and cool

locations in a future under climate change, according to the climatic predictions for the area of Israel.

Future research should experimentally address the contributions of environmental and genetic variation (and their interaction) on plant performance under various stresses using ecological experiments, such as reciprocal transplantation. Significant home versus away advantages may reflect local specialization by genetic adaptation (Joshi et al., 2001; Franks & Hoffmann, 2012) and may enable us to make predictions about the evolutionary responses of *Brachypodium* spp. to climate change.

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Supplementary Material

The following supplementary material is available online for this article at http://onlinelibrary.wiley.com/doi/10.1111/jse.12489/suppinfo:

Doc. S1. Chromosome counts method.

Fig. S1. Common garden using rain shelters in courtesy of the Tel Aviv University Botanical Garden.

Fig. S2. Principal component analysis (PCA) of 5 morphological traits measured in populations of *Brachypodium* spp. in Israel. Diploid plants are represented by empty circles and

allotetraploid plants by full circles. We measured five morphological traits that account for the difference between diploid and polyploid cytotypes in *Brachypodium* (Catalán et al., 2012). These traits included the following: 1) Inflorescence length, measured as the length of the inflorescence on the primary stem, from the bottom of the lower spikelet to the top of the upper one (no awns included). 2) Spike length, measured as the length of the upper spikelet on the primary stem (no awns included). 3) Stem length, measured as the length of the primary stem from the ground to the bottom of the lower spikelet. 4) Stems number, counted as the number of branches at the base of the plant. 5) Aboveground biomass, measured as the dry weight of the shoot, without the roots and the spikelets. Morphological differentiation between ploidy levels was tested using the first two principal components of the multivariate analysis which explained 75.7% of the morphological variance. We found no difference between the morphology of diploid and allotetraploid plants, as PCA plot did not show any clustering of the two ploidy levels.

Table S1. Mean values (±SE) of phenological traits across individuals in diploid and allotetraploid plants of *Brachypodium* from all populations collected along the Mediterranean–desert gradient in Israel. Differences were tested for significance using student's *t*-test.