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Distribution of polyploid plants in the common annual *Brachypodium distachyon* (s.l.) in Israel is not linearly correlated with aridity

Nils Bareither^a, André Scheffel^b and Johannes Metz^a

^aPlant Ecology & Nature Conservation Group, University of Potsdam, Potsdam, Germany; ^bMax Planck Institute of Molecular Plant Physiology, Potsdam, Germany

ABSTRACT

The ecological benefits of polyploidy are intensely debated. Some authors argue that plants with duplicated chromosome sets (polyploids) are more stress-resistant and superior colonizers and may thus outnumber their low ploidy conspecifics in more extreme habitats. *Brachypodium distachyon* (*sensu lato*), for example, a common annual grass in Israel and the entire Mediterranean basin, comprises three cytotypes of differing chromosome numbers that were recently proposed as distinct species. It was suggested that increased aridity increases the occurrence of its polyploid cytotype. Here, we tested at two spatial scales whether polyploid plants of *B. distachyon* s.l. are more frequently found in drier habitats in Israel. We collected a total of 430 specimens (i) along a large-scale climatic gradient with 15 thoroughly selected sites (spanning 114–954 mm annual rainfall), and (ii) from corresponding Northern (more mesic) and Southern (more arid) hill slopes to assess the micro-climatic difference between contrasting exposures. Cytotypes were then determined via flow cytometry.

Polyploid plants comprised 90% of all specimens and their proportion ranged between 0% and 100% per site. However, this proportion was not correlated with aridity along the large-scale gradient, nor were polyploids more frequently found on Southern exposures.

Our results show for both spatial scales that increasing aridity is not the principal driver for the distribution of polyploids in *B. distachyon* s.l. in Israel. Notably, though, diploid plants were restricted essentially to four intermediate sites, while polyploids dominated the most arid and the most mesic sites. This, to some degree, clustered pattern suggests that the distribution of cytotypes is not entirely random and calls for future studies to assess further potential drivers.

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Introduction



Polyploidization, i.e. the duplication of entire chromosome sets, is an important mechanism of speciation that has played a significant role in the origin and evolution of many plant species (de Wet 1971; Levin 2001; Wendel & Doyle 2005). It can result from either *intra*-specific genome duplication (autopolyploidy) or the acquisition of at least two complete chromosome sets via *interspecific* hybridization (allopolyploidy) (Stebbins 1947; te Beest et al. 2012). Despite major advances in molecular tools and corresponding insights in the genomic and genetic mechanisms of polyploidy, understanding of its ecological consequences is still limited (Soltis et al. 2010; Ramsey & Ramsey 2014).

An often suggested yet debated notion is that polyploids possess larger ecological amplitudes and

tolerate more extreme conditions than their diploid ancestors (reviewed in te Beest et al. 2012; del Pozo & Ramirez-Parra 2015). This is supported by the fact that polyploidization increases the genetic potential for evolutionary (pre-)adaptation to diverse environmental conditions, e.g. due to augmented heterozygosity, genome rearrangements and gene redundancy with higher chances for beneficial mutations (Comai 2005; te Beest et al. 2012). This may also contribute to explain why some polyploid lineages are highly successful colonizers and invaders (Soltis & Soltis 2000; Pandit et al. 2011; te Beest et al. 2012). However, whether polyploids generally inhabit distinct habitat ranges than their diploid relatives is inconclusive (Soltis et al. 2010; te Beest et al. 2012), with strong evidence for wider and/or divergent distributions of

CONTACT Johannes Metz  johmetz@uni-potsdam.de

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polyploids (e.g. Brochmann et al. 2004; Krejčíková et al. 2013; Lopez-Alvarez et al. 2015) but also against it (e.g. Stebbins & Dawe 1987; Petit & Thompson 1999; Martin & Husband 2009).

A more specific case is the relationship between polyploidy and aridity, where several lines of evidence support an association of polyploidy with more arid conditions. One line is the above-mentioned enlarged genetic potential for environmental adaptations (Comai 2005; te Beest et al. 2012). Furthermore, polyploids usually possess larger cells due to their duplicated genomes and therefore fewer but bigger stomata (te Beest et al. 2012; del Pozo & Ramirez-Parra 2015). Consistent with that, polyploid lineages showed decreased transpiration rates in *Betula papyrifera* (Li et al. 1996) as well as higher tolerance to water stress in the perennial herb *Chamerion angustifolium* (Maherali et al. 2009). Polyploids of *C. angustifolium* were also shown to inhabit drier habitats than their diploid conspecifics (Thompson et al. 2014) and the same was reported for polyploids of *Centaurea maculosa*, especially within its invasive North American range (Treier et al. 2009). These various findings support the claim that differential drought adaptation may be of major importance for the spatial distribution and niche differentiation between diploid and polyploid plants (Weiss-Schneeweiss et al. 2013; Thompson et al. 2014).

However, a more intricate study system regarding polyploidy and aridity is the annual grass complex *Brachypodium distachyon* (*sensu lato*), a common species in Israel and the entire Mediterranean basin (Feinbrun-Dothan & Danin 1998; Lopez-Alvarez et al. 2015). It has also emerged as a powerful model system for functional genomics in Poaceae (Draper et al. 2001; Vogel et al. 2010), including some studies on polyploidy. Three different cytotypes have been identified for this grass (Robertson 1981) which were recently described as distinct species: the older diploid lineage of *B. stacei* ($2n = 20$), the more recent and likewise diploid lineage of *B. distachyon* (*sensu stricto*) ($2n = 10$) and their subsequent allopolyploid hybrid *B. hybridum* ($2n = 30$) (Catalan et al. 2012, 2016; Lopez-Alvarez et al. 2015). In their pivotal paper, Manzaneda et al. (2012) reported for the Iberian Peninsula that polyploids ($2n = 30$) outnumbered diploids ($2n = 10$) towards drier climates and suggested that aridity is an important driver for polyploid distribution also within the *B. distachyon* *s.l.* complex. However, although this

claim was substantiated by ecophysiological differences between $2n = 10$ and $2n = 30$ plants for a subset of populations (Manzaneda et al. 2015), other factors such as Holocene re-colonization processes could possibly underlie the observed polyploid distribution (Manzaneda et al. 2012). A more complex relation between aridity and polyploidy was also suggested by environmental niche models that were calculated from the (still limited) location records of the three cytotypes/species: the most divergent niches regarding aridity existed between the two *diploids* ($2n = 10$, $2n = 20$), yet the polyploid *B. hybridum* ($2n = 30$) was intermediate and showed substantial niche overlap with both diploids (Lopez-Alvarez et al. 2015). While this questions the idea that polyploids of *B. distachyon* *s.l.* are generally more drought-resistant and gradually outnumber diploids with increasing aridity, additional studies are missing that tested the reported distribution pattern from the Iberian Peninsula (Manzaneda et al. 2012) for other regions.

Large-scale distribution patterns of ancestral lineages, however, remain generally prone to bias by (re-)colonization history because they can hardly disentangle two alternatives: is a certain lineage missing in particular regions because it cannot exist there, or because it has not yet arrived there? As a promising approach to disentangle these two alternatives and to relate distribution patterns more closely to environmental drivers, we propose to combine large-scale distribution studies with tests at much smaller spatial scale where colonization history is unlikely to bias distributions. For example, large-scale aridity gradients are mirrored by opposing North vs. South exposed hillslopes at only a few hundred meters distance. South slopes typically possess more arid micro-climates than corresponding North slopes (northern hemisphere), and this difference in abiotic conditions is reflected in the resident plant communities, with South slopes harboring species compositions and standing biomass similar to macro-climatically more arid sites (Kutiel 1992; Kutiel & Lavee 1999; Nevo 2012; Guierrez-Jurado et al. 2013). It would therefore be intriguing to test whether potential shifts in polyploid frequency along large-scale aridity gradients are matched by corresponding frequency shifts at smaller spatial scale, e.g. between North vs. South exposures.

The present study followed this approach and tested whether the distribution of polyploids within *B. distachyon* *s.l.* in Israel is associated with increasing

aridity at both large and small spatial scale. To this aim, we analyzed the cytotype of a total of 430 specimens that originated from 15 sites along a steep natural rainfall gradient (large spatial scale) and from North and South hill slopes within single sites (small spatial scale). In particular, we tested the hypotheses that polyploids are more frequent compared to diploids (i) towards drier sites along the large-scale gradient and (ii) also on Southern than on Northern hill slopes.

Materials and methods

Study organism

Brachypodium distachyon (*sensu lato*) (L.) Beauv. is a common annual grass of approximately 15–40 cm height with a circum-Mediterranean and Irano-Turanian distribution (Feinbrun-Dothan & Danin 1998; Catalan et al. 2012). It is widespread across Israel from the Upper Galilee to the Negev Desert where it covers a substantial climatic range from >1000 to 100 mm annual rainfall and inhabits a wide variety of habitats, e.g. semi-natural shrublands, road sides and abandoned fields (Feinbrun-Dothan & Danin 1998).

B. distachyon s.l. comprises three different cytotypes that were recently proposed as distinct species: the diploid *B. stacei* ($2n = 20$), the likewise diploid *B. distachyon* (*sensu stricto*) ($2n = 10$) and their allopolyploid hybrid *B. hybridum* ($2n = 30$) (Catalan et al. 2012, 2016). The genome sizes of both diploids are very similar despite their different chromosome numbers, with c -values of 0.564 pg/2C DNA ($2n = 20$ type) and 0.631 pg/2C DNA ($2n = 10$); consequently, their polyploid hybrid *B. hybridum* ($2n = 30$) has approximately twice the genome size of the diploids with $c = 1.265$ pg/2C (Vogel et al. 2006; Wolny & Hasterok 2009; Catalan et al. 2012). Moreover, all three species can be separated via morphological details (Catalan et al. 2016).

Sampling sites

As large-scale aridity gradient, we utilized 15 sites along the exceptionally steep natural rainfall gradient from Northern to Southern Israel (Figure 1). It spanned about 215 km in total and we kept at least 4 km distance between single sites. Notably, these sites comprised the largest part of the principal aridity gradient in Israel and spanned from mesic Mediterranean to

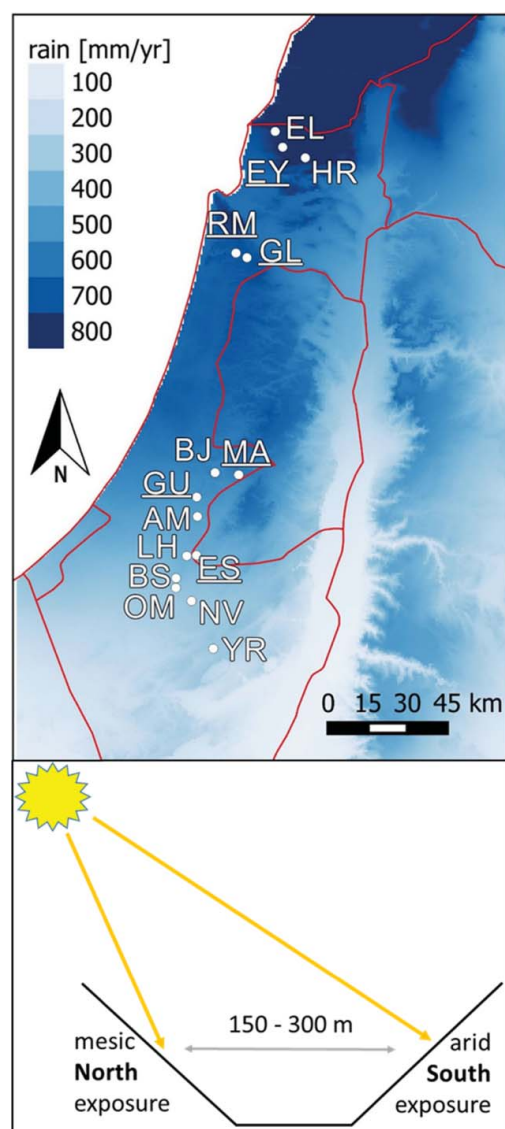


Figure 1. Top: Rainfall map of Israel and adjacent areas showing mean annual precipitation and the location of single study sites; underlined sites comprised both North and South exposures. Bottom: schematic illustration of the small-scale aridity gradient between corresponding North and South exposures within single sites.

arid conditions with parallel vegetation changes from dense Mediterranean woodlands to sparse desert shrublands (Table 1, Figure 1). Mean annual precipitation per site ranged between 114 and 954 mm, as calculated across the 30-year data record prior to our research (1984–2013) of the nearest available rainfall station of the Israel Meteorological Service (0.5–3.0 km distance to sampling sites; www.ims.gov.il). Single sites were furthermore chosen to have minimal differences in environmental conditions other than rainfall. Namely, all sites were located in comparable habitats (semi-natural shrub or woodlands), on

Table 1. Characterization of the 15 sampling sites with their contrasting North and South exposures (if available). Sites were named after the nearest town or village.

Site	Exposure	Rain ^a (mm/ year)	Temp. (°C) ^b	Altitude (m a.s.l.)	Slope (°inclination)	Coordinates	Brachyp. abundance ^c	No. indiv. ^d 2n = 10/20/30	Vegetation
HR, Harashim	N	954	17.8	669	22	32.9646°N, 35.3287°E	4	0/0/20	Dense woodland, about 80% tree cover of <i>Quercus calliprinos</i> , <i>Calicotome villosa</i> , <i>Rhamnus lycioides</i> ; <25% cover of herbaceous layer
EY, Ein Yaacov	N	838	18.9	480	13	33.0057°N, 35.2394°E	4	0/0/20	Dense woodland, about 80% tree cover of <i>Q. calliprinos</i> , <i>C. villosa</i> ; <25% cover of herbaceous layer
	S			490	12	33.0067°N, 35.2394°E	4	2/0/28	Dense shrubland, about 75% shrub cover of <i>C. villosa</i> , <i>Sarcopoterium spinosum</i> , <i>Cistus</i> spp.; 25–75% herbaceous cover between shrubs
EL, Eilon	N	811	19.8	225	12	33.0689°N, 35.1968°E	4	0/0/20	Woodland, about 60% woody cover of <i>Q. calliprinos</i> , <i>C. villosa</i> , <i>S. spinosum</i> , <i>Salvia fruticosa</i> ; 25–50% herbaceous cover between trees/shrubs
RM, Ramot Menashe	N	661	19.7	158	15	32.5878°N, 35.0613°E	4	0/0/20	Dense grassland, about 75% cover of annual and perennial herbaceous vegetation, 10% shrub cover of <i>C. villosa</i>
	S			140	10	32.5885°N, 35.0558°E	4	0/0/20	Similar to N-slope; about 70% herbaceous cover
GL, Gelad	N	636	19.5	230	15	32.5677°N, 35.1003°E	4	0/0/20	Dense grassland, about 70% cover of annual and perennial herbaceous vegetation, 10% shrub cover of <i>Majorana syriaca</i> , <i>C. villosa</i>
	S			230	13	32.5703°N, 35.0986°E	4	0/0/20	Similar to N-slope, about 60% herbaceous cover
MA, Mata	N	589	18.1	606	17	31.7117°N, 35.0691°E	4	1/0/19	Shrubland, about 70% shrub cover of <i>C. villosa</i> , <i>S. spinosum</i> , <i>Cistus</i> spp., <i>Q. calliprinos</i> ; 50–75% herbaceous cover between shrubs
	S			610	17	31.7130°N, 35.0665°E	4	0/3/17	Shrubland, about 50% shrub cover of <i>C. villosa</i> , <i>S. spinosum</i> , <i>Cistus</i> spp.; about 50% herbaceous cover between shrubs
BJ, Bet Jimal	N	505	19.8	335	19	31.7212°N, 34.9735°E	2	0/20/0	Dense shrubland, about 70% shrub cover of <i>Cistus</i> spp., <i>Q. calliprinos</i> , <i>S. fruticosa</i> , <i>S. spinosum</i> ; 25–50% herbaceous cover between shrubs
GU, Bet Guvrin	N	393	19.8	302	14	31.6223°N, 34.9009°E	2	2/4/14	Shrubland, about 50% shrub cover of <i>S. spinosum</i> , <i>Pistacia lentiscus</i> , <i>Q. calliprinos</i> , <i>R. lycioides</i> ; about 50% herbaceous cover between shrubs
	S			306	14	31.6238°N, 34.9014°E	2	0/1/18	Similar to N-slope
AM, Amatziya	N	380	19.6	329	19	31.5467°N, 34.9041°E	2	0/11/9	Shrubland, about 40% shrub cover of <i>R. lycioides</i> , <i>P. lentiscus</i> , <i>S. spinosum</i> , <i>Q. calliprinos</i> ; 25–50% herbaceous cover between shrubs
LH, Lahav	N	298	18.9	428	17	31.3884°N, 34.8532°E	5	0/0/20	Shrubland, about 70% shrub cover of <i>S. spinosum</i> , <i>Euphorbia hierosolymitana</i> ; 50–75% herbaceous cover between shrubs
ES, Eshkolot	N	298	18.4	555	17	31.3929°N, 34.9008°E	5	0/0/20	Shrubland, about 70% shrub cover of <i>S. spinosum</i> ; about 50% herbaceous cover between shrubs
	S			555	13	31.3938°N, 34.9005°E	4	0/0/20	Shrubland, 30% shrub cover of <i>S. spinosum</i> ; about 25% herbaceous cover between shrubs
BS, Beer Sheva North	N	240	18.9	420	14	31.3034°N, 34.8198°E	4	0/0/20	Open shrubland, about 10% shrub cover of <i>Thymelea hirsuta</i> , <i>S. spinosum</i> ; about 25% herbaceous cover between shrubs
OM, Omer	N	214	19.2	342	17	31.2653°N, 34.8192°E	1	0/0/20	Open shrubland, about 10% shrub cover of <i>T. hirsuta</i> , <i>Ballota undulata</i> , <i>Teucrium</i> spp.; about 25% herbaceous cover between shrubs
NV, Nevatim	N	155	19.2	381	15	31.2131°N, 34.8809°E	4	0/0/20	Open shrubland, about 25% shrub cover of <i>T. hirsuta</i> , <i>Echinops</i> spp.; <25% herbaceous cover between shrubs
YR, Yeruham	N	114	18.4	567	5	31.0251°N, 34.9666°E	1	0/0/20	Open shrubland, about 10% shrub cover of <i>Raetama raetam</i> , <i>T. hirsuta</i> ; about 10% herbaceous cover between shrubs

^aMean annual rainfall; ^bmean annual temperature; ^cabundance of *B. distachyon* s.l. individuals among the herbaceous vegetation: 1 = very rare (<0.1% of herb. individuals), 2 = rare (<1%), 3 = frequent (1–5%), 4 = common (5–40%), 5 = dominant (>40%); ^dnumber of individuals recorded for each of the three species *B. distachyon* s.s. (2n = 10), *B. stacei* (2n = 20), *B. hybridum* (2n = 30).

calcareous bedrock, and North-facing slopes. They shared the overall Mediterranean climate type where annual plants (including *B. distachyon s.l.*) grow only during the mild, rainy winter season and die before the hot, rainless summers. Mean annual temperatures differed only marginally among sites (17.8–19.8°C; obtained from the worldclim database, www.worldclim.org) and were uncorrelated with rainfall across sites ($r^2 < 0.01$, $p > 0.9$, $n = 15$; Table 1). This careful site selection across an exceptionally steep aridity gradient isolated rainfall as the key aspect of aridity and as the single major difference among sites. It provided an experimental set-up that minimized other environmental factors that might influence the frequency of the three *Brachypodium* species among sites and thus maximized the power for detecting aridity effects. Note also that *B. distachyon s.l.* could not be found in more arid locations than our driest site, despite substantial search. Its occurrence under extreme desert conditions (<100 mm annual rainfall (Feinbrun-Dothan & Danin 1998) thus appears scarce and restricted to specific microhabitats. This suggests that our gradient extended towards the driest conditions inhabitable for *B. distachyon s.l.*

As small-scale aridity gradient, we used the microclimatic difference between North and South exposed hill slopes in six of our 15 sites (Figure 1). In the other sites no *B. distachyon s.l.* was found on Southern slopes (namely in drier sites) or analogous South exposures were unavailable in close vicinity. Contrasting exposures were approximately 150–300 m apart, separated only by a small wadi (dry valley) (Figure 1). They shared the same bedrock, altitude and inclination (Table 1). Despite generally similar vegetation on both slopes, there was a tendency for lower vegetation cover on South slopes that mirrored the decreasing vegetation cover towards arid sites along the large-scale rainfall gradient (Table 1).

Seed collection and plant cultivation

In May 2014 *B. distachyon s.l.* seeds were collected and bagged separately from 20 mother plants from each of the 15 North exposures and the six additional South exposures (exception: 30 plants from site EY South exposure for another experiment). This sampling effort per site was comparably high (Manzaneda et al. 2012) and ensured a more precise estimate for cyto-type frequencies for each single site and increased

our power to detect also subtle frequency changes. These in total 430 mother plants were selected by random walks within 100 m × 50 m areas per site and exposure, leaving at least 3 m space between single plants. Cytotypes of *B. distachyon s.l.* were not discriminated during sampling.

Collected seeds were raised in a greenhouse in Potsdam, Germany, from January to May 2015. They were planted separately per mother in 430 pots (400 ml Deepot Cells, Stuewe & Sons, Oregon, USA) filled with a mixture of humus soil and loamy sand. Pots were arranged randomly in the greenhouse and rearranged every two weeks to ensure equal growth conditions for all plants. In most pots several seeds germinated; we thus randomly thinned plants to one per pot (i.e. one offspring per mother) after 4 weeks.

Ploidy determination

The ploidy level of all 430 greenhouse individuals was determined between April and May 2015 by flow cytometry, using the established protocol of Galbraith and co-workers (Galbraith et al. 1983; Fuentes et al. 2014). Fresh leaf material (a leaf blade piece of approximately 3cm length) from each plant was harvested in the greenhouse and kept wet by wrapping in wet paper towels. The leaflets were immersed into 1 ml freshly prepared ice cold Galbraith's buffer supplemented with 10 mM dithiothreitol (DTT), 50 µg/ml RNase A and 50 µg/ml propidium iodide (PI) and chopped with a razor blade. The suspension was mixed thoroughly, filtered through a 20 µm filter (CellTrics® Partec, Germany) and kept in the dark for 10 min. DNA content of the nuclei was determined with a FACS Aria II flow cytometer (BD Bioscience, California, USA). The PI fluorescence of each sample was compared to that of a preselected internal *B. stacei* reference plant from our collection and the fluorescence of the reference nuclei was determined multiple times for all FACS sessions. PI fluorescence was excited at 488 nm using a blue laser and recorded using a 585 nm/42 band-pass filter and a 556 nm long-pass dichroic mirror. On average 2000 nuclei (600–7000) were recorded per plant to reach an unequivocal signal of its ploidy level.

The flow cytometric measurements were accompanied by assessing morphological differences among plants. The two diploid species show generally the most distinct phenotypes (Catalan et al. 2016).

We focused on the following traits where this distinction is clearest: *B. stacei* ($2n = 20$) possesses clearly wider and curled leaf blades, a flag leaf that overlaps/overtops the panicle, and larger caryopses and spikelets than *B. distachyon* ($2n = 10$); their hybrid *B. hybridum* ($2n = 30$) is intermediate (Catalan et al. 2016).

As additional information, we determined the absolute genome sizes (in pg/2C) of all three species for our study region. After the above-described ploidy determination, we selected randomly a subset of 10 *B. hybridum*, 10 *B. stacei*, and five *B. distachyon s.s.* individuals from our collection. Flow cytometry was repeated for these plants with tomato (*Solanum lycopersicum*, 1.96 pg/2C) and *Arabidopsis thaliana* col0 (0.32 pg/2C) as external standards to calculate genome sizes from measured fluorescence intensities (for details, see Supplementary Material, Figure S1).

Data analyses

As dependent variable for statistical analyses we used the proportion of diploid to polyploid plants per site and exposition (defined by their absolute numbers). Generalized linear models were calculated in R 3.2.3 (R Core Team 2015) with logit link function and quasi-binomial error structure (accounting for observed overdispersion rates of 2–12 in the analyses below). The first model tested whether the proportion of polyploids shifted gradually along our large-scale aridity gradient: we included all 15 sites, yet only their North exposures (i.e. 300 plants), and fitted rainfall per site as continuous predictor. The second model tested whether the proportion of polyploids differed between North and South exposures: we included only the six sites comprising both exposures (i.e. 250 plants) and fitted “site” and “exposure” as categorical main factors. The significance of single predictors was then assessed via stepwise model reduction.

Because these statistical analyses did not discriminate between the two diploid species, both models were re-calculated without the rare diploid *B. distachyon s.s.* (only five plants of 430, see Results) to assess whether results were influenced by mixing both diploids.

Results

Polyploid plants ($2n = 30$, *B. hybridum*) had roughly twice the genome size of the two diploid species *B.*

distachyon s.s. ($2n = 10$) and *B. stacei* ($2n = 20$), which had similar genome sizes (Figure 2, Figure S1). The absolute genome sizes determined from a subset of plants were for *B. hybridum* 1.231 ± 0.021 pg/2C DNA (mean \pm SD), *B. stacei* 0.611 ± 0.017 pg/2C, and *B. distachyon s.s.* 0.631 ± 0.009 pg/2C (Figure S1). These values were very similar to genome sizes reported previously for *B. hybridum* (1.265 pg/2C) and *B. distachyon s.s.* (0.631 pg/2C), and about 8% higher than earlier values for *B. stacei* (0.564 pg/2C) (Vogel et al. 2006; Wolny & Hasterok 2009; Catalan et al. 2012). Moreover, morphological details separated well the two diploids and allowed unequivocal cytotype identification for all diploid plants (Catalan et al. 2016): *B. stacei* ($2n = 20$) possessed clearly wider and curled leaf blades, larger caryopses and spikelets and a flag leaf that overlaps/overtops the panicle as compared to *B. distachyon* ($2n = 10$).

The proportion of polyploid *B. hybridum* ($2n = 30$) plants per site ranged between 0% (one site) and 100% (10 sites) while four sites harbored mixed populations (Figures 3 and 4). *B. hybridum* comprised in total 385 plants (89.5%) that occurred on both exposures and across the entire gradient except for one site (Figures 3 and 4). Forty-four plants were diploid (10.2%), of which 39 belonged to *B. stacei* ($2n = 20$), occurring in the sites MA, BJ, GU and AM, and five belonged to *B. distachyon s.s.* ($2n = 10$), occurring in the sites EY, MA and GU (Figures 3 and 4, Table 1). For one plant from site GU-South, ploidy determination failed.

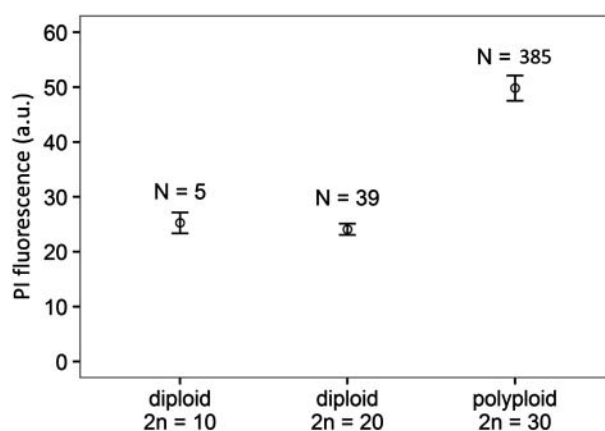


Figure 2. Cytotype frequency and relative genome sizes among all *Brachypodium distachyon s.l.* samples as determined via flow cytometry by propidium iodide (PI) fluorescence (in arbitrary units, error bars indicate 1 SD).

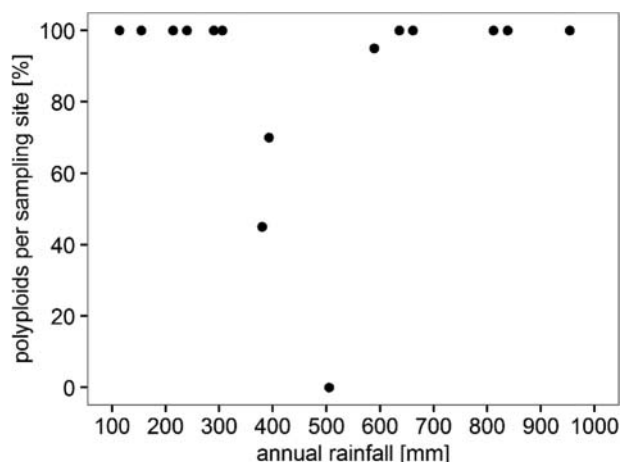


Figure 3. The proportion of polyploid plants of *Brachypodium distachyon s.l.* in 15 sites along the large-scale climatic aridity gradient in Israel. Note that the sites ES and LH (both 298 mm rainfall) were projected slightly detached for improved readability.

Along our large-scale aridity gradient (Figure 3), the proportion of polyploids compared to all diploids was not linearly correlated to average rainfall per site ($F_{1,13} = 0.015$, $p = 0.90$). Instead, lower proportions of polyploids were largely restricted to sites with intermediate rainfall. Also at the micro-climatic scale (Figure 4), North and South exposures harbored no significantly different proportions of polyploids (exposure: $F_{1,5} = 0.104$, $p = 0.76$), while the analysis had accounted for marginally significant differences in polyploid proportions between single sites (site: $F_{5,5} = 3.51$, $p = 0.097$).

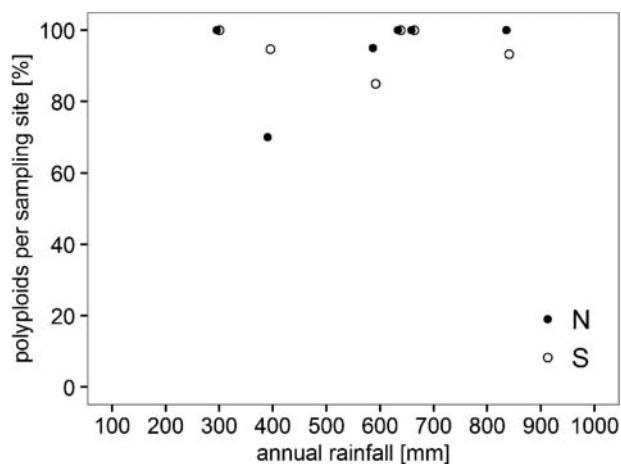


Figure 4. The proportion of polyploid plants at corresponding North (N) and South (S) exposures in six sites along the large-scale rainfall gradient in Israel. Exposures within a site were projected slightly detached for improved readability.

The same patterns remained when the rare diploid *B. distachyon s.s.* was excluded from analyses. The proportion of polyploid *B. hybridum* to diploid *B. stacei* was not correlated to average rainfall along the large-scale gradient ($F_{1,13} = 0.014$, $p = 0.91$), and North and South exposures harbored no significantly different proportions of polyploids (exposure: $F_{1,5} = 0.006$, $p = 0.94$; site: $F_{5,5} = 3.73$, $p = 0.088$).

Discussion

The proportion of polyploid plants within *B. distachyon sensu lato* did not increase linearly with decreasing rainfall along our large-scale gradient, although this gradient covered the largest part of the principal aridity gradient across Israel. Likewise, at the micro-climatic scale, polyploids were not more frequent on South than on North exposures. Both spatial scales therefore showed a different pattern than a study from the Iberian Peninsula on the same species complex where the proportion of polyploids gradually increased with aridity (Manzaneda et al. 2012) and also than studies on other species that reported increasing polyploid frequency towards drier sites (Treier et al. 2009; Thompson et al. 2014). Within the scope of our study region, these results challenge the generality of the claim that aridity is an important driver for polyploid distributions (Weiss-Schneeweiss et al. 2013; Thompson et al. 2014). However, the high dominance of polyploids in almost all sites across the gradient – including the driest sites – agrees with the notion that polyploids profit from their increased genetic potential and can thus adapt to a wide range of environmental conditions (Comai 2005; te Beest et al. 2012; del Pozo & Ramirez-Parra 2015).

The more intriguing aspect of our results and their contrast to the Iberian trend is therefore the “vice-versa” (te Beest et al. 2012): why were diploids so rare, particularly at rainier sites and the micro-climatically more mesic North exposures? It seems unlikely that this occurred primarily because the rainfall gradient in this study (114–954 mm) was too short to test aridity effects on polyploid frequencies. The average rainfall niche reported for *B. distachyon s.s.* was 602 mm mean annual precipitation, 482 mm for *B. stacei* and 554 mm for *B. hybridum*, as calculated by environmental niche models across the Mediterranean basin (Lopez-Alvarez et al. 2015). Therefore, the rainfall requirements of all three species seemed well covered

by our study and, indeed, all three species were recorded. Moreover, diploids occurred mostly at intermediate sites in Israel, i.e. their occurrence appeared not primarily limited by the captured rainfall range. Finally, it is unlikely that the number of sites was too low to detect consistent trends. One strength of our study was that sites were chosen carefully to share major characteristics such as habitat type, bedrock, altitude and temperature. This set-up isolated rainfall as the key aspect of aridity and as the single major difference between sites and strengthened the power to detect aridity effects – if it was indeed a key driver.

However, the earlier study across the Iberian Peninsula was located at slightly higher latitudes, further away from the Saharo-Arabian desert belt. Its gradient was generally less arid but extended to more humid conditions (250–1773 mm) than the span of rainfall conditions occurring in Israel (Manzaneda et al. 2012). Interestingly, this Iberian gradient also included mostly the other diploid, *B. distachyon s.s.* (Manzaneda et al. 2012), which was very rare in Israel (5 plants of 430, found between 398 and 838 mm). The dominant diploid in Israel was *B. stacei*, which is otherwise considered the rarest species among the *B. distachyon s.l.* complex across the Mediterranean basin (Catalan et al. 2012, 2016; Lopez-Alvarez et al. 2015). The shifting polyploid frequencies across the Iberian Peninsula thus occurred between the polyploid *B. hybridum* and diploid *B. distachyon*, while the missing shift across the gradient in Israel chiefly reflected *B. hybridum* and the other diploid *B. stacei*. This suggests that potential frequency shifts among the *B. distachyon s.l.* complex are not attributable to polyploidy per se, but are species-specific.

These results for our particular study gradient corresponded remarkably well with the broad environmental niche models that Lopez-Alvarez et al. (2015) calculated across the entire Mediterranean basin. Based on thus far available occurrence records, they reported a more mesic niche for *B. distachyon s.s.* compared to *B. hybridum*, in line with the trend found among these two species across the Iberian gradient (Manzaneda et al. 2012). In contrast, Lopez-Alvarez et al. (2015) suggested little niche segregation between *B. hybridum* and the other diploid *B. stacei*, which agrees with the missing shift in polyploidy frequency along the aridity gradient in Israel, as well as with our finding that *B. stacei* co-occurred with *B. hybridum* in three of its four sites. It is furthermore noteworthy that all 39 *B. stacei* specimens in our study

occurred in only four sites of intermediate rainfall, where they comprised up to 100% of all collected specimens. This narrow distribution pattern observed at the scale of our study gradient matched again Lopez-Alvarez et al. (2015), where *B. stacei* exhibited the narrowest niche and the most restricted distribution range of the *B. distachyon s.l.* complex. The congruency of both independent studies suggest rather specific habitat requirements for *B. stacei* and render it less likely that its restricted distribution resulted chiefly from limited migration/colonization processes.

We conclude that there is no linear shift in the proportion of polyploids per se within the *Brachypodium distachyon s.l.* complex in Israel that is correlated with aridity – whether at large or at small spatial scale. Instead, each of the three species showed a rather unique distribution. The polyploid *B. hybridum* was by far the most abundant species and apparently spans the entire distribution range reported for *B. distachyon s.l.* in Israel (Feinbrun-Dothan & Danin 1998). Of the two diploids, *B. stacei* was more common and mainly occurred under intermediate rainfall conditions in central Israel. The other diploid, *B. distachyon s.s.*, was generally very rare and occurred scarcely in three sites between 398 and 838 mm annual rainfall.

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Disclosure statement

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