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RESEARCH ARTICLE

Crossbreeding reveals the scale and genetic architecture of local adaptation in a predominantly selfing *Hordeum spontaneum*

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ABSTRACT

Environmental variation can be large across a wide range of spatial scales resulting in complex patterns of local adaptation across species ranges. We analyzed the scale, genetic mechanism and direct climatic causes of local adaptation in a widely distributed grass *Hordeum spontaneum*. We performed artificial crosses of maternal plants representing the same Negev desert population with plants originating elsewhere. Pollen donors were plants from other Negev desert populations, non-desert Israeli populations sampled along an aridity gradient, and accessions covering the entire species range. Our study included planting of inter-population hybrids under favorable and simulated desert experimental conditions, followed by analysis of their performance, variation in adaptive traits and relationship with climatic parameters at sampling locations. The combined results of parental phenotypic variation and performance of hybrids were consistent with local selection, reflecting the importance of both regional and local climates. The adaptive genetic differentiation of barley desert populations had a complex architecture. None of the three effects (additive, dominance and epistasis) were fully responsible for this differentiation. Although genetic effects not related to extrinsic selection appear to contribute to genetic differentiation in barley, epistatic effects arising from local selection clearly predominated. The short-term effect of gene flow by pollen was generally negative, indicating that a majority of the new allele combinations created by recombination were maladaptive. However, the long-term effect of occasional pollen flow from other desert populations appears to be positive, as some new recombined genotypes were superior in fitness to the maternal plants even in the F_2 generation.

Key words: population differentiation; local adaptation; outbreeding depression; genetic architecture; experimental hybridization; wild barley

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Introduction

Plants distributed over heterogeneous or clinally changing environments are often genetically and/or phenotypically differentiated (Bradshaw 1984; Linhart & Grant 1996; Hedrick 2006). This differentiation can arise as a result of drift, due to limited gene flow (Bradshaw 1984; Endler 1986), or as a result of natural selection (Garcia-Ramos & Kirkpatrick 1997; Kirkpatrick & Barton 1997; Doebeli & Dieckmann 2003). Recognition of the adaptive significance of genetic/phenotypic differentiation and its relationship to climate and geographic scale, as well as understanding the genetic basis of adaptation, has long been a challenge in evolutionary biology (Endler 1977; Conover & Schultz 1995; Linhart & Grant 1996; Hufford & Mazer 2003; 2004b; Rasanen & Hendry 2008; Volis 2008; Hereford 2009; Tiffin & Ross-Ibarra 2014; Rellstab et al. 2015, 2015; Forester et al. 2016; Hoban et al. 2016).

The strength of local adaptation and the probability of detecting its presence are expected to increase with geographic distance between compared locations, due to increasing both environmental differences and genetic isolation (Galloway & Fenster 2000). However, although this pattern has been observed in some studies (e.g. Joshi et al. 2001; Etterson 2004; Galloway & Etterson 2005; Becker et al. 2008; Volis 2009), meta-analysis of local adaptation in plants found no effect of geographic distance on the extent of local adaptation (Leimu & Fisher 2008). This lack of a relationship has been hypothesized to be due to the independence of the magnitude of environmental variation and geographic scale (Galloway & Fenster 2000) or the non-linearity of this relationship (Thompson 2005). Environmental variation can be large across a wide range of spatial scales producing multi-scale patchiness in the distribution and abundance of species (Pelletier 1997; Kunin 1998; Richardson et al. 2014) resulting in a complex pattern of local adaptation across species ranges.

Taken together, the aforementioned studies illustrate the difficulty of identifying the geographic scale at which direct environmental causes drive the local adaptation of populations. This is because sampling populations at multiple geographic scales with proper coverage of the species geographic range and adequate coverage of the range of environmental conditions to which populations are exposed to is challenging. Even more challenging in that task is the need to capture the relationship between variation in adaptive traits and relevant environmental parameters in multiple sampling locations. Wild barley, *Hordeum vulgare ssp. spontaneum* Koch (hereinafter *H. spontaneum*) is an ideal system for studying adaptive evolution across multiple spatial scales for several reasons. The species occupies a broad geographic range across southwest Asia, with stable populations in desert environments receiving 1/10 of the annual rainfall received by Mediterranean populations. This species exhibits large genetic differentiation at both large and small spatial scales (Brown et al. 1978a; Morrell et al. 2003; Turpeinen et al. 2003; Volis et al. 2005; 2010) and a distinct ecotypic differentiation (Snow & Brody 1984; van Rijn et al. 2000) despite considerable gene flow (Morrell et al. 2003). Because of the importance of wild barley as a source for cultivated barley improvement, its genetic variation and the selective role of the environment in this variation have been the subject of intensive research (Nevo et al. 1979; Turpeinen et al. 2001; Volis et al. 2002a; Morrell et al. 2003, 2003, 2003, 2005; Hubner et al. 2009; Jakob et al. 2014; Pournosrat et al. 2018). However, the scale and genetic architecture of local adaptation in this species are still poorly understood.

Wild barley is primarily distributed in the Mediterranean climatic zone with peripheral populations in less favorable desert environments (Harlan & Zohary 1966). Aridity is the major environmental limit to the species distribution, and deserts determine to the large extent the

current species range. These include the Ad Dahna desert in northern Saudi Arabia, the Syrian desert in Syria and the Persian Gulf, the Dasht-e Kavir desert in eastern Iran and the Caspian Sea, the Karakum desert in Central Asia to the north and the Sahara Desert in Africa to the south. The second environmental factor limiting species distribution is cold. Wild barley has limited cold tolerance and is rare at altitudes above 1,500 m; this limits the species distribution in the mountains of Southwest Asia and Tibet (Shulgina et al. 2006).

The barley populations from the Negev Desert, which covers southern Israel, represent the periphery of the species and, as is typical of marginal populations, are small and isolated. Furthermore, Israeli desert plants have been found to possess morphological, phenological and life history traits that distinguish them from plants originating from other climatic zones in Israel (Snow & Brody 1984; Volis et al. 2002a; 2004c) and also from plants originating in Central Asia (Volis et al. 2000). Evidence of local adaptation was found when plants were reciprocally transplanted between one desert and one Mediterranean site (Volis et al. 2002b) and among four locations along the aridity gradient (Volis et al. 2002c). These studies employed cross-relocation of individuals originating from different habitats, which is the classic approach to testing for spatially heterogeneous selection (Turesson 1922; Clausen et al. 1940) but did not involve cross-breeding. Unfortunately, although reciprocal transplanting is a powerful tool for detecting adaptive differentiation, it does not allow inferences about the genetic architecture of local adaptation, that is, about the genetic effects responsible for fitness differences. However, when combined with experimental hybridization between ecotypically differentiated populations, reciprocal field or laboratory experiments can elucidate the contribution of dominance, genetic linkage and pleiotropic effects of genes under selection to phenotype (e.g. Fenster & Galloway 2000b; Erickson & Fenster 2006; Johansen-Morris & Latta 2006; Leinonen et al. 2011). Introduction of hybrids together with parents into the field or simulated natural conditions is also the method of choice when testing for the scale of local adaptation, as it allows testing across large numbers of populations, habitats or distance classes (e.g. Waser & Price 1994; Galloway & Fenster 2000; Montalvo & Ellstrand 2001; Heiser & Shaw 2006; Wright & Stanton 2007).

In experimental hybridization, if crossed individuals are genetically divergent as a result of local adaptation, the fitness of their offspring will be reduced compared to their parents (hybrid breakdown or outbreeding depression effect) (e.g., Waser & Price 1991; Fischer & Matthies 1997; Montalvo & Ellstrand 2001; Becker et al. 2006). Two mechanisms of outbreeding depression caused by divergent natural selection have been recognized. The first one operates when selection makes populations fixed for different alleles, and F_1 hybrids are heterozygous for different locally adapted alleles at different loci. Thus, F_1 individuals have a "diluted" set of locally adapted alleles, leading to under-dominance (heterozygote disadvantage). The latter is evident as genotype-by-environment interaction with hybrids being outperformed by parents in the parental environment (Galloway & Fenster 2000; Montalvo & Ellstrand 2001; Tallmon et al. 2004). Under this scenario, the initial decline in F_1 fitness is expected to be quickly restored in subsequent hybrid generations with an increase in homozygosity. The second mechanism applies when local adaptation in populations is achieved through the creation of co-adapted gene combinations (intrinsic co-adaptation) (Templeton 1986). This scenario attributes the outbreeding depression to the breakup of epistatic interactions among loci as a result of recombination and therefore is not expressed until the F_2 generation (Hufford & Mazer 2003). Reduced fitness of hybrids in comparison with parents beyond F_1 is also predicted as a result of Dobzhansky-Muller incompatibilities, i.e. intrinsic interactions among alleles that have become fixed in populations as a result of drift (Dobzhansky 1936; Muller 1942; Turelli & Orr 2000; Johansen-Morris & Latta 2006). The model predicts that recombination will reduce the fitness effects of some alleles because of a new genetic background, regardless of extrinsic (e.g. environmental) selective forces.

Since intrinsic coadaptation and extrinsic selection can affect the outcome of hybridization in a similar way (Bordenstein & Drapeau 2001; Campbell & Waser 2001; Willett & Burton 2003; Fritz et al. 2006), the distinction between these two evolutionary forces, divergent selection or drift, requires testing for genotype-by-environment interaction in performance of F_2 and subsequent generations. If co-adaptation is caused by the population structuring due to drift, no genotype-by-environment interaction is expected in the expression of epistasis, i.e. the fitness ranking of parents and their offspring will not change from one common environment to another. However, if co-adaptation is due to local selection, the loss of fitness in hybrids relative to the parents will exhibit genotype-by-environment interactions across common environments, that is, the fitness ranking of parents and their offspring will correspond to how close the environmental conditions of a common environment are to the parental environment. This means that the distinction between the two mechanisms requires testing the fitness of hybrids beyond the F_1 generation and in at least two common gardens that differ in environmental conditions. Although the fitness effects of interpopulation crosses are known to depend on the environment in which plants are grown, little is known about how stressful vs. favorable environments affect the strength of heterosis or hybrid breakdown (Fenster & Galloway 2000a; Edmands & Deimler 2004; Edmands 2007).

The use of common garden experiments can also provide several additional insights on a scale and pattern of adaptation across a species range. It can identify involvement of morphological, phenological or life-history traits in adaptation to the local environment by assessing phenotypic divergence among populations/accessions (McKay & Latta 2002; Volis et al. 2002d; Latta et al. 2004; Rutter & Fenster 2007; Gardner & Latta 2008; 2016). Common gardens can also be used for analyzing climatic histories of accessions and ranking environmental variables in their effects on plant performance (Rutter & Fenster 2007).

The aim of our study was to analyze the scale and mechanism of local adaptation in a wildly distributed plant species. Although the used methodology is not novel, the study is unique in its multi-scale geographic range coverage. We performed targeted inter-population crossing in wild barley to analyze the genetic architecture of adaptation to the Negev desert environment and the effect of gene flow from plants that originated elsewhere. The maternal plants represented the same Negev population, while pollen donors were plants from 1) three other Negev populations; 2) nine non-desert Israeli populations sampled along the aridity gradient; 3) eight Turkmenian populations from locations of different aridity; and 4) 17 accessions from different countries covering the entire range of wild barley (from Morocco to Tibet). Our study included planting inter-population hybrids and their parents under favorable and simulated desert experimental conditions, followed by analysis of their performance, variation in adaptive traits, and relationship with relevant climatic parameters at sampling locations.

We asked the following questions: i) what is the role of geographic scale in adaptation of wild barley to desert climate? ii) what is the genetic architecture of this adaptation? and iii) how do particular climatic parameters contribute to the adaptation in this species?

Materials and Methods

Study species and sampling

Wild barley, *Hordeum vulgare* ssp. *spontaneum* Koch (=*Hordeum spontaneum*), is a diploid annual grass (x = n = 7) whose range corresponds to the distribution of open park forests and herbaceous formations of the Mediterranean part of the Near East, the Zagros mountains and continental plateaus in Turkey and Iran, and Southwest Asia. Although represented by smaller and more isolated populations, wild barley is also found in semiarid and arid regions, primarily loess and sandy deserts, where barley can exist only in wadies or ravines which accumulate runoff

water. *Hordeum spontaneum* is a predominantly self-pollinating species (Brown et al. 1978b), whose spikes have a brittle rachis and contain two rows of spikelets, and seed dispersal is usually limited to within a few meters of the mother plant, although they can be carried in the fur of animals over longer distances (Zohary 1969).

The plant material used in this study had different geographic origins (Fig. 1).

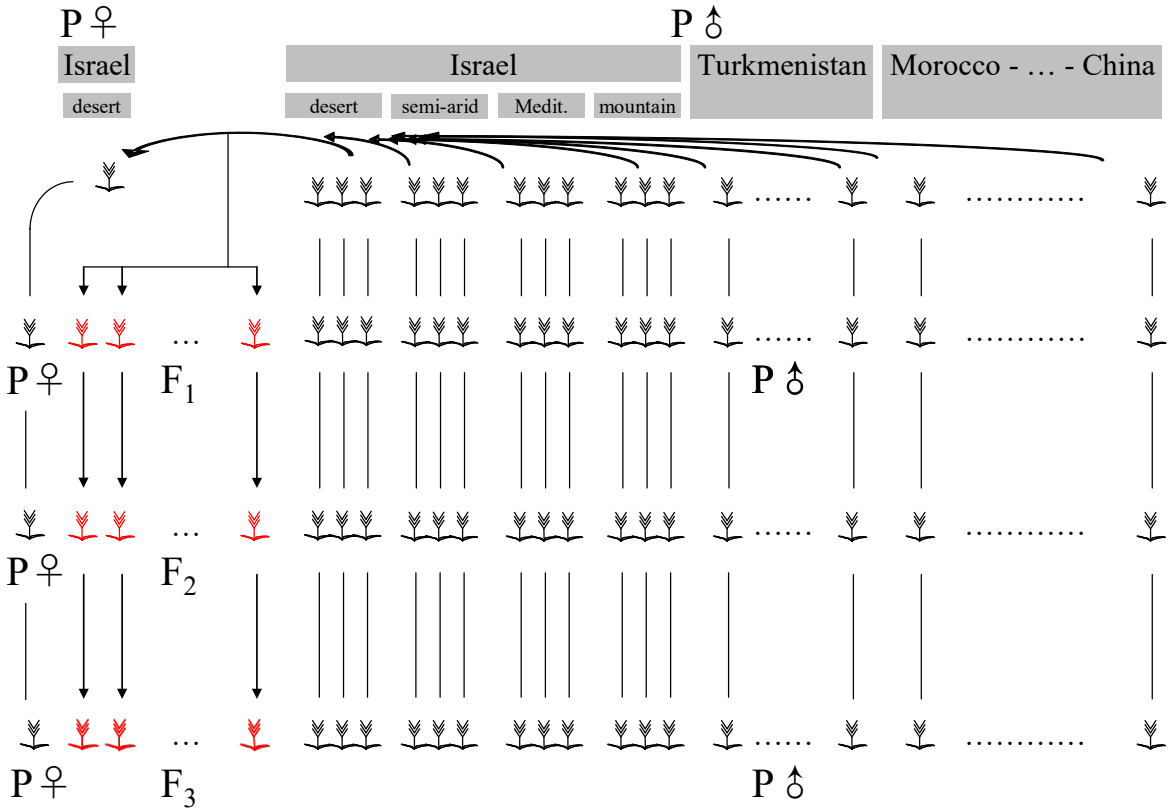

Fig. 1. Diagrammatic presentation of the cross-breeding experiment in which crosses (F_1) derived through pollination of the maternal plant with pollen of different geographic origin, or the progeny of self-pollinated F_1 (F_2 and F_3) were grown together with the progeny of parents ($P_♀$ and $P_♂$) derived through self-pollination.

Plants from Israel included nine accessions separated by at least 100 m in a wadi (= Arabic for ephemeral river valley) near kibbutz Sede Boqer in the Negev Desert which acted as pollen recipients, and 12 accessions of different population origin that acted as pollen donors. The latter accessions were sampled employing a nested sampling design, in which populations were nested within climatic zones of different aridity. Three populations were collected in 1996 in each of the following climatic zones (in order of increasing mean annual rainfall and predictability): desert, semi-desert, Mediterranean and mountain. Relief, slope exposition, vegetation and soil types were kept constant in sampling localities within the group (for population geographic coordinates and detailed description of the environments, see Suppl. Table 1). The populations comprising the group were ≤ 5 km (mountain) or ≤ 20 km (three other groups) from each other. Eight accessions (one accession per population from a known location) were from Turkmenistan, collected in 1992-1993. The other 17 accessions of different country origin, covering the whole range of wild barley distribution (from Morocco to Tibet), were obtained from barley germplasm collections such as the National Small Grains

Collection (USDA-ARS); Leibniz Institute of Plant Genetics and Crop Plant Research (IPK); Research Institute for Bioresources, Okayama University (OUH); and the International Center for Agricultural Research in the Dry Areas (ICARDA) (Suppl. Table 2). Although there is a debate whether accessions from North Africa are truly "wild" (von Bothmer et al. 1995), they were included in the analysis because even if they are feral types that have acquired wild type seed dispersal phenotype, they are, nevertheless, adapted to their respective areas.

Crossing design and common garden experiments

Crosses were performed in 2005 in a greenhouse at the Bergman Campus, Beer Sheva, by artificial pollination of a maternal plant of desert origin with pollen of plants originating elsewhere (P plants) (Fig. 1). Crossings were performed using the protocol developed for *Hordeum vulgare* ssp. *vulgare* as described in Volis et al. (2011). In this procedure, immature anthers are emasculated at the bolting stage, and the inflorescence (spike) is enclosed in a paper bag, which completely prevents self-pollination. Mature flowers of an emasculated inflorescence were pollinated with pollen of a particular accession and enclosed again within the paper bag.

Using this method, we crossed nine maternal plants with plants of different origins and analyzed the produced hybrids (F_1) regarded as half-sib families, and their self-pollinated offspring (F_2 and F_3) for their relative performance as compared with the self-pollinated parental plants in subsequent experiments (Fig. 1). The latter were two common gardens simulating environmental conditions of the maternal plants (i.e. desert) and one common garden with favorable conditions in terms of water supplied. Due to the limited number of F_1 seeds because of the relatively low success of artificial pollination, and also because of space limitations, it was not possible to plant simultaneously F_1 and F_2 generations under simulated desert conditions. However, in 2011, F_1, F_2 and F_3 plants and plants derived from self-pollinated parents were planted simultaneously under favorable conditions. In these experiments, conducted in a greenhouse at the Bergman Campus, Beer Sheva, seeds were simultaneously germinated in an incubator at 24°C and transferred to 3-liter pots filled with the sieved loess soil from the original desert population location.

Two common gardens with simulated desert conditions, conducted in two consecutive seasons, 2008-2009 and 2009-2010, had identical experimental designs and differed only in the amount of water supplied. Plants were arranged using a block design. There were nine blocks representing families that differed in maternal origin. Within the block, half-sibs (either F_1 or F_2) differed only in paternal origin. The half-sibs were grown in each block with the corresponding maternal and paternal plants, and the block comprised 37 hybrids, one $P_{♀}$ and 37 $P_{♂}$. A total of 675 plants were grown in each of the two experiments (75 plants per block x 9 blocks). During these experiments, the plants received an amount of water close to the multiyear average rainfall recorded in 1961-2010 at the Sede Boqer meteorological station, located about 1 km from the population that supplied the maternal plants (95.2 mm). The amount of water applied was adjusted to compensate for the higher evaporation rate in the greenhouse due to different ambient temperatures. The winter season 2008-2009 was warmer than the next one and this is reflected in the amount of water supplied to the plants (221 and 150 mm, respectively). Watering was done twice a week using a drip-irrigation system. All spikes were harvested upon maturation, but before shattering, and used to obtain estimates of plant fitness, such as the total number of seeds and the total mass of seeds produced by a plant.

In the experiment with favorable conditions, plants were arranged using a completely randomized design. There were 333 hybrid plants of each F_1, F_2 and F_3 that resulted from nine maternal and 37 paternal origins (37 x 9 = 333) that were not replicated, and three replications for each parent that participated in the crossing, i.e. 9 x 3 = 27 $P_{♀}$ and 37 x 3 = 111 $P_{♂}$ plants. Since 999 hybrids were not replicated, we used

for the analysis the average P♀ and P♂ values. All the plants were measured for the total number of seeds and the total mass of seeds produced. Parent plants in this experiment were also measured for the following traits: days to anthesis, spike length, awn length, individual spikelet weight, and the number of spikelets per spike.

An additional experiment was set up to estimate fitness at the early stage of plant development. In this experiment, P, F_2 and F_3 seeds were arranged in a grid of 4 x 4 seeds in boxes of 8 x 8 x 10 cm filled with soil from the original desert population location. A seed was considered viable if it germinated and developed true leaves. The water was applied twice, simulating two events of heavy rain (20 mm rainfall) separated by one week. Precipitation of 10 mm is considered the threshold for mass germination of wild barley (Gutterman 1993).

Data analyses

To estimate outbreeding depression or heterosis effects in hybrids, relative performance (RP) was calculated for each generation/family following (Volis et al. 2011) as $RP = (w_i - w_p)/\max(w_i, w_p)$ where w_p was the fitness of progeny of either maternal or paternal plant (P♀ or P♂) derived through selfing, and w_i was the fitness of either F_1, F_2 or F_3. The use of RP values bounded by –1 and +1, allowed standardization of the data obtained in two seasons. The huge balanced ANOVA of Statistica (StatSoft Inc. 2004) was used to analyze three performance traits: seed number, total seed mass, and germination fraction. Prior to analysis, the raw values were converted to RP values. For the desert conditions experiments, RP values were calculated within each block because the blocks differed from each other in maternal plants and probably in the micro-environmental conditions of the greenhouse. For the favorable conditions experiment, thanks to a completely randomized design, RP values were calculated for each maternal family using the average values of the replicated maternal and paternal plants of a corresponding origin. The model included as terms Generation (either F_1, F_2 or F_3) (fixed), Pollen geographic origin (fixed), Referent parent (either maternal P♀ or paternal P♂ plant used to calculate RP) (fixed) and Maternal effect (different maternal plants) (random). For a particular combination of Generation and Referent parent, we used One-way ANOVA followed by Newman-Keuls test to compare geographic regions or Israel climatic zones. One-sided t-test was used to compare the RP values of a particular generation of hybrids with the parents, and the paired t-test was used for between-generation comparisons of hybrid RPs.

To understand the role of dominance in the genetic architecture of adaptation of wild barley to drought, we calculated the degree of dominance (d/a) estimated from F_1 and midparent values obtained for plants grown in the desert conditions experiments. Values of $d/a = 0$ correspond to additivity, between -1 and +1 to full or partial dominance, and beyond -1 and +1 (i.e. the hybrid trait values lie outside the parental trait range) to overdominance. Negative and positive d/a values correspond to the poorer and better performance of F_1 in comparison with the midparent value. Since the d/a values were highly skewed (with a long tail of values beyond unity), instead of the mean and confidence intervals, we used median and 25-75% quartiles.

The effect of paternal original environment on hybrid (F_1, F_2 and F_3) performance was analyzed by multivariate ordination. For the latter, the RP values of the hybrids relative to the maternal plant (F_1 and F_2 under desert conditions, and F_1, F_2 and F_3 under favorable conditions) were subjected to Redundancy Analysis (RDA), a multivariate constrained ordination technique able to extract the major gradients in data (dependant variables) that can be attributed to the measured explanatory (independent, usually environmental) variables. This analysis was performed using CANOCO ver. 4.02; (ter Braak & Smilauer 1998). The environmental effects were eight climatic variables, humidity at 14:00 (Hu), annual rainfall (Rn), number of rainy days (Rd), annual temperature (Tm), temperature in August (Ta), temperature in January (Tj), diurnal temperature range (DTR) in March, April and May. The climate data were obtained from

meteorological stations closest to the sampling location of each accession. Each hybrid was provided with climatic variables' values corresponding to its paternal environment of origin. The statistical significance of each environmental effect was determined by the Monte Carlo permutation test with 999 permutations. Environmental variables explaining most of the variation in dependent variables were selected using the forward selection procedure in CANOCO, with a cutoff point of p=0.10 (default).

To analyze the relationship between variation in the five phenotypic traits and the accession geographic origin, traits measured on parent plants in the favorable conditions experiment were averaged per accession, standardized and used in Two-way Cluster Analysis performed with Statistica (StatSoft Inc. 2004). In the resulting two-dimensional color/shading graph, color codes/shading intensities are determined by the magnitude of the values in the original data matrix. By doing joint classification of objects (accessions) and variables (phenotypic traits), the analysis allowed visualizing which traits and accessions within classes correlate with each other to a greater extent than with members of other classes.

Results

Performance in the simulated desert environment

There was a highly significant generation effect (F_1 vs. F_2) on both seed number and total seed mass, but not on germination (F_2 vs. F_3) (Table 1). The maternal effect, that is, genetic and non-genetic differences between maternal plants representing the same population, was not significant, while paternal (pollen origin) and especially referent parent (whether RP values were calculated relative to $P_♀$ or $P_♂$) effects were highly significant (Table 1). The generation effect, i.e. the overall decrease in performance of plants from F_1 to F_2, was independent of the referent parent (Table 1 and Fig. 2), while the interactions generation x pollen origin, pollen origin x referent parent and generation x pollen origin x referent parent were significant for all three traits (Table 1).

Germination of F_2 and F_3 was significantly lower than $P_♀$ and $P_♂$ (one-sample t-test, Table 2). There was no significant difference between F_2 and F_3 in the average RP calculated relative to either $P_♀$ or $P_♂$ (difference = 0.02 and 0.02, $t = 1.7$ and 1.6, $p > 0.05$, paired t-test).

For seed number and total seed weight, the performance of F_1 was significantly lower than $P_♀$ and higher than $P_♂$ (one-sample t-test, Table 2). Similarly, the performance of F_2 was lower than $P_♀$ and higher than $P_♂$ for these traits (one-sample t-test, Table 2). The RP of F_1 plants was higher than of F_2 plants in reference to $P_♀$ (difference = 0.24 and 0.30, $t = 15.1$ and 18.3, $p < 0.001$, paired t-test) and to $P_♂$ (difference = 0.29 and 0.30, $t = 4.1$ and 4.0, $p < 0.001$, paired t-test).

In most crosses, F_1 plants had reduced performance (seed number and total seed mass) compared to their corresponding half-sibs derived through self-pollination of maternal plants (Table 3). However, the fitness of F_1 plants was almost ubiquitously higher than the of the progeny of self-pollinated paternal plants (Fig. 2).

The degree of dominance (d/a) estimated from the F_1 and midparent values, varied greatly between the half-sibs, but positive values prevailed for both, seed number and total seed weight (median d/a = 0.41 and 0.66, 25% quartiles -0.05 and 0.13, 75% quartiles 0.85 and 1.28, number of seeds and total seed mass, respectively), indicating that the alleles conferring adaptation to the desert environment in these traits are mostly (but not exclusively) dominant.

Table 1. Analysis of variance of seed number and total seed weight used to estimate performance of hybrids relative to the corresponding $P_♀$ or $P_♂$ plants (referent parent). Under desert conditions, the generation effect included the difference in seed number and total seed weight between F_1 and F_2 and the difference between F_2 and F_3 in germination. Under favorable conditions, generation effect included the difference in seed number and total seed weight between F_1, F_2 and F_3. Maternal effect (different maternal plants) was treated as a random effect and the other three factors as fixed effects.

		Desert conditions			Favorable conditions		
Source	df	*F* Seed number	*F* Total seed weight	*F* Germination	df	*F* Seed number	*F* Total seed weight
Generation (G)	1	46.1***	39.2***	0.5 ns	2	0.8 ns	8.3**
Maternal effect (M)	8	2.2 ns	2.4***	1.0 ns	8	6.9***	8.0***
Pollen geographic origin (O)	36	7.5***	6.9***	2.8***	36	5.0***	4.4***
Referent parent (P)	1	929.4***	639.2***	5.5*	1	14.9**	13.6**
G x O	36	6.3***	5.7***	1.8**	72	1.2 ns	1.3 ns
G x P	1	0.6 ns	0.1 ns	0.1 ns	2	1.1 ns	0.2 ns
O x P	36	69.5***	80.3***	334.0***	36	324.0***	386.2***
G x O x P	36	15.8***	17.2***	2.1***	72	2.4***	2.0***
G x O x P x M	288				576		

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns not significant.

On average, both F_1 and F_2 plants of North African origin were superior, and plants of Southwest Asian/Tibetan origin were inferior in the number of seeds and total seed mass over F_1 and F_2 plants of other origins (Table 3). Within the Fertile Crescent, hybrids of Israeli desert origin had superior fitness. In crosses with Israeli plants as both parents, the performance of the hybrids gradually decreased with an increase in the difference in aridity between the original locations of the maternal and paternal plants in the following order: desert, semi-arid, Mediterranean, mountain. This effect was consistent across generations (F_1 and F_2), and referent parent ($P_♀$ and $P_♂$).

Of the plants of non-Israeli paternal origin, only the plants of Ethiopian origin and their F_1 offspring had a higher fitness than the maternal plants. In crosses that involved plants from Israeli populations, a higher fitness of parents and F_1 was observed only in crosses with plants of desert origin (Fig. 2).

Performance in the favorable environment

The average number of seeds and total seed mass per individual were 469±10 and 21.0±0.5 g ($P_♀$), and 421±22 and 24.4±1.4 g ($P_♂$), which indicates far more favorable conditions than in the experiments simulating a desert environment (34±2 and 1.12±0.08 g, $P_♀$; 13±1 and 0.52±0.04 g, $P_♂$).

There was no effect of generation on the number of seeds, but there was a significant decrease in the total mass of seeds from F_1 to F_3 (Table 1), despite the high variation of RP of hybrid plants of different origins. Three other effects, maternal, paternal and referent parent effects, were significant (Table 1). The effect of pollen origin strongly depended on the referent parent for both seed number and total seed mass. The generation effect was independent of pollen origin and referent parent, but the interaction generation x pollen origin x referent parent was significant for both traits (Table 1).

Performance of F_1, F_2 and F_3 relative to $P_♀$ and $P_♂$ is shown in Table 2. The RP of F_1 was lower than $P_♀$ for seed number but not total seed weight, and was lower than $P_♂$ for both traits (one-sample t-test). In the next two generations, RP of F_2 and F_3 was lower than both $P_♀$ and $P_♂$ (one-sample t-test).

The RP of F_1 and F_2 plants did not differ for seed number, but differed for total seed weight in

reference to both $P_♀$ (difference = 0.015 and 0.032, t = 1.2 and 2.3, p > 0.05 and < 0.01, paired t-test) and $P_♂$ (difference = 0.015 and 0.033, t = 1.1 and 2.5, p > 0.05 and < 0.01, paired t-test). The same was true for the RP of F_2 vs. F_3 ($P_♀$, difference = 0.001 and 0.034, t = 0.1 and 2.2, p > 0.05 and < 0.05; $P_♂$, difference = 0.005 and 0.035, t = 0.3 and 2.4, p > 0.05 and < 0.01) and F_1 vs. F_3 ($P_♀$, difference = 0.020 and 0.068, t = 1.1 and 4.4, p > 0.05 and < 0.001; $P_♂$, difference = 0.005 and 0.035, t = 1.4 and 4.7, p > 0.05 and < 0.001).

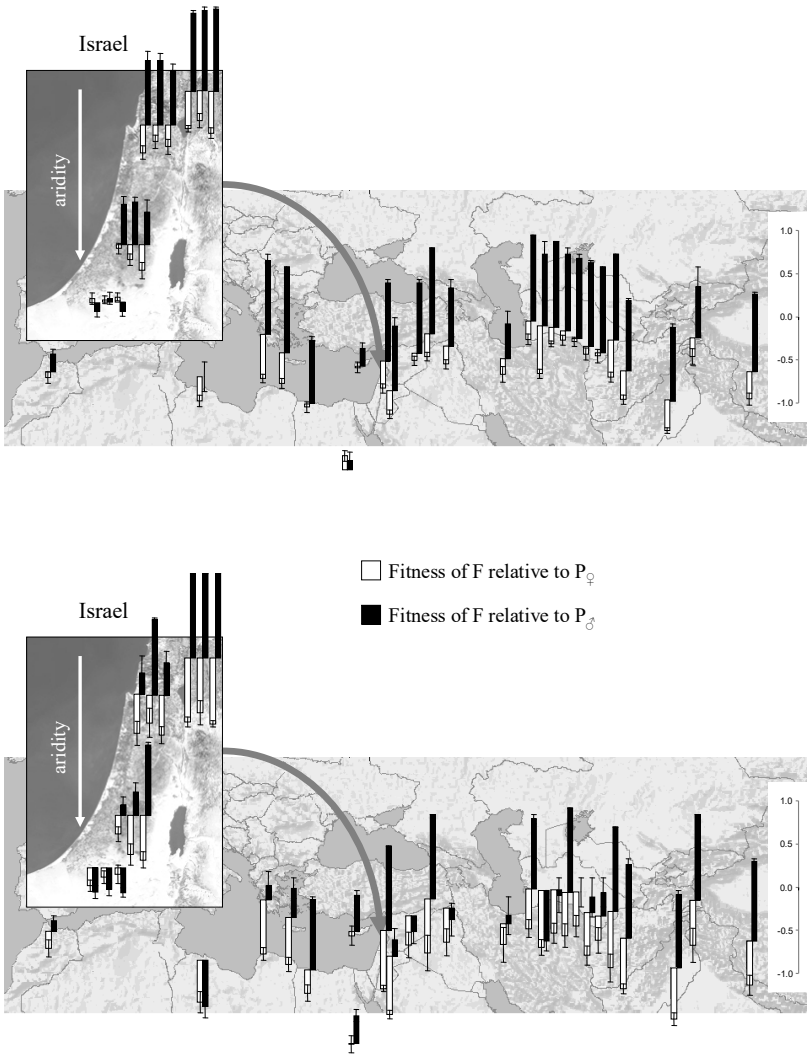

Fig. 2. Relative performance (RP), estimated using seed number, of F_1 (top) and F_2 (bottom) plants (±SE) under simulated desert conditions. In the bars, positive and negative values denote better and poorer performance of the hybrids, respectively, compared to the self-pollinated $P_♀$ and $P_♂$ plants. The bars are located on the map according to the paternal origin of the hybrid plants. A separate box shows performance of the hybrids of Israeli paternal origin, with aridity of original paternal locations increasing from north to south.

The F_1 – F_3 plants derived from the pollen of Fertile Crescent origin produced fewer seeds and a lower total seed biomass than hybrids of other geographic origins, compared to the paternal plant. A similar, albeit not significant, trend was also observed in reference to the maternal plant (Table 4). On a smaller geographical scale, within Israel, in reference to

the maternal plant, F_1 plants of desert origin produced more seeds than plants of the three other origins, but did not differ in total seed weight. No advantage of F_1 plants of desert origin was observed in reference to the paternal plant and no clear differences were observed among the F_2 and F_3 plants of different origin in reference to either $P_♀$ or $P_♂$ parent.

Table 2. Mean performance (SE) of F_1, F_2 and F_3 plants relative to the corresponding $P_♀$ or $P_♂$ plants in the desert and favorable conditions experiments, and results of one-sample t-test

	Referent parent/generation					
	Relative to $P_♀$			Relative to $P_♂$		
	F_1	F_2	F_3	F_1	F_2	F_3
Desert conditions						
Seed number	-0.210 (0.014) t = 7.4***	-0.452 (0.022) t = 13.3***		0.659 (0.024) t = 11.7***	0.381 (0.033) t = 4.8***	
Total seed weight	-0.092 (0.014) t = 3.1**	-0.393 (0.024) t = 10.5***		0.635 (0.025) t = 10.8***	0.349 (0.034) t = 4.3***	
Germination		-0.103 (0.010) t = 8.0***	-0.087 (0.010) t = 5.6***		-0.088 (0.011) t = 7.9***	-0.071 (0.010) t= 4.7***
Favorable conditions						
Seed number	-0.154 (0.015) t = 9.9***	-0.170 (0.015) t = 11.0***	-0.170 (0.015) t = 11.3***	-0.040 (0.019) t = 2.1*	-0.055 (0.019) t = 2.9**	-0.060 (0.018) t = 3.3**
Total seed weight	-0.017 (0.014) t = 1.2 ns	-0.050 (0.015) t = 3.4***	-0.084 (0.014) t = 5.7***	-0.089 (0.018) t = 4.9***	-0.122 (0.018) t = 6.7***	-0.157 (0.017) t = 9.2***

Hybrid performance and paternal original environment

Under simulated desert conditions, RDA revealed, using Monte Carlo permutation tests, a significant effect of Annual Rainfall and Temperature in January on hybrid performance ($F = 4.7$ and 2.3, $p < 0.001$ and 0.034, respectively). Analogous analysis of the hybrid performance under favorable conditions revealed the effect of Rainy days and Diurnal Temperature Range in May ($F = 5.0$ and 3.5, $p = 0.010$ and 0.021, respectively).

The relationship among hybrid performance, hybrid paternal origin and climatic data corresponding to the paternal origin can be seen in the ordination (RDA) triplot (Fig. 3). The triplot shows the general similarity in performance of hybrids among families (maternal plants) and generations (F_1-F_3) under desert, but not under favorable conditions.

Under desert conditions, the performance of hybrids (both F_1 and F_2) was negatively associated with Rainy days, Annual Rainfall and Humidity, and positively associated with the other variables. Among the four Israeli origins, the desert origin of pollen donors was associated with the highest, and mountain origin with the lowest performance of F_1 and F_2 plants. Among other origins, Ethiopia, Morocco and Egypt were associated with the highest hybrid performance. Under favorable

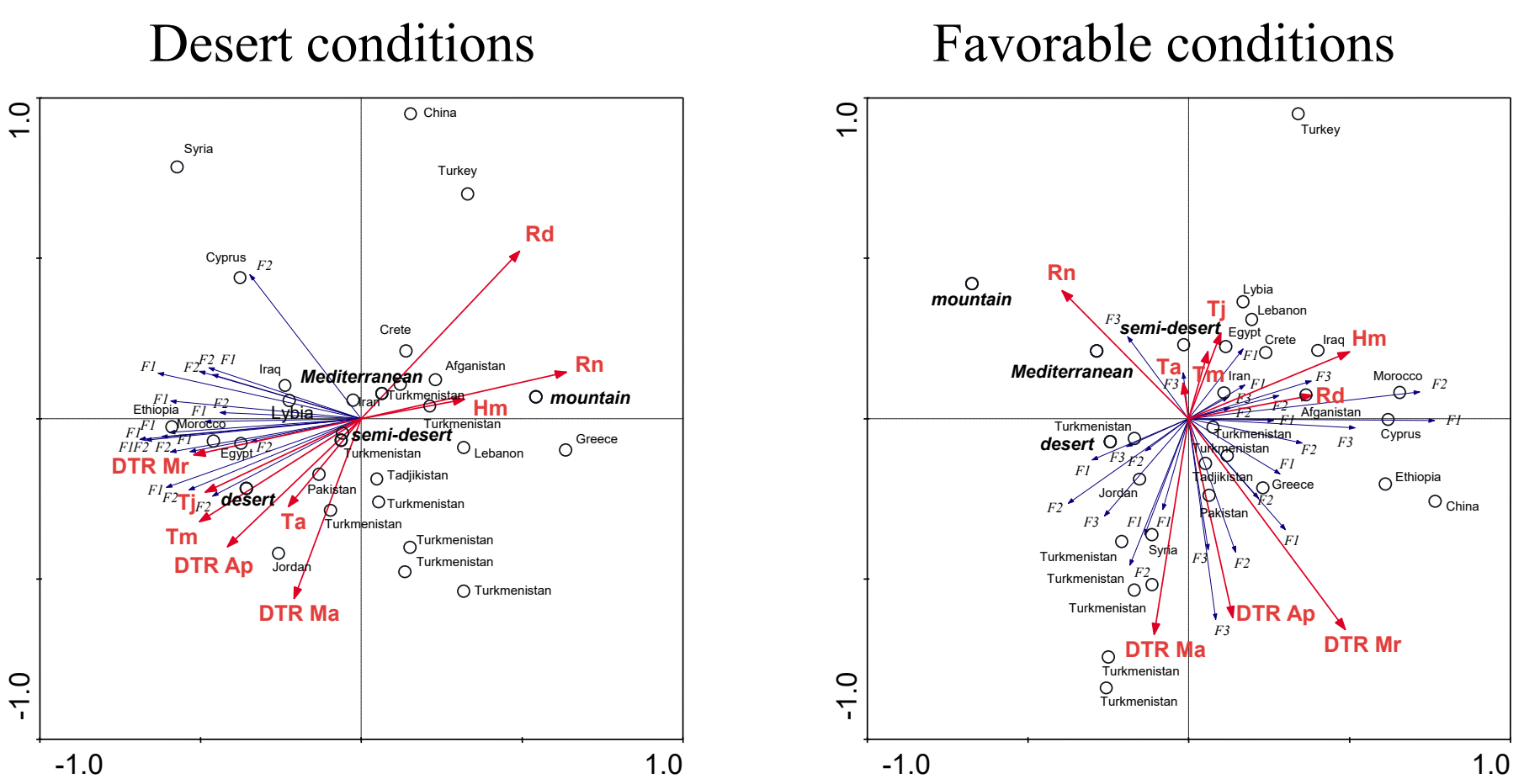

Fig. 3. Ordination (RDA) triplot showing the variation in the relative performance of hybrids (blue arrows) among the pollen donor sites of origin (dots), and the effect of climatic variables (red arrows). The relative performance of F_1, F_2 and F_3 plants was estimated as differences in the yield (number of seeds) of hybrids and self-pollinated P_{φ} plants under desert and favorable conditions. The four Israeli climatic zones are in bold. Desert conditions: axis 1 explains 26.7% of the hybrid performance variance and 70.2% of the hybrid performance-environment relationship; axis 2 explains a cumulative 30.8% of the hybrid performance variance and 81.1% of the hybrid performance-environment relationship. Favorable conditions: axis 1 explains 15.1 % of the hybrid performance variance and 42.1% of the hybrid performance-environment relationship; axis 2 explains a cumulative 21.4% of the hybrid performance variance and 59.5% of the hybrid performance-environment relationship. Abbreviations: Hu humidity at 14:00, Rn annual rainfall, Rd number of rainy days, Tm annual temperature, Ta temperature in August, Tj temperature in January, DTR Mr, DTR Ap and DTR Ma diurnal temperature range in March, April and May.

conditions, despite some significant associations of hybrid performance with climatic variables, there was no clear relationship between the performance of F_1, F_2 and F_3 and the origin of pollen donors (Fig. 3).

Phenotypic variation across the species range

In the tree that resulted from Two-way cluster analysis, all the accessions of the Negev desert origin that acted in cross-breeding as either maternal plants or pollen donors were clustered together. They had a distinct set of traits such as early flowering and many but small seeds in a spike (Fig. 4). These accessions were close to the accessions from North Africa, Iran and China, and, to a lesser extent, to many other accessions, but were very different from the Jordanian and Israeli Mediterranean steppe accessions, separated by less than 400 km, from the Negev desert populations. The latter accessions had another distinct set of traits, such as large spikes with long awns, and very large but few seeds per spike.

Discussion

Scale and role of local adaptation in population phenotypic structuring across the species range

Our study of the adaptation of wild barley to climate differs from the large bulk of studies devoted to this question (e.g. Nevo et al. 1979; Volis et al. 2000; Turpeinen et al. 2001; 2001; Ivandic et al. 2002, 2003, 2003; Hubner et al. 2009; Pournosrat et al. 2018; Hosseini et al. 2019) in that we not only used a correlative approach, but combined it with a much more powerful analytical approach through experimental cross-breeding and testing of progeny fitness. Previously, we have demonstrated adaptive differentiation to climate among wild barley ecotypes using reciprocal introduction into natural environments (Volis et al. 2002d). Furthermore, the desert ecotype from the Negev Desert showed not only a home advantage, but also a specific combination of traits that distinguished it from non-desert ecotypes (Volis et al. 2002d, 2004a; Volis 2007).

The present study investigated the role of the geographic scale in adaptation of wild barley to the desert climate (i.e. whether it is adapted to specific conditions of the Negev Desert or to the broadly defined desert climate), analyzed the contribution of particular climatic parameters to this adaptation, and allowed elucidating the underlying genetic mechanisms.

Experimental cross-breeding followed by planting of offspring under simulated desert conditions strongly supports the adaptation of plants from the Negev desert to their local desert environment, as evident in superior performance of the plants having both parents from the Negev desert. On the other hand, plants from the Southwestern part of the range of the species distribution (Ethiopia, Morocco and Lybia) appear to have similar adaptations to the desert climate (Fig. 2). At least one of these adaptations is early flowering to avoid summer drought (Aronson et al. 1992; 2004c; Volis 2007). The accessions that performed best in the simulated desert environment possessed this trait (Figs. 2 and 4). Second, F_1 hybrid plants of the Negev desert origin exhibited substantial (and often superior to maternal plants) variation in fitness, indicating a high potential for natural and artificial selection (Fig. 2). These results agree with the fact that distribution of wild barley comprises two regions having very different climates: The Middle East (including the Eastern Mediterranean and Northern Africa) and Southwest Asia (including Tibet). The former has primarily a Mediterranean climate with short wet mild winters and long hot summers, whereas Southwest Asia has a continental or mountainous climate with cold long winters, relatively shorter summers and much greater differences between winter and summer temperatures (Volis et al. 2000).

The combined results of our analysis of parental phenotypic variation and performance of offspring of different origins clearly show the importance of local climate for wild barley.

Table 3. Mean performance (SE) of F_1, F_2 and F_3 plants of different origin relative to the corresponding $P_♀$ or $P_♂$ plants in the desert conditions experiment. Number is parenthesis is a group sample size. Letters denote the results of Newman-Keuls test after one-way ANOVA with either geographic region or Israel climatic zone as a factor.

Generation / Referent parent	Geographic region				Israel climatic zone			
	North Africa (4)	Southern Europe (3)	Fertile Crescent (18)	Southwest Asia and Tibet (12)	Desert (3)	semi-arid (3)	Mediterranean (3)	Mountain (3)
Relative to $P_♀$								
F_1 seed number	-0.055 (0.040)^{a}	-0.294 (0.047)^{b}	-0.208 (0.019)^{b}	-0.249 (0.025)^{b}	0.045 (0.031)^{a}	-0.173 (0.047)^{b}	-0.257 (0.048)^{b}	-0.422 (0.038)^{c}
total seed weight	0.130 (0.038)^{a}	-0.122 (0.044)^{bc}	-0.070 (0.018)^{b}	-0.206 (0.024)^{cd}	0.105 (0.028)^{a}	-0.018 (0.49)^{a}	-0.030 (0.040)^{a}	-0.272 (0.043)^{b}
F_2 seed number	-0.243 (0.064)^{a}	-0.420 (0.073)^{b}	-0.460 (0.031)^{b}	-0.525 (0.039)^{b}	-0.146 (0.045)^{a}	-0.396 (0.064)^{b}	-0.427 (0.079)^{b}	-0.732 (0.054)^{c}
total seed weight	-0.132 (0.070)^{a}	-0.301 (0.079)^{b}	-0.387 (0.034)^{b}	-0.524 (0.042)^{c}	-0.135 (0.060)^{a}	-0.294 (0.071)^{a}	-0.250 (0.093)^{a}	-0.675 (0.066)^{b}
Relative to $P_♂$								
F_1 seed number	0.273 (0.061)^{a}	0.690 (0.069)^{b}	0.587 (0.029)^{b}	0.934 (0.039)^{c}	-0.051 (0.039)^{a}	0.449 (0.058)^{b}	0.710 (0.055)^{c}	0.936 (0.018)^{d}
total seed weight	0.211 (0.062)^{a}	0.590 (0.071)^{b}	0.568 (0.030)^{b}	0.940 (0.040)^{c}	0.012 (0.049)^{a}	0.419 (0.058)^{b}	0.630 (0.064)^{c}	0.930 (0.019)^{d}
F_2 seed number	0.203 (0.097)^{a}	0.315 (0.110)^{ab}	0.359 (0.048)^{ab}	0.498 (0.059)^{b}	-0.280 (0.073)^{a}	0.378 (0.080)^{b}	0.502 (0.098)^{b}	1.000^{c}
total seed weight	0.205 (0.099)^{a}	0.242 (0.113)^{a}	0.308 (0.049)^{a}	0.498 (0.061)^{a}	-0.251 (0.046)^{a}	0.240 (0.104)^{b}	0.419 (0.109)^{b}	1.000^{c}
Relative to $P_♀$								
F_2 germination	-0.061 (0.030)^{a}	-0.077 (0.035)^{a}	-0.100 (0.014)^{a}	-0.131 (0.018)^{a}	-0.078 (0.022)^{a}	-0.121 (0.048)^{a}	-0.111 (0.037)^{a}	-0.139 (0.033)^{a}
F_3 germination	-0.064 (0.029)^{a}	-0.061 (0.033)^{a}	-0.091 (0.014)^{a}	-0.099 (0.018)^{a}	-0.124 (0.038)^{a}	-0.034 (0.014)^{a}	-0.066 (0.021)^{a}	-0.122 (0.055)^{a}
Relative to $P_♂$								
F_2 germination	-0.073 (0.032)^{a}	-0.073 (0.037)^{a}	-0.076 (0.015)^{a}	-0.115 (0.019)^{a}	-0.071 (0.022)^{a}	-0.130 (0.048)^{a}	-0.028 (0.052)^{a}	-0.109 (0.037)^{a}
F_3 germination	-0.079 (0.031)^{a}	-0.056 (0.035)^{a}	-0.066 (0.015)^{a}	-0.081 (0.019)^{a}	-0.118 (0.038)^{a}	-0.047 (0.015)^{a}	0.025 (0.041)^{a}	-0.092 (0.056)^{a}

The latter is reflected in which climatic variables and how were associated with higher performance of F_1 and F_2 hybrids in the experimental desert environment. In our study, F1 plants inherited half of the genes from the maternal plant of the Negev desert origin, and another half from the pollen donor originating elsewhere, therefore contribution of the parental genes adapted to particular climatic conditions is what makes a difference in hybrid plant performance.

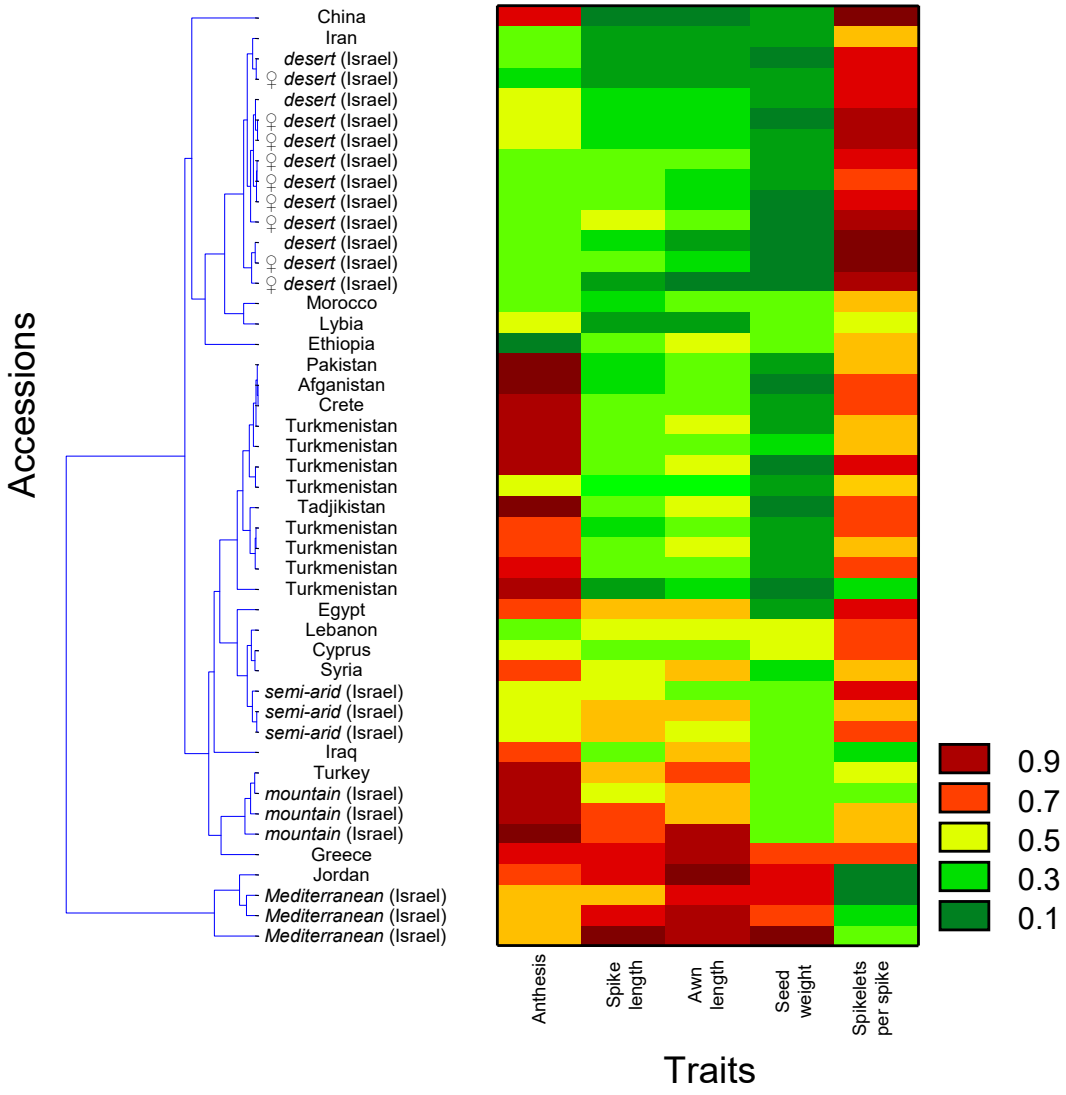

Fig. 4. Two-way clustering of the standardized data matrix of five phenotypic traits measured on P plants of different geographic origin (accessions used as maternal plants have the ♀ sign, accessions used as pollen donors have a geographic origin). The tree on the left illustrates geographic clustering of the accessions based on phenotypic similarity. The bar shows the scale and magnitude of the standardized trait values.

Higher performance of hybrids was associated with less rainfall and humidity, and fewer rainy days, but higher temperature in August and throughout the year, and a wider diurnal temperature range (Fig. 3). Not only hybrids having both parents descended from the Negev desert have been highly successful under desert growing conditions, but also those having a father from the Northern Africa, where the climate is very similar to the Negev desert. On

the other hand, the importance of the regional climate is reflected in the positive relationship between hybrid performance with winter temperature. In accord with the known distribution of wild barley, which is limited by low winter temperature, the performance of the two studied generations of hybrids (F_1 and F_2) was positively associated with temperature in January. Thus natural selection in wild barley appears to occur at several scales, reflecting the importance of both regional and local climate. In some parts of the species range, the local climate is more important, while in others, regional climate effects predominate. This is evident from the fact that the Israeli accessions, which all come from the Mediterranean climatic region, but from zones of different aridity, were clustered separately, while the Turkmenian accessions collected in environments with an annual rainfall in the wide range of 185-460 mm, but all having a continental climate, were clustered together (Fig. 4).

Genetic architecture of adaptive population differentiation

The genetic architecture of adaptation in wild barley was analyzed in two common garden environments: one simulating environmental conditions of the maternal plants (i.e. desert), and another one with favorable conditions. Comparison of the performance of F_2 relative to F_1 and P in simulated maternal (desert) and favorable (i.e. giving no advantage to plants of the desert origin) environments should allow distinguishing additive effects from effects of dominance and gene interactions, and identifying the nature of these effects (selection or drift).

In the simulated desert environment, hybrid breakdown (reduced performance as compared with the maternal plant) was observed in most of F_1 plants. Only F_1 plants that resulted from pollination of the maternal plant with the pollen of a plant originating from other Negev desert populations and an accession from Ethiopia exhibited moderate heterosis (improved performance as compared to the maternal plant). At the same time, F_1 plants strongly outperformed (with a few exceptions) the paternal plants.

Under favorable conditions, a decrease in the performance of F_1 plants was also observed, although it had much smaller magnitude than under simulated desert conditions. The F_1 plants outperformed the paternal plants under simulated desert conditions, but were inferior to them under favorable conditions, and at the same time were inferior to the maternal plants under any conditions. From these findings we can conclude that i) cross-pollination in this species has a negative effect already in F_1; and ii) this negative effect is more deleterious in the environment to which one of the parents is adapted (that is, in the desert).

In the selfed progeny of F_1 (F_2 generation), a further increase in the outbreeding depression effect was observed, including the selfed progeny of F_1 plants with heterosis, under both simulated desert and favorable conditions. The hybrid breakdown did not stop at the F_2 generation and got escalated under favorable conditions in F_3. These results are inconsistent with underdominance which is expected to lead to a rapid restoration of fitness with an increase in homozygosity from the F_1 generation (Hufford & Mazer 2003). The observed hybrid breakdown effect in F_2 can result from breakup of co-adapted gene complexes due to recombination (Price & Waser 1979; Campbell & Waser 1987; Hufford & Mazer 2003), but this mechanism cannot explain the presence of hybrid breakdown already in F_1.

If the basis of genetic differentiation between the two parent populations is additive, in the test of plant performance in desert environment, the relative fitness of F_1 should be half that of $P_{♀}$ having desert origin, while the performance of F_2 should not differ from F_1. The observed relative fitness of F_1 varied depending on the origin of $P_{♂}$, being close to half or less than half of the $P_{♀}$ having desert origin for pollen of mountain Israeli origin (the most extreme contrast to the desert environment in Israel) and most geographically remote accessions from Afghanistan, Pakistan and China. On the other hand, the relative fitness of F_2 was lower than

Table 4. Mean performance (SE) of F_1, F_2 and F_3 plants of different origin relative to the corresponding $P_♀$ or $P_♂$ plants in the favorable conditions experiment. Number in parenthesis is a group sample size. Letters denote the results of Newman-Keuls test after one-way ANOVA with either geographic region or Israel climatic zone as a factor.

Generation	Geographic region				Israel climatic zone			
Referent parent	North Africa (4)	Southern Europe (3)	Fertile Crescent (18)	Southwest Asia and Tibet (12)	Desert (3)	semi-arid (3)	Mediterranean (3)	Mountain (3)
Relative to $P_♀$								
F_1 seed number	-0.087[a] (0.055)	-0.135[a] (0.062)	-0.210[a] (0.020)	-0.097[a] (0.027)	-0.138[b] (0.034)	-0.254[a] (0.045)	-0.363[a] (0.026)	-0.265[a] (0.044)
total seed weight	0.043[a] (0.050)	0.078[a] (0.055)	-0.042[a] (0.019)	-0.025[a] (0.025)	-0.103[a] (0.034)	-0.065[a] (0.050)	-0.045[a] (0.032)	-0.074[a] (0.043)
F_2 seed number	-0.076[a] (0.055)	-0.132[a] (0.056)	-0.242[b] (0.020)	-0.102[a] (0.026)	-0.219[b] (0.030)	-0.302[ab] (0.037)	-0.376[a] (0.034)	-0.241[b] (0.042)
total seed weight	0.033[a] (0.054)	0.052[a] (0.057)	-0.079[a] (0.019)	-0.059[a] (0.026)	-0.164[a] (0.033)	-0.126[ab] (0.029)	-0.111[ab] (0.029)	-0.032[b] (0.045)
F_3 seed number	-0.102[a] (0.046)	-0.190[a] (0.059)	-0.221[a] (0.020)	-0.112[a] (0.027)	-0.138[b] (0.048)	-0.253[ab] (0.031)	-0.344[a] (0.033)	-0.246[a] (0.028)
total seed weight	0.014[a] (0.042)	-0.058[a] (0.065)	-0.093[a] (0.019)	-0.108[a] (0.027)	-0.113[a] (0.045)	-0.066[a] (0.032)	-0.096[a] (0.034)	-0.090[a] (0.037)
Relative to $P_♂$								
F_1 seed number	0.076[bc] (0.051)	0.166[c] (0.061)	-0.099[a] (0.027)	-0.041[ab] (0.031)	-0.181[ab] (0.051)	-0.280[a] (0.054)	-0.089[b] (0.045)	-0.076[b] (0.066)
total seed weight	-0.010[b] (0.048)	0.075[b] (0.057)	-0.198[a] (0.025)	0.008[b] (0.030)	-0.156[b] (0.035)	-0.373[a] (0.043)	-0.356[a] (0.026)	-0.049[b] (0.058)
F_2 seed number	0.091[b] (0.061)	0.181[b] (0.053)	-0.134[a] (0.026)	-0.043[a] (0.029)	-0.261[ab] (0.046)	-0.323[a] (0.043)	-0.117[b] (0.042)	-0.053[c] (0.069)
total seed weight	-0.007[b] (0.059)	0.047[b] (0.062)	-0.237[a] (0.024)	-0.030[b] (0.029)	-0.221[b] (0.029)	-0.411[a] (0.034)	-0.407[a] (0.021)	-0.023[c] (0.056)
F_3 seed number	0.070[b] (0.052)	0.115[b] (0.057)	-0.118[a] (0.025)	-0.060[a] (0.032)	-0.171[ab] (0.056)	-0.273[a] (0.039)	-0.068[b] (0.052)	-0.049[b] (0.071)
total seed weight	-0.039[b] (0.048)	-0.062[b] (0.064)	-0.251[a] (0.023)	-0.080[b] (0.029)	-0.162[b] (0.044)	-0.363[a] (0.035)	-0.380[a] (0.033)	-0.064[b] (0.057)

F_1 in all crosses under desert conditions. These results cannot be explained solely by the additive mechanism of gene action. The observed decline in fitness from F_1 to F_2 contrasts with several methodologically similar studies in which it was not observed (Taylor et al. 2009; Leinonen et al. 2011).

If dominance is present and alleles associated with adaptation are mostly dominant (in which case *d/a* should be positive), F_1 should be close to $P_♀$ in performance, and F_2 should be intermediate in performance compared to F_1 and $P_♀$, because the heterozygosity in F_2 should be half that of F_1 due to segregation. We found a predominantly positive degree of dominance in the two performance traits, assessed for F_1 plants, which suggests the contribution of dominance to the maintenance of adaptive genetic differentiation in wild barley. However, the inferiority of F_2 to F_1 suggests that it is not the major mechanism here.

If the two parental populations differ in gene interactions (i.e., if epistasis is present), the fitness of segregating and recombining F_2 generation should be less than $(F_1 + P_♀)/2$ (Lynch 1991; Lynch & Walsh 1998). The observed decrease in performance with segregation supports the presence of epistatic effects, but epistasis cannot explain the reduced performance of F_1. This indicates that there must be other mechanisms underlying adaptive genetic differentiation in barley.

Planting hybrids and their parents under favorable conditions provided additional information on the genetic architecture of genetic differentiation of desert populations. Under favorable conditions, F_1 plants had reduced performance as compared with both parents, indicating an immediate negative effect of cross-breeding regardless of the planting environment. This effect is small in magnitude and can be partially or completely offset by heterosis. The heterosis effect was not evident in hybrids having both parents from Israel, but was present in most hybrids of non-Israel paternal origin. This heterosis effect did not diminish in subsequent segregating generations in the favorable environment. In contrast, in the simulated desert environment, the negative intrinsic effect of cross-breeding was greatly enhanced by extrinsic selection (as evidenced by the many-fold difference in performance values in these two experimental environments), completely swamping the heterosis effect in F_1 with exacerbation in the following generations due to segregation. The exacerbating effect of stressful conditions on the expression of the outbreeding effect was also observed by Walisch et al. (2012). Our results can give a clue why several studies did not observe a reduction in fitness from F_1 to F_2 (Verhoeven et al. 2004; Taylor et al. 2009; Leinonen et al. 2011). It appears that in these studies, the experimental environmental conditions did not impose extrinsic selection strong enough.

In summary, none of the three effects (i.e. additive, dominance and epistasis) is fully responsible for the adaptive genetic differentiation of barley desert populations, and this differentiation has a very complex architecture. Although genetic effects unrelated to extrinsic selection, such as the presence of deleterious alleles or Dobzhansky-Muller incompatibilities (Dobzhansky 1936; Muller 1942; Turelli & Orr 2000; Johansen-Morris & Latta 2006) appear to contribute to the genetic differentiation in barley, epistatic effects that result from local selection clearly predominate. On the other hand, the calculated *d/a* values suggest the involvement of dominance with the probable presence of not only additive-by-additive epistasis (expected for highly homozygous species), but also dominance-by-additive epistasis (expected for outcrossing species with high heterozygosity) (Rousselle et al. 2010). The strong effect of epistatic interactions distinguishes our results from several methodologically similar studies done on outcrossing (Erickson & Fenster 2006; Campbell et al. 2008; Taylor et al. 2009; Leinonen et al. 2011) and predominantly selfing species including wild barley (Verhoeven et al. 2004; Johansen-Morris & Latta 2006) which found no hybrid breakdown in the F_2 or F_3 generation. Our explanation for this discrepancy is a much wider range of environmental dissimilarities of crossed accessions in our study than in any previous study. For example, in a study by Verhoeven et al. (2004), the two parental populations of wild barley, the coastal Mediterranean and the steppe inland, had 270 and 424 mm of rainfall, respectively, while in our study, the geographically closest ecotypes, the desert and the semi-arid, had 93 and 400 mm of rainfall, respectively.

Importance of gene flow

Experimental crossbreeding, besides revealing the scale and genetic architecture of adaptation, can provide valuable information on the effects of gene flow on a locally adapted population. These effects can be negative (introduction of maladaptive genes) or positive (restoration of depleted heritable variation and adaptive potential) (Holt & Gomulkiewicz 1997; Kirkpatrick & Barton 1997; Boulding & Hay

2001; Garant et al. 2007; Rasanen & Hendry 2008; Whiteley et al. 2015). The positive effect of occasional pollen flow can be especially important in predominantly self-pollinating species, since recombination will create new combinations of alleles with enhanced fitness, preventing the accumulation of mildly deleterious mutations (Fritsche & Kaltz 2000; Johansen-Morris & Latta 2006) and fixation of beneficial alleles in different genetic neighborhoods (Hill & Robertson 1966; Felsenstein 1974). Although most new combinations of alleles among and within loci created by occasional sexual recombination will have lower fitness than existing combinations, and therefore a low chance of persisting through generations (Volis et al. 2011), sexual recombination appears to be an important mechanism by which the beneficial mutations are brought together within the same individual. Occasional gene flow can restore genetic variation and improve performance of peripheral populations that are depleted by bottlenecks, and provide a basis for adaptation to new or changing environmental conditions characteristic for the species range edge (Pujol & Pannell 2008; Pujol et al. 2009; Facon et al. 2011; Kottler et al. 2021).

The Negev desert is one of the desert areas where wild barley reaches the southern edge of its range, either as a result of range expansion with no current gene flow from more favorable core environments, or where re-colonization and gene influx from the core occur regularly. In any case, the isolation of populations and genetic drift in this part of the species range should be stronger, and the spatial genetic structuring should be higher than at the species core. We found some positive effects of gene exchange via pollen between Israeli desert populations, but this effect was limited to F_1 only, and disappeared already in the F_2 generation. As for the effect of genes from other environments, it was predominantly negative already in the F_1 generation. Thus, the short-term effect of gene flow by pollen in wild barley appears to be predominantly negative because the majority of new allelic combinations created by recombination are maladaptive. As a result, the proportion of new recombined genotypes with enhanced fitness resulting from a pollen or seed flow from the other range edge populations is usually low and cannot significantly change the overall level of genetic variation in range edge populations, as was found by Pujol et al. (2010). Nevertheless, what we found in our study, namely that some (though not many) of new recombined genotypes created by pollen flow from other desert populations outperformed mother plants even in the F2 generation, supports a conclusion of (Volis et al. 2011) that the long-term effect of occasional pollen flow is positive.

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References

Aronson J, Kigel J, Shmida A, Klein J (1992) Adaptive phenology of desert and Mediterranean populations of annual plants grown with and without water stress. *Oecologia* 89: 17-26.

Becker U, Dostal P, Jorritsma-Wienk LD, Matthies D (2008) The spatial scale of adaptive population differentiation in a wide-spread, well-dispersed plant species. *Oikos* 117: 1865–1873.

Becker U, Reinhold T, Matthies D (2006) Effects of pollination distance on reproduction and offspring performance in *Hypochoeris radicata*: Experiments with plants from three European regions. *Biological Conservation* 132: 109-118.

Bordenstein SR, Drapeau MD (2001) Genotype-by-environment interaction and the Dobzhansky-Muller model of post-zygotic isolation. *Journal of Evolutionary Biology* 14: 490-501.

Boulding EG, Hay T (2001) Genetic and demographic parameters determining population persistence after a discrete change in the environment. *Heredity* 86: 313-324.

Bradshaw AD (1984) Ecological significance of genetic variation between populations. In: Dirzo R, Sarukhan J (eds), Perspectives on plant population ecology. Sinauer, Sunderland, MA, pp. 213-228.

Brown AHD, Nevo E, Zohary D, Dagan O (1978a) Genetic variation in natural populations of wild

barley (*Hordeum spontaneum*). *Genetica* 49: 97-108.

Brown AHD, Zohary D, Nevo E (1978b) Outcrossing rates and heterozygosity in natural populations of *Hordeum spontaneum* Koch in Israel. *Heredity* 41: 49-62.

Campbell DR, Waser NM (1987) The evolution of plant mating systems - multilocus simulations of pollen dispersal. *American Naturalist* 129: 593-609.

Campbell DR, Waser NM (2001) Genotype-by-environment interaction and the fitness of plant hybrids in the wild. *Evolution* 55: 669-676.

Campbell DR, Waser NM, Aldridge G, Wu CA (2008) Lifetime fitness in two generations of *Ipomopsis* hybrids. *Evolution* 62: 2616-2627.

Clausen J, Keck DD, Hiesey WM (1940) Experimental studies on the nature of species. I. Effect of varied environments on western North American plants. Carnegie Inst. Washington Publ. No. 520.

Conover DO, Schultz ET (1995) Phenotypic similarity and the evolutionary significance of countergradient variation. *Trends in Ecology and Evolution* 10: 248-252.

Dobzhansky T (1936) Studies on hybrid sterility. II. Localization of sterility factors in *Drosophila pseudoobscura* hybrids. *Genetics* 21: 113-135.

Doebeli M, Dieckmann U (2003) Speciation along environmental gradients. *Nature* 421: 259-264

Edmands S (2007) Between a rock and a hard place: evaluating the relative risks of inbreeding and outbreeding for conservation and management. *Molecular Ecology* 16: 463-475.

Edmands S, Deimler JK (2004) Local adaptation, intrinsic coadaptation and the effects of environmental stress on interpopulation hybrids in the copepod *Tigriopus californicus*. *Journal of Experimental Marine Biology and Ecology* 303: 183 – 196

Endler JA (1977) Geographic variation, speciation, and clines. Princeton Univesity Press, Princeton, New Jersey.

Endler JA (1986) Natural selection in the wild. Princeton University Press, Princeton, New Jersey.

Erickson DL, Fenster CB (2006) Intraspecific hybridization and the recovery of fitness in the native legume *Chamaecrista fasciculata*. *Evolution* 60: 225-233.

Etterson JR (2004) Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change. I. Clinal patterns of selection along an environmental gradient in the Great Plains. *Evolution* 58: 1446-1458.

Facon B, Hufbauer RA, Tayeh A, Loiseau A, Lombaert E, Vitalis R, Guillemaud T, Lundgren JG, Estoup A (2011) Inbreeding depression is purged in the invasive insect *Harmonia axyridis*. *Current Biology* 21: 424-427.

Felsenstein J (1974) The evolutionary advantage of recombination. *Genetics* 78: 737-756.

Fenster CB, Galloway LF (2000a) Inbreeding and outbreeding depression in natural populations of *Chamaecrista fasciculata* (Fabaceae). *Conservation Biology* 14: 1406-1412.

Fenster CB, Galloway LF (2000b) Population differentiation in an annual legume: genetic architecture. *Evolution* 54: 1157-1172.

Fischer M, Matthies D (1997) Mating structure and inbreeding and outbreeding depression in the rare plant *Gentianella germanica* (Gentianaceae). *American Journal of Botany* 84: 1685-1692.

Forester BR, Jones MR, Joost S, Landguth EL, Lasky JR (2016) Detecting spatial genetic signatures of local adaptation in heterogeneous landscapes. *Molecular Ecology* 25: 104-120.

Fritsche F, Kaltz O (2000) Is the *Prunella* (Lamiaceae) hybrid zone structured by an environmental gradient? Evidence from a reciprocal transplant experiment. *American Journal of Botany* 87: 995-1003.

Fritz RS, Hochwender CG, Albrectsen BR, Czesak ME (2006) Fitness and genetic architecture of parent and hybrid willows in common gardens. *Evolution* 60: 1215-1227.

Galloway LF, Etterson JR (2005) Population differentiation and hybrid success in *Campanula americana*: Geography and genome size. *Journal of Evolutionary Biology* 18: 81-89.

Galloway LF, Fenster CB (2000) Population differentiation in an annual legume: local adaptation. *Evolution* 54: 1173-1181.

Garant D, Forde SE, Hendry AP (2007) The multifarious effects of dispersal and gene flow on contemporary adaptation. *Functional Ecology* 21: 434-443.

Garcia-Ramos G, Kirkpatrick M (1997) Genetic models of adaptation and gene flow in peripheral populations. *Evolution* 51: 21-28.

Gardner KM, Latta RG (2008) Heritable variation and genetic correlation of quantitative traits within and between ecotypes of *Avena barbata*. *Journal of Evolutionary Biology* 21: 737-748.

Gutterman Y (1993) Seed germination in desert plants. Springer Verlag, Berlin.

Harlan RJ, Zohary D (1966) Distribution of wild wheats and barley. *Science* 153: 1074-1080.

Hedrick PW (2006) Genetic polymorphism in heterogeneous environments: the age of genomics. *Annual Review in Ecology and Systematics* 37: 67-93.

Heiser DA, Shaw RG (2006) The fitness effects of outcrossing in *Calylophus serrulatus*, a permanent translocation heterozygote. *Evolution* 60: 64-76.

Hereford J (2009) A quantitative survey of local adaptation and fitness trade-offs. *American Naturalist* 173: 579-588.

Hill WG, Robertson A (1966) The effect of linkage on limits to artificial selection. *Genetical Research* 8: 269-294.

Hoban S, Kelley JL, Lotterhos KE, Antolin MF, Bradburd G, Lowry DB, Poss ML, Reed LK, Storfer A, Whitlock MC (2016) Finding the genomic basis of local adaptation: pitfalls, practical solutions, and future directions. *American Naturalist* 188: 379-397.

Holt RD, Gomulkiewicz R (1997) How does immigration influence local adaptation? A reexamination of a familiar paradigm. *American Naturalist* 149: 563-572.

Hosseini M, Ghorbani R, Mohassel MHR, Yassaie M (2019) Correlation of environmental factors and phenotypic diversity of iranian wild barley (*Hordeum spontaneum* Koch) populations. *Acta Oecologica* 95: 19-25.

Hubner S, Hoffken M, Oren E, Haseneyer G, Stein N, Graner A, Schmid K, Fridman E (2009) Strong correlation of wild barley (*Hordeum spontaneum*) population structure with temperature and precipitation variation. *Molecular Ecology* 18: 1523–1536.

Hufford KM, Mazer SJ (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends in Ecology & Evolution* 18: 147-155.

Ivandic V, Hackett CA, Nevo E, Keith R, Thomas WTB, Forster BP (2002) Analysis of simple sequence repeats (SSRs) in wild barley from the Fertile Crescent: associations with ecology, geography and flowering time. *Plant Molecular Biology* 48: 511–527.

Jakob SS, Rödder D, Engler JO, Shaaf S, Özkan H, Blattner FR, Kilian B (2014) Evolutionary history of wild barley (*Hordeum vulgare* subsp. *spontaneum*) analyzed using multilocus sequence data and paleodistribution modeling. *Genome Biology and Evolution* 6: 685-702.

Johansen-Morris AD, Latta RG (2006) Fitness consequences of hybridization between ecotypes of *Avena barbata*: hybrid breakdown, hybrid vigor, and transgressive segregation. *Evolution* 60: 1585-1595.

Joshi J, Schmid B, Caldeira MC, Dimitrakopoulos PG, Good J, Harris R, Hector A (2001) Local adaptation enhances performance of common plant species. *Ecology Letters* 4: 536-544.

Kirkpatrick M, Barton NH (1997) Evolution of a species' range *American Naturalist* 150: 1-23.

Kottler EJ, Dickman EE, Sexton JP, Emery NC, Franks SJ (2021) Draining the swamping hypothesis: little evidence that gene flow reduces fitness at range edges. *Trends in Ecology & Evolution* (in press).

Kunin WE (1998) Extrapolating species abundance across spatial scales. *Science* 281: 1513-1515.

Latta RG, MacKenzie JL, Vats A, Schoen DJ (2004) Divergence and variation of quantitative traits between allozyme genotypes of *Avena barbata* from contrasting habitats. *Journal of Ecology* 92: 57-71.

Leimu R, Fisher M (2008) A meta-analysis of local adaptation in plants. *Plos One* 3: e4010.

Leinonen PH, Remington DL, Savolainen O (2011) Local adaptation, phenotypic differentiation, and hybrid fitness in diverged natural populations of *Arabidopsis lyrata*. *Evolution* 65: 90-107.

Linhart YB, Grant MC (1996) Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* 27: 237-277.

Lynch JM (1991) The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* 45: 622-629.

Lynch M, Walsh B (1998) Genetics and analysis of quantitative traits. Sinauer Associates, Sunderland, M.A.

McKay JK, Latta RG (2002) Adaptive population divergence: markers, QTL and traits. *Trends in Ecology & Evolution* 17: 285-291.

Montalvo AM, Ellstrand NC (2001) Nonlocal transplantation and outbreeding depression in the subshrub *Lotus scoparius* (Fabaceae). *American Journal of Botany* 88: 258-269.

Morrell PL, Lundy KE, Clegg MT (2003) Distinct geographic patterns of genetic diversity are maintained in wild barley (*Hordeum vulgare* ssp. *spontaneum*) despite migration. *Proceedings of the National Academy of Sciences* 100: 10812–10817.

Muller HJ (1942) Isolating mechanisms, evolution, and temperature. *Biological Symposia* 6: 71-125.

Nevo E, Zohary D, Brown ADH, Haber M (1979) Genetic diversity and environmental associations of wild barley, *Hordeum spontaneum* in Israel. *Evolution* 33: 815-833.

Pelletier JD (1997) Analysis and modeling of the natural variability of climate. *Journal of Climatology* 10: 1331–1342.

Pournosrat R, Kaya S, Shaaf S, Kilian B, Ozkan H (2018) Geographical and environmental determinants of the genetic structure of wild barley in southeastern Anatolia. *Plos One* 13: e0192386.

Price MV, Waser NM (1979) Pollen dispersal and optimal outcrossing in *Delphinium nelsoni*. *Nature* 277: 294-297.

Pujol B, Obbard DJ, Pannell JR (2010) Symptoms of population range expansion: lessons from phenotypic and genetic differentiation in hexaploid *Mercurialis annua*. *Plants Ecology & Diversity* 3: 103-108.

Pujol B, Pannell J (2008) Reduced responses to selection after species range expansion. *Science* 321: 96.

Pujol B, Zhou S, Sanchez VJ, Pannell J (2009) Reduced inbreeding depression after species range expansion. *Proceedings of the National Academy of Sciences* 106: 15379-15383.

Rasanen K, Hendry AP (2008) Disentangling interactions between adaptive divergence and gene flow when ecology drives diversification. *Ecology Letters* 11: 624-636.

Rellstab C, Gugerli F, Eckert AJ, Hancock AM, Holderegger R (2015) A practical guide to environmental association analysis in landscape genomics. *Molecular Ecology* 24: 4348-4370.

Richardson JL, Urban MC, Bolnick DI, Skelly DK (2014) Microgeographic adaptation and the spatial scale of evolution. *Trends in Ecology & Evolution* 29: 165-176.

Rousselle Y, Thomas M, Galic N, Bonnin I, Goldringer I (2010) Inbreeding depression and low between-population heterosis in recently diverged experimental populations of a selfing species. *Heredity* 106: 289-299.

Rutter MT, Fenster CB (2007) Testing for adaptation to climate in *Arabidopsis thaliana*: A calibrated common garden approach. *Annals of Botany* 99: 529-536.

Shulgina I, Yakubov B, Orlovsky N, Mendlinger S, Volis S (2006) Genetic (RAPD) diversity across species range: core vs. peripheral populations of wild barley in Israel and Turkmenistan. *Israel Journal of Ecology and Evolution* 52: 93-109.

Snow L, Brody T (1984) Genetic variation of *Hordeum spontaneum* in Israel: eco-geographical races, detected by trait measurements. *Plant Systematics and Evolution* 145: 15-28.

StatSoft Inc. (2004) STATISTICA (data analysis software system), version 7. www.statsoft.com.

Tallmon DA, Luikart G, Waples RS (2004) The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology & Evolution* 19: 489-496.

Taylor SJ, Arnold M, Martin, N.H. (2009) The genetic architecture of reproductive isolation in Louisiana irises: hybrid fitness in nature. *Evolution* 63: 2581–2594.

Templeton AR (1986) Coadaptation and outbreeding depression. In: Soule ME (ed), Conservation biology: The science of scarcity and diversity Sinauer, Sunderland, MA, pp. 105-116.

ter Braak CJF, Smilauer P (1998) CANOCO for Windows version 4.02. Centre for Biometry Wageningen, CPRO-DLO, Wageningen, the Netherlands.

Thompson JN (2005) Local adaptation I. Geographic selection mosaics. The geographic mosaic of coevolution. University of Chicago Press, Chicago.

Tiffin P, Ross-Ibarra J (2014) Advances and limits of using population genetics to understand local adaptation. *Trends in Ecology & Evolution* 29: 673-680.

Turelli M, Orr HA (2000) Dominance, epistasis and the genetics of post-zygotic isolation. *Genetics* 154: 1663-1679.

Turesson G (1922) The genotypical response of the plant species to the habitat. *Hereditas* 3: 211-350.

Turpeinen T, Tenhola T, Manninen O, Nevo E, Nissila E (2001) Microsatellite diversity associated with ecological factors in *Hordeum spontaneum* populations in Israel. *Molecular Ecology* 10: 1577-1591.

Turpeinen T, Vanhala T, Nevo E, Nissila E (2003) AFLP genetic polymorphism in wild barley (*Hordeum spontaneum*) populations in Israel. *Theoretical and Applied Genetics* 106: 1333-1339.

van Rijn CPE, Heersche I, van Berkel YEM, Nevo E, Lambers H, Poorter H (2000) Growth characteristics in *Hordeum spontaneum* populations from different habitats. *New Phytologist* 146: 471–481.

Verhoeven KJF, Vanhala TK, Biere A, Nevo E, Van Damme JMM (2004) The genetic basis of adaptive population differentiation: A quantitative trait locus analysis of fitness traits in two wild barley populations from contrasting habitats. *Evolution* 58: 270-283.

Volis S (2007) Correlated patterns of variation in phenology and seed production in populations of two annual grasses along an aridity gradient. *Evolutionary Ecology* 21: 381-393.

Volis S (2008) Detection of signatures of positive selection in naturally occurring genetic variation. In: Koven VT (ed), Population genetics research progress. Nova Science Publishers pp. 279-310.

Volis S (2009) Seed-related traits and their adaptive role in population differentiation in *Avena sterilis* along an aridity gradient *Israel Journal of Plant Sciences* 57: 79-90.

Volis S, Mendlinger S, Orlovsky N (2000) Variability in phenotypic traits in core and peripheral populations of wild barley *Hordeum spontaneum* Koch. *Hereditas* 133: 235-247.

Volis S, Mendlinger S, Turuspekov Y, Esnazarov U (2002a) Phenotypic and allozyme variation in Mediterranean and desert populations of wild barley, *Hordeum spontaneum* Koch. *Evolution* 56: 1403-1415.

Volis S, Mendlinger S, Ward D (2002b) Adaptive traits of wild barley plants of Mediterranean and desert origin. *Oecologia* 133: 131-138.

Volis S, Mendlinger S, Ward D (2002c) Differentiation in populations of *Hordeum spontaneum* along a gradient of environmental productivity and predictability: life history and local adaptation. *Biological Journal of the Linnean Society* 77: 479-490.

Volis S, Mendlinger S, Ward D (2002d) Differentiation in populations of *Hordeum spontaneum* Koch along a gradient of environmental productivity and predictability: plasticity in response to water and nutrient stress. *Biological Journal of the Linnean Society* 75: 301-312.

Volis S, Mendlinger S, Ward D (2004a) Differentiation in populations of Hordeum spontaneum Koch along a gradient of environmental productivity and predictability: Intra- and interspecific competitive responses. *Israel Journal of Plant Sciences* 52: 223-234.

Volis S, Olsvig-Whittaker L, Anikster Y, Mendlinger S (2004b) The influence of space in genetic-environmental relationships when environmental heterogeneity and seed dispersal occurs at similar scale. *American Naturalist* 163: 312-327.

Volis S, Ormanbekova D, Shulgina I (2016) Role of selection and gene flow in population differentiation at the edge vs. interior of the species range differing in climatic conditions. *Molecular Ecology* 25: 1449-1464.

Volis S, Ormanbekova D, Yermekbayev K, Song M, Shulgina I (2015) Multi-approaches analysis reveals local adaptation in the emmer wheat (*Triticum dicoccoides*) at macro- but not micro-geographical Scale. *Plos One* 10: e0121153.

Volis S, Shulgina I, Ward D, Mendlinger S (2003) Regional allozyme variation in wild barley, *Hordeum spontaneum*: adaptive or neutral? *Journal of Heredity* 94: 341-351.

Volis S, Shulgina I, Zaretsky M, Koren O (2011) Epistasis in natural populations of a predominantly selfing plant *Heredity* 106: 300-309.

Volis S, Verhoeven K, Mendlinger S, Ward D (2004c) Phenotypic selection and regulation of reproduction in different environments in wild barley. *Journal of Evolutionary Biology* 17: 1121-1131.

Volis S, Yakubov B, Shulgina I, Ward D, Mendlinger S (2005) Distinguishing adaptive from non-adaptive genetic differentiation: comparison of Q_{ST} and F_{ST} at two spatial scales. *Heredity* 95: 466-475.

Volis S, Yakubov B, Shulgina I, Ward D, Zur V, Mendlinger S (2001) Tests for adaptive RAPD variation in population genetic structure of wild barley, *Hordeum spontaneum* Koch. *Biological Journal of the Linnean Society* 74: 289-303.

Volis S, Zaretsky M, Shulgina I (2010) Fine-scale spatial genetic structure in a predominantly selfing plant: role of seed and pollen dispersal. *Heredity* 105: 384-393.

von Bothmer R, Jacobsen N, Baden C, Jorgensen RB, Linde-Laursen I (1995) An ecogeographical study of the genus *Hordeum*. 2nd edition. International Plant Genetic Resources Institute, FAO, Rome.

Walisch TJ, Colling G, Poncelet M, Matthies D (2012) Effects of inbreeding and interpopulation crosses on performance and plasticity of two generations of offspring of a declining grassland plant. *American Journal of Botany* 99: 1300-1313.

Waser NM, Price MV (1991) Outcrossing Distance Effects in Delphinium-Nelsonii - Pollen Loads,

Pollen Tubes, and Seed Set. *Ecology* 72: 171-179.

Waser NM, Price MV (1994) Crossing-distance effects in *Delphinium nelsonii* - outbreeding and inbreeding depression in progeny fitness. *Evolution* 48: 842-852.

Whiteley AR, Fitzpatrick SW, Funk WC, Tallmon DA (2015) Genetic rescue to the rescue. *Trends in Ecology & Evolution* 30: 42-49.

Willett CS, Burton RS (2003) Environmental influences on epistatic interactions: viabilities of cytochrome c genotypes in interpopulation crosses. *Evolution* 57: 2286–2292.

Wright JW, Stanton ML (2007) *Collinsia sparsiflora* in serpentine and nonserpentine habitats: using F2 hybrids to detect the potential role of selection in ecotypic differentiation. *New Phytologist* 173: 354-366.

Zohary D (1969) The progenitors of wheat and barley in relation to domestication and agricultural dispersal in the old world. In: Ucko PJ, Dimbleby GW (eds), The Domestication and Exploitation of Plants and Animals. Gerald Duckworth, London.

Supplementary data.

Suppl. Table 1. Ecogeographical data for the Israeli populations used in crossbreeding

Climatic zone/ Population	Region		Soil	Slope exposition	Altitude	Climatic parameters						
	Lon	Lat				Tm	Tj	Hu	Rd	Rn	CV	n
Desert	Negev Desert		Loess	-	450-470	19	9	36	15	93	44	58
SB*	34.46	30.51										
ARU	34.46	30.55										
HAT	34.51	30.55										
NN	34.49	30.52										
Sparse desert vegetation dominated by shrubs and semi-shrubs *Retama raetam*, *Tymelaea hirsuta, Zygophyllum dumosum*, *Hammada scoparia* with patchily distributed *H. spontaneum*.												
Semi-arid	Shefela Hills		Rendzina	North	250-300	19	11	47	37	400	32	57
BG	34.53	31.36										
NEH	34.57	31.37										
AMZ	34.54	31.33										
Semi-steppe batha with mosaic of shrub - semishrub cover (*Sarcopoterium spinosum* , *Calicotome villosa* , *Cistus salvifolius* and *C. creticus*) and dense stands of *H. spontaneum* among other grasses												
Mediterranean	Northern Galilee		Terra Rossa	North	300-500	19	10	48	50	587	28	48
AM	35.33	32.57										
BAR	35.28	33.04										
ZAL	35.27	32.52										
Mediterranean grassland without strong dominance of any species												
Mountain	Mount Hermon		Terra Rossa	South	1300-1550	11	1	52	70	>1000	-	-
MH	35.46	33.17										
BM	35.45	33.18										
SJ	35.46	33.18										
Mountain forest with deciduous *Quercus boissieri*, *Q. libani* and a few Rosaceae tree species accompanied by mainly perennial grasses and other herbaceous plants												

Abbreviations: * used for crossbreeding as $P_{♀}$, Hu - humidity at 14:00, Tm - annual temperature, Tj - temperature in January, Rn - annual rainfall (mm), Rd - number of rainy days, CV - coefficient of variation in Rn (%), n - years of observation, - no data

Suppl. Table 2. Sources of wild barley germplasm used as pollen donors in the crossing experiment.

Country	Source
Israel	Personal collection
Turkmenistan	Personal collection
China	ICARDA
Pakistan	USDA
Afghanistan	USDA
Tajikistan	IPK
Iran	IPK
Iraq	USDA
Turkey	USDA
Syria	ICARDA
Lebanon	USDA
Jordan	USDA
Egypt	ICARDA
Ethiopia	USDA
Lybia	IPK
Morocco	OUH
Cyprus	OUH
Crete	José Luis Molina Cano
Greece	IPK

Abbreviations: The International Center for Agricultural Research in the Dry Areas (ICARDA); National Small Grains Collection (USDA-ARS); The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK); Research Institute for Bioresources, Okayama University (OUH).