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Inter-population variability in seed dormancy, seed mass and germination in *Helianthemum salicifolium* (Cistaceae), a hard-seeded annual herb

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Abstract Many plant traits show variability within species in response to ecological gradients. In this study, we examined the variability in seed traits among ten populations of *Helianthemum salicifolium* (L.) Miller in Anatolia, Turkey. Since the Anatolian Peninsula has a geographic structure exhibiting longitudinal, elevational and climatic gradients, we hypothesized that seed dormancy, seed mass and germination show inter-population variability. We also postulated that seed traits are correlated to climatic and geographic characteristics of the populations. Since *H. salicifolium* is a species with a hard seed coat, we conducted germination experiments using heat shock and mechanical scarification. The germination percentage was improved significantly by mechanical scarification but remained unaltered by heat shock treatments in all sampled populations. As expected, seed traits exhibited substantial variation among the populations. We found no association between germination traits (dormancy level and germination rate in different treatments) and environmental variables. By contrast,
seed mass showed an elevational/longitudinal/climatic pattern: Populations in cooler and higher-elevation areas had larger seed mass. However, when the influence of climate was controlled, the geographic location became an important factor affecting seed mass. We conclude that seed mass variation among populations of *H. salicifolium* in Anatolia is shaped by the climate at the regional scale, but at the same time, geographically closer populations exhibit more similarity in seed mass.

Keywords elevational gradient · Anatolia · climate variability · geographic variation · scarification · seed mass

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Introduction

Many plant traits show variability within species in response to ecological gradients (Jung et al. 2010; Cochrane et al. 2015). Using the data about plant traits at the species level enables the testing of many ecological hypotheses (Hanley et al. 2003; Moreira et al. 2010; Çatav et al. 2014) and to identify ecological patterns in response to climatic or geographic variables at higher taxonomic and geographic scales (Moles et al. 2007; Qi et al. 2014). On the other hand, every trait of a species includes a variability component (Violle et al. 2012). Studying the variability among populations of a species is crucial to understand the ecological and evolutionary drivers shaping a trait (Karlsson and Milberg 2008; Moreira et al. 2012), to analyse the biotic and abiotic filtering processes responsible for the community composition and structure (Violle

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et al. 2012; Marteinsdóttir and Eriksson 2014), and to predict the ecological interactions within a community (Bolnick et al. 2011).

Seed traits such as dormancy level, seed size and germination vary among populations (Michaels et al. 1988; Giménez-Benavides et al. 2005; Karlsson and Milberg 2008; Cochrane et al. 2015; Santo et al. 2015). Variability in seed traits among and within populations is of critical importance for the persistence of a species under variable environmental conditions (Cruz et al. 2003; Tavşanoğlu and Çatav 2012). Since many seed traits are important for the regeneration of plant populations (Leishman et al. 2000; Baskin and Baskin 2014), they are under selective pressure owing to various environmental factors such as predation (Martínez et al. 2007), fire (Moreira et al. 2012) and climatic conditions (Karlsson and Milberg 2008). Such environmental factors are therefore expected to shape the variation in seed traits among populations of a species. For example, post-dispersal seed predation by animals (e.g. ants, rodents) creates a selective pressure over larger seeds, favouring smaller seeds in environments with abundant seed predators (Martínez et al. 2007). The alternative strategy to escape from predation can be mast seeding that allows the satiation of predators in a single pulse of seed production (Kelly 1994). In environments with predictable fire regimes, many non-resprouter species tend to have dormant seeds (to form a persistent soil seed bank) and fire-stimulated germination (for successful post-fire germination – Pausas and Keeley 2014). The existence of these trait combinations allows seed germination after fire in an environment with high resources and low competition (Tormo et al. 2014). Moreover, a low dormancy level and small seed size are selected in temporally predictable (i.e. less temporal variation in resources) and favourable (i.e. less competition with other plants due to high resources levels) environments (Volis and Bohrer 2013). On the other hand, larger seeds have an advantage in the seedling establishment stage since they include more resources than smaller ones and allow seedlings to grow more independently of resources levels at the microsite (Moles and Westoby 2004). In annual species, larger seed size has an extra survival advantage from seedling establishment to reproduction (Metz et al. 2010). Consequently, larger and dormant seeds are more advantageous under harsher environmental conditions (i.e. temporally variable environments with low resource levels – Metz et al. 2010; Volis and Bohrer 2013).

Cistaceae is one of the plant families characterized by physical dormancy (Baskin and Baskin 2014). Heat shock and mechanical scarification are known to break dormancy in species from Cistaceae (Thanos et al. 1992). Heat shock-stimulated germination has been observed in several genera of Cistaceae including *Cistus* (Trabaud and Oustric 1989; Thanos et al. 1992; Tavsanoglu 2011; Moreira and Pausas 2012), *Fumana* (Moreira et al. 2010), *Halimium* (Thanos et al. 1992; Herranz et al. 1999) and *Tuberaria* (Thanos et al. 1992; Luna et al. 2007; Gonçalves et al. 2009). Contrary to the general trend in this family, however, seed germination in *Helianthemum* is stimulated by mechanical scarification (Thanos et al. 1992; Pérez-García and González-Benito 2003; Zaidi et al. 2010) but not by heat shock (Thanos et al. 1992; Robles and Castro 2002; Luna et al. 2007; Paula et al. 2009; Moreira et al. 2010). Consequently, the germination behavior of *Helianthemum* species differs from other genera in Cistaceae and warrants more attention.

The effect of heat shock and mechanical scarification on germination has often been tested in Cistaceae at the species level. Corral et al. (1989) showed that seed size exhibits significant variability among populations of many *Cistus* species, and Thanos et al. (1992) revealed considerable variation in germination and dormancy properties among seed lots of species of Cistaceae originating from various botanical gardens. Recently, Moreira et al. (2012) showed that germination response in *Cistus* species to heat shock treatments varies at the population level, possibly as a result of local fire regimes. One unexplored question regarding the seed traits in Cistaceae family is whether the populations of *Helianthemum* species differ in seed traits such as seed mass, dormancy level and germination. Since the members of Cistaceae family have impermeable (hard) seed coats, germination experiments including pretreatments (i.e. heat shock and mechanical scarification) should be performed to understand whether the populations differ in germination properties.

In this study we examined the variability in seed traits among populations of *Helianthemum salicifolium* (L.) Miller. *H. salicifolium* is a cosmopolitan annual herb lacking any specific habitat requirements. It is distributed in the Circum-Mediterranean region in various habitats such as openings in Mediterranean woodlands and shrublands, steppes and deserts (Yeşilyurt 2014). Similar to all the members of the Cistaceae family, *H. salicifolium* seeds have a hard seed coat impermeable to water and

gasses, imposing physical dormancy (Thanos et al. 1992). The widespread distribution of *H. salicifolium* in various habitat types renders it an ideal model organism to study inter-population variability of seed traits in the Cistaceae. Since the geographic structure of the Anatolian Peninsula exhibit longitudinal, elevational and climatic gradients, we hypothesized that seed dormancy, seed mass and germination show substantial variability among populations of *H. salicifolium* in Anatolia, Turkey. We also predicted that climatic (temperature and precipitation) and geographic (elevation, latitude and longitude) characteristics of the study locations explain most of the variation in seed traits. From the patterns reported in the literature mentioned above, we hypothesized that populations growing in locations with lower temperature and precipitation (i.e. under harsher environmental conditions) will have larger seed mass and higher dormancy level. Finally, we also examined whether seed germination in populations of different origin has differential response to heat shock and mechanical scarification treatments, and if these responses are correlated to climate and geography.

Material and methods

Study areas, populations and seed collection

We selected ten populations of *H. salicifolium* naturally growing on the Anatolian Peninsula, Turkey. Selected populations represented both latitudinal and longitudinal gradients from the north (40.4° N) to the south (37.2° N) and from the western (27.0° E) to the central (34.7° E) parts of Anatolia. The elevation varied among the populations, from ca. sea level to 1,450 m above sea level (a.s.l; Table 1). The population locations had a dichotomous structure in respect to elevation and could be classified into two elevation classes (< 200 m and > 750 m), varying in climatic properties (higher temperatures at lower elevations and vice versa; Table 1). Moreover, coefficient of variation (CV) values of the monthly mean temperatures at higher elevations were higher than those at lower elevations (mean ± *SE*; 0.72 ± 0.04 and 0.46 ± 0.02, respectively). Dominant vegetation at the study sites varied from open shrublands to grasslands, depending on the elevation and geographic location. Climatic data for the study sites were obtained from the WorldClim database (Hijmans et al. 2005). The populations differed considerably in climatic variables such as the mean (9.5–16.8°C), maximum (15.2–22.1°C) and minimum (3.7–11.5°C) annual temperatures (hereafter T_{mean}, T_{max}, T_{min}, respectively), and the total annual precipitation (398–870 mm; Table 1).

Fruit capsules of *H. salicifolium* were collected from at least 20 individuals from each of the selected populations between May and July 2011. Fruit collection time in each population varied depending on the seed maturation time in each population due to the difference in elevation. Collected fruits from different individuals in each population were pooled to serve as a seed source for the population and stored in paper envelopes under

Table 1 Climatic properties and location of seed collection sites for each population included in the study.

Population	Location				Climate			
	Place	Lat	Long	Elev	T_{mean}	T_{max}	T_{min}	Prec
P1	Aşağıyurtçu (Ankara)	39.813	32.534	1,030	10.7	17.0	4.7	398
P2	Osmaneli (Bilecik)	40.312	30.068	174	13.9	19.0	8.8	542
P3	Erdek (Balıkesir)	40.387	27.800	140	13.8	18.1	9.4	676
P4	Foça (İzmir)	38.851	27.034	15	16.8	22.1	11.5	658
P5	Milas (Muğla)	37.303	27.900	17	15.1	20.9	9.4	870
P6	Dağbeli (Antalya)	37.195	30.498	800	13.1	19.6	7.0	543
P7	Keçiborlu (Isparta)	37.965	30.299	1,050	11.2	17.1	5.3	545
P8	Beynam (Ankara)	39.675	32.915	1,420	9.7	15.3	4.0	422
P9	Kalecik (Ankara)	40.094	33.399	770	11.3	17.3	5.2	413
P10	Gülek (Adana)	37.312	34.744	1,450	9.5	15.2	3.7	498

Lat and Long – latitude and longitude (decimal degrees) respectively; Elev – elevation (m a.s.l.); Prec – total annual precipitation (mm); T_{mean}, T_{max} and T_{min} – mean, maximum and minimum annual temperatures (°C), respectively.

room conditions for eight months until the germination experiments in March 2012.

We measured the maximum height of individuals in five of the populations studied (two located at > 750 m a.s.l. and three located at < 200 m a.s.l.) in the field and the mean seed number per fruit of those populations was determined in the laboratory. The populations at higher elevations (> 750 m a.s.l.) were smaller in height and had fewer seeds per fruit than did those at lower elevations (< 200 m a.s.l.; the mean maximum height was 22.5 cm and 28.3 cm, the mean seed number per fruit was 45.2 and 81.2, respectively). These preliminary measurements suggested that the increase in elevation imposed harsher growing conditions on *H. salicifolium* individuals.

The experiment

Although the germination experiment was conducted eight months after the seeds were collected, we expected no or very little loss in dormancy or viability due to the laboratory storage period since hard-coated seeds of the Cistaceae persist in soil seed banks and during laboratory storage for extended periods of time (Cerabolini et al. 2003; Probert et al. 2009). Just before the start of the germination experiment, the seeds were separated from fruit parts by hand and placed in aluminum pockets for subsequent treatment. For each population and treatment combination, four aluminum pockets, each containing 25 seeds were prepared. Before the treatments were applied, five replicates of 10 seeds were weighed using a digital scale to determine the mean seed mass for each population.

For heat shock treatments, the aluminum pockets were kept in a temperature-controlled oven preheated to the desired temperature (60, 80, 100 and 120°C) for 5 min. For the mechanical scarification treatment, the seeds were rubbed six times between two pieces of sandpaper with grid size of 500 μm. Each treatment had four replicates, and each replicate was subjected independently to the treatments. Four replicates of aluminum pockets, each including 25 seeds not subjected to any treatment, served as a control.

For each replicate per treatment and population, the seeds were sown in Petri dishes containing agar (0.7 %) as a substrate. Agar was selected for practical reasons and to create a standard germination medium for seeds. Sown Petri dishes were placed in an incubator at 20°C in the dark, the favourable conditions for germination of

Helianthemum species (Thanos et al. 1992). The germination was monitored every two or three days during the first two weeks of incubation, and once a week after this period. Germination was confirmed by observations under a stereomicroscope and scored in the presence of a visible radicle protrusion 0.5–1.0 mm long. The germinated, rotten, and empty seeds were counted and removed from dishes at every check. At the end of the experiment, the viability of non-imbibed and non-germinated seeds was determined by the cut test with scalpel, and the seeds with an intact embryo were assumed viable and dormant (Kenny 2000; Baskin and Baskin 2014; Çatav et al. 2014). The experiment commenced in March 2012 and was concluded after 38 days of incubation when no germination was recorded for one week.

Data analysis

Before statistical analysis, the initial number of seeds in each Petri dish was corrected by excluding the number of empty seeds. Dormancy level was estimated only for the control, as a percentage of non-germinated viable seeds, and germination percentage was estimated for the control and treatment groups as a percentage of germinated seeds in each group. For each population, the final germination of each treatment was compared to that in the control using an analysis of deviance (generalized linear model, GLM) assuming a binomial error distribution and considering the treatment as the fixed factor.

Linear and generalized linear models were performed to assess the differences in seed and germination characteristics among the populations, as well as among the treatments in each population. For continuous data (seed mass and dormancy level), we used general linear models and the differences among the populations were tested by the analysis of variance (ANOVA). Dormancy level data were arcsine-transformed prior to the analysis to meet the requirements of normality. For binary data (germinated vs. non-germinated seeds in the control and treatments), we used generalized linear models with binomial error distribution and the differences were tested by an analysis of deviance. In this analysis, we considered the population and the treatment as fixed factors and compared each treatment with the control separately to elucidate which treatment affected germination and to determine the interaction between the treatment and the population.

The mean germination time (MGT) was determined for treatments with sufficient number of germinated seeds using the formula $\sum(nD)/\sum n$, where n is the number of seeds germinated on day D, and D is the number of days from the beginning of the incubation period. Differences in MGT among the populations were examined using one-way ANOVA and Tukey's honestly significant difference post-hoc test. The MGT values were log-transformed before the analysis.

Pearson correlation analysis was performed to explore the associations between seed traits and environmental variables as well as between the environmental variables. Since all the variables, excluding germination data, had a normal distribution, germination percentages in each treatment were arcsine-transformed before the analysis. When the correlation between any seed trait and an environmental variable was significant ($P < 0.05$) or critically significant ($0.10 < P < 0.05$), linear regression analysis was conducted by fitting the linear model to observed data to understand the nature of the relationship between two variables. A one-way ANOVA was also used to test whether seed mass significantly differed among populations within different elevation groups. Additionally, a principal component analysis (PCA) was performed to create a composite 'geography plus climate' variable from highly correlated variables (i.e. elevation, longitude, and temperature), and its correlation with seed traits was tested. All data were standardized before PCA processing. The relationship between the outcome variable of the PCA (the first principal component, PC1) and seed traits was investigated using Pearson correlation and linear regression analyses.

A partial Mantel test (1,000 permutations) was performed to understand whether the germination and seed traits of the populations were structured in the climatic or geographic space. Three matrices were created for the analysis: (1) pairwise geographic distance between populations, (2) climatic dissimilarity, and (3) seed trait dissimilarity. We preferred to use only T_{min} data to create a climatic dissimilarity matrix since the rest of climatic variables (and the elevation) were highly correlated with T_{min} (Table S1 in the Electronic Supplementary Material). Bray-Curtis dissimilarity index was used to create dissimilarity matrices for seed mass, T_{min} (climate), and germination of mechanically scarified and heat-shock treated seeds. For each seed and germination trait, we performed two independent partial Mantel tests using Pearson's correlation coefficient: one to assess the association between a trait and the climate while controlling for difference in

the geographic location, and the other to evaluate the relationship between a trait and the geographic location while controlling for the difference in the climate. Using this approach, we could identify the effects of the climate and geographic location on a particular trait. For this analysis, statistical significance was estimated by permutation test (1,000 simulations).

The partial Mantel tests were performed using the 'vegan' (Oksanen et al. 2015) package. All analyses were conducted in R version 3.2.2 (R Core Team 2015).

Results

Among the ten populations included in this study, five exhibited less than 10 % germination and three less than 22 % germination in the control (Table 2). Mechanical scarification significantly improved the germination percentage in all populations, including two populations (P3 and P7) with relatively high germination percentage in the control (Table 2). In six populations, mechanical scarification enhanced germination percentage more than 90 %. However, heat shock treatments did not improve germination in any of the populations studied. The heat shock treatment at 80°C significantly reduced germination percentage in two populations, and no germination occurred in the heat shock treatment at 100°C in eight populations (Table 2). No difference in germination percentage was found between the heat shock treatment at 60°C and that in the control in all populations, and no seed germination was recorded in the heat shock treatments with 120°C.

Seed traits such as seed mass, dormancy level, and germination percentages in the treatments significantly varied among the populations (Table 3, Table S1 and Table S2 in the Electronic Supplementary Material). A significant effect of the treatment was present in mechanical scarification, 80°C, and 100°C heat shock treatments, and the interaction between the population and the treatment was significant only in the mechanical scarification treatment (Table 3, Table S2 in the Electronic Supplementary Material).

Since only mechanical scarification treatment had a sufficient number of germinations, we only tested the difference among populations by MGT only for this treatment. The MGT in most populations did not differ although the overall analysis presented a significant result (one-way ANOVA; $F = 3.3$, $P < 0.05$); the significant difference was observed only between the

Table 2 Mean seed mass [mg] and mean germination percentage in the control and treatments. The results of the statistical analysis (binomial generalized linear models) of the germination in treatments in comparison to that in the control are also given.

| Pop | S_{mass} | Cont | SC | Heat-shock treatments | | | |
				60°C	80°C	100°C	120°C
P1	0.208 ± 0.007	1.0	93.0****	0 [ns]	0 [ns]	0 [ns]	0 [ns]
P2	0.082 ± 0.007	14.1	97.0****	16.2 [ns]	10.2 [ns]	0**	0**
P3	0.088 ± 0.005	63.0	97.5****	65.9 [ns]	47.2*	7.3****	0****
P4	0.148 ± 0.004	9.2	79.8****	6.2 [ns]	4.7 [ns]	0*	0*
P5	0.144 ± 0.005	0	94.0****	1.0 [ns]	2.0 [ns]	0 [ns]	0 [ns]
P6	0.180 ± 0.009	20.0	95.0****	23.4 [ns]	8.7*	0**	0**
P7	0.114 ± 0.002	47.6	82.6****	62.0 [ns]	38.6 [ns]	0****	0****
P8	0.208 ± 0.006	1.7	28.3**	0 [ns]	1.7 [ns]	0 [ns]	0 [ns]
P9	0.288 ± 0.009	21.7	98.3****	16.7 [ns]	10.0 [ns]	5.2*	0**
P10	0.240 ± 0.006	8.3	85.0****	8.3 [ns]	6.2 [ns]	0 [ns]	0 [ns]

ns – non-significant, * – $P < 0.05$, ** – $P < 0.01$, *** – $P < 0.001$, **** – $P < 0.0001$; Pop – population code as in Table **1**; S_{mass} – seed mass expressed as mean ± SE; Cont – control; SC – mechanical scarification.

populations with fastest (3.23 days in population P10) and slowest (8.14 days in population P8) germination (Tukey HSD test, $P < 0.05$). Therefore, the variation among the populations in germination rate was limited (Table **S3** in the Electronic Supplementary Material).

Climatic variables were correlated significantly with longitude and elevation (Table **S4** and Fig. **S1** in Electronic Supplementary Material), but no correlation was found between latitude and climatic variables. There was also a strong positive correlation between

Table 3 Probability values for the general or generalized linear models testing the effect of population and treatment on seed mass – S_{mass} [mg], dormancy level – Dorm [%] and germination stimulation by mechanical scarification (SC) or different heat shock temperatures. Full model parameters are indicated in Table **S1** and Table **S2**.

| | Fixed effects | | |
	Population	Treatment	Population × Treatment
S_{mass} *	< 0.0001	–	–
Dorm *♀	< 0.0001	–	–
SC †	< 0.0001	< 0.0001	< 0.0001
60°C †	< 0.0001	ns	ns
80°C †	< 0.0001	0.0017	ns
100°C †	< 0.0001	< 0.0001	ns

*The results are based on general linear models (see the text).

♀ Dorm data were arcsine transformed prior to the analysis.

† The results are based on generalized linear models (see the text).

longitude and elevation (Table **S4** in the Electronic Supplementary Material) because of the natural elevation gradient across the longitudinal transect in Anatolia from the Aegean Sea coast in the west to the eastern Anatolian plateau. The 'geography plus climate' variable (PC1) extracted by the PCA explained 94.7 % of the total variance (eigenvalue = 2.84). All variables, namely T_{min}, elevation and longitude, were highly correlated with PC1 (with loadings −0.99, 0.96, and 0.95, respectively). PC1 was positively correlated with seed mass ($P < 0.05$), but not with other seed traits ($P > 0.05$; Table **S5** in the Electronic Supplementary Material). The positive relationship between the seed mass and PC1 followed a linear model ($R^2 = 0.48$, $P = 0.026$; Fig. **1**). Loadings of the PCA and the significant relationship between the PC1 and seed mass indicated that seed mass increased with increasing elevation, lowering temperatures and toward eastern longitudes.

No association between germination and environmental variables (geographic and climatic data) was found. Moreover, the relationship between germination traits and seed mass was not significant ($P > 0.05$; Table **S5** in the Electronic Supplementary Material). By contrast, significant associations were observed between seed mass and longitude and seed mass and T_{min} ($P < 0.05$; Table **S5** in the Electronic Supplementary Material). A positive relationship between seed mass and longitude ($R^2 = 0.59$, $P = 0.009$) and a negative relationship between seed mass and T_{min} ($R^2 = 0.42$, $P = 0.042$) followed a linear model (Fig. **S2** in the Electronic Supplementary Material).

Fig. 1 Correlation between seed mass and principal component 1 (PC1) comprising longitude, elevation and annual minimum temperature ($R^2 = 0.48$, $P = 0.026$). Each point represents a population. Note that the elevation is inversely related to the temperature. Altitude increases toward the eastern end of the longitudinal gradient of PC1, while temperature is higher towards the western end of the gradient.
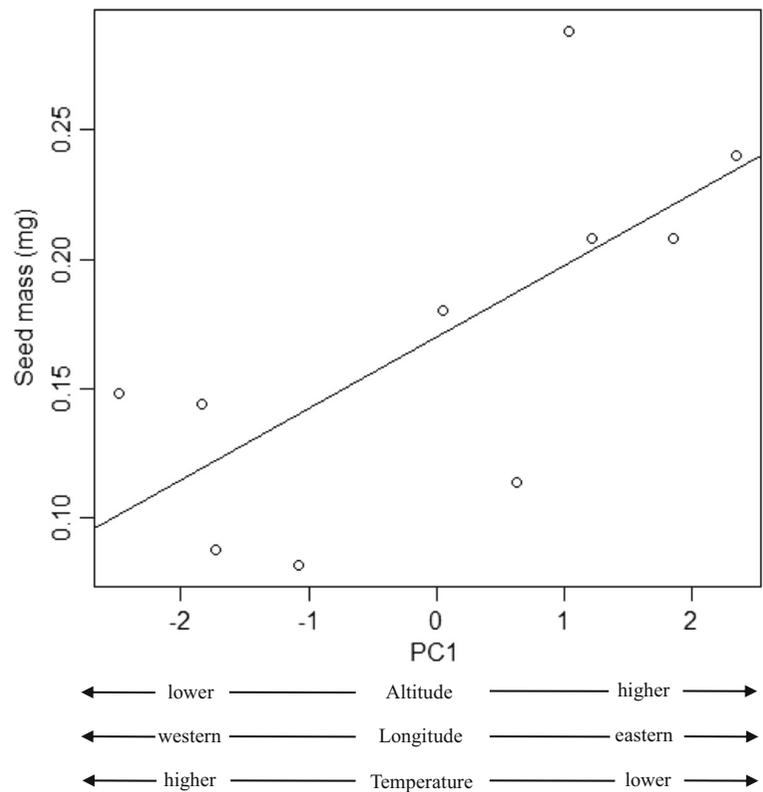
Furthermore, the correlation between seed mass and elevation (positive relationship) and T_{mean} (negative relationship) were critically significant ($R^2 = 0.37$, $P = 0.062$ and $R^2 = 0.33$, $P = 0.082$, respectively; Fig. **S2** in the Electronic Supplementary Material). The relationship between precipitation and seed mass was also critically significant ($0.10 < P < 0.05$, Table **S5** in the Electronic Supplementary Material). Further analysis of the relationship between seed mass and elevation showed that seeds in the populations found at elevations < 200 m a.s.l. were 2-fold smaller than those of the populations found at elevations > 750 m a.s.l. (0.108 and 0.207 mg, respectively; $F = 11.1$, $P = 0.010$).

The results of the partial Mantel tests showed that climatic properties at the study sites affected significantly the germination percentage after mechanical scarification treatment regardless of the geographic proximity of the populations (i.e. distance between the populations; $R = 0.31$, $P < 0.05$; Table **S6** in the Electronic Supplementary Material). Moreover, the mean seed mass in populations was significantly associated with the geographic location of the populations ($R = 0.32$, $P < 0.05$; Table **S6** in the Electronic Supplementary Material) if the

effect of the climate was controlled. This result suggested that the closer the populations in geographical space, the more similar the seed mass. Other traits were not structured in geographical or climatic space ($P > 0.05$, Table **S6** in the Electronic Supplementary Material).

Discussion

Our study revealed that dormancy level, germination properties, and seed mass show substantial inter-population variation in *H. salicifolium*. Longitude, elevation and climate explained the variability in seed mass of different populations, but not the germination traits. Moreover, our results pertaining to the germination patterns showed that mechanical scarification, not heat shock (fire temperature) stimulates germination in *H. salicifolium*.

A potential drawback of our study was the use of seeds that were stored under laboratory conditions for eight months. The seeds of the Cistaceae family, which are characterized by water-impermeable seed coats and physical dormancy, can persist in soil seed banks or

stored under laboratory conditions for long periods (more than five years, in some cases more than twenty years) without any loss of dormancy or viability (Cerabolini et al. 2003; Probert et al. 2009). Therefore, seeds of Cistaceae species do not show any viability or dormancy loss during a lab storage period that is shorter than the duration of potential seed persistence (e.g. Pérez-García 1997; Moreira et al. 2010; Chamorro et al. 2017). Hence, we do not think that our results would be considerably different if we had conducted the experiments within the first month after seed collection.

The climatic and geographic variables considered in our study did not explain the observed variability in dormancy and germination properties among the populations, and these traits in *H. salicifolium* did not show climatic or geographic gradient within Anatolia. The lack of such relationships can be explained by the predominant effect of local habitat properties (such as maternal effects and edaphic factors) on dormancy and germination traits (Schütz and Millberg 1997; Vandelook et al. 2008; Huang et al. 2015; Chamorro et al. 2017). On the other hand, the seed mass in *H. salicifolium* showed a significant elevational/longitudinal/climatic pattern throughout Anatolia. The triple relationship among the longitude, elevation and T_{min} is due to the three-dimensional topographic structure of the Anatolian Peninsula – the Anatolian plateau rises in the west-to-east direction (Miliaresis 2006), and this gradient is accompanied by a decrease in the mean temperature. Therefore, the suitable period is shorter and the environmental conditions are harsher for plant growth towards the central and eastern parts of Anatolia (Türkeş 1996; Atalay and Efe 2010). Germination after heat shock treatments was found positively related to seed mass in *Cistus ladanifer* (Delgado et al. 2001) and *Cistus salviifolius* (Tavşanoğlu and Çatav 2012). Positive seed mass-germination relationship is not restricted to the Cistaceae family; for example, Skarpaas et al. (2011) found a strong correlation between these two traits in two *Carduus* species (Asteraceae). Seed mass has a positive effect on the survival of seedlings and saplings. Moles and Westoby (2006) in their meta-analysis showed that seed mass is positively related to the percentage of seedlings surviving through the first week of establishment in 113 species and sapling survival in the first three years in 76 species from around the world. A similar conclusion was reached by Metz et al. (2010) for 49 annual plant species from semi-arid and Mediterranean habitats in Israel. In many

plant species, larger seed mass affects the overall success of seedlings by increasing germination, survival and growth. These general trends can be observed in many species because more resources stored in larger seeds allow seedlings to cope with unfavourable conditions better (Moles and Westoby 2004). However, the relationship among seed traits can be more complex. Although the climate is one of the main drivers in the evolution of seed traits (Cochrane et al. 2015), specific combinations of dormancy and seed size are favoured by natural selection in areas that have different environmental conditions (Volis and Bohrer 2013). The short growing season in northern populations of *Amaranthus retroflexus* (McWilliams et al. 1968) and *Reynoutria japonica* (Mariko et al. 1993) resulted in the selection of larger seeds in comparison to southern populations. Larger seed size is also advantageous in shady and stable plant associations with high competition for resources in a Peruvian forest (Foster and Janson 1985). Therefore, considering the positive effects of larger seed mass to seedling survival, it can be expected that environments harsh for plant growth favour larger seed size (Leishman et al. 2000). In several cases, however, this relationship can be inverse (since dispersal ability is important in shaping seed size – Harel et al. 2011) or absent (due to gene flow – Pluess et al. 2005). Our results on dormancy level and seed mass in Anatolian *H. salicifolium* populations fit a model proposed by Volis and Bohrer (2013), which predicts for which seed dormancy is selected in harsh and temporally variable environments, but at the same time, larger seeds have an advantage over smaller ones. Our study also supports that among-population variation in seed traits are common and should be studied for the better conservation, restoration and management of plant species (Cochrane et al. 2015).

Although climate shapes the seed mass variation among populations of *H. salicifolium* at the regional scale, when controlling for the influence of the climate, the geographic location becomes an important factor affecting seed mass. This difference between regional and local patterns of seed mass variation may be because the role of climate in shaping seed traits is overridden by higher gene flow between closer populations (Lenormand 2002; Pluess et al. 2005). However, more studies are warranted to elucidate whether such a pattern exists in Anatolian populations of *H. salicifolium*. Seed size variation in plants is a result of complex interactions of several ecological and genetic factors (Wolfe 1995;

Alonso-Blanco et al. 1999). In conclusion, our results suggest that seed mass variation among populations of *H. salicifolium* in Anatolia is shaped by climate at the regional scale, but at the same time, geographically closer populations exhibit more similarity in seed mass.

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