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Genetic and environmental variation of seed weight in *Trichloris* species (Chloridoideae, Poaceae) and its association with seedling stress tolerance

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Background: Seed weight is a key fitness-related trait associated with plant adaptation and is commonly targeted in plant breeding.

Aims: We evaluated seed weight variation within and between *Trichloris crinita* and *Trichloris pluriflora* across their geographical ranges in Argentina.

Methods: Genetic variation in seed weight was evaluated through a common garden experiment. To examine the possible role of such variation in local adaptation, we compared the seed weight of plants of populations raised in the common garden with seed weight variation and ecogeographical variables across their original habitats. We also evaluated experimentally the effects of seed weight variation upon osmotic stress tolerance at germination.

Results: Variation in seed weight existed in both species. Such variation had a genetic basis in *T. crinita* related to several ecogeographical variables. Larger seeds of *T. crinita* were associated with more stressful environments and produced larger seedlings under both osmotic stress and non-stress conditions.

Conclusions: Our results suggest that seed weight variation in *T. crinita* is likely adaptive, with large seed having an advantage during early developmental stages, particularly under stressful conditions. Such knowledge should prove helpful in selecting the most suitable populations for restoration and plant breeding.

Keywords: Argentina; ecogeography; germination; grasslands; local adaptation; osmotic stress; seed weight variation; *Trichloris crinita*; *Trichloris pluriflora*

Introduction

Seed weight is a critical attribute of a plant's life history related to fitness (Cohen 1966; Venable and Brown 1988) and an essential aspect of its ecological strategy (Westoby et al. 2002). Seed weight variation can be an important adaptation to temporally and spatially heterogeneous environments (Venable 2007). Spatial variation in phenotypic traits *in situ* may reflect genetic differentiation (Linhart and Grant 1996) and adaptation to local climate and soils (Davis et al. 2005) but may also reflect environmentally induced phenotypic plasticity (Nicotra et al. 2010). Although it is often difficult to separate genetic differentiation from the effects of environmental variation, much of the variation among plant populations within species appears to reflect the direct effects of the environment (Ackerly et al. 2000). Hence, reciprocal transplant or common garden experiments are required to detect genetic differentiation that may be adaptive and result from an evolutionary response to natural selection (Baskin and Baskin 1973; Roach and Wulff 1987; Schmitt et al. 1992; Cochrane et al. 2015).

Several studies have shown that seed weight varies greatly across species (Westoby et al. 1992; Moles et al. 2005), within species (Münzbergová and Plačková 2010;

Cochrane et al. 2015) and even within individual plants (Michaels et al. 1988). Such variability reflects plant adjustment to different environmental conditions affecting germination, growth, survival and competitive ability (Westoby et al. 1992; Susko and Lovett-Doust 2000; Murray et al. 2004; Violle et al. 2009), suggesting that seed weight is an adaptive trait (Silvertown 1989; Davis et al. 2005; Violle et al. 2009; Nicotra et al. 2010). In this sense, increases in seed weight have been correlated with higher germination rates and seedling size under stressful conditions (Leishman and Westoby 1994; Milberg and Lamont 1997; Khan and Ungar 2001; Daws et al. 2008; Easton and Kleindorfer 2009; Qiu et al. 2010; Zabala et al. 2011).

Because of the association between environmental conditions, genes and phenotypic expression as a consequence of natural selection and local adaptation (Greene and Hart 1999), ecogeographical principles can be used to predict patterns of genetic variation across natural populations according to their origin (Peeters et al. 1990). Some ecogeographical variables may vary over small spatial scales and fluctuate temporally, so environmental differences should be sought at a large enough spatial scale to encompass seed dispersal and repeated between generations, providing

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appropriate conditions for phenotypic adaptation to local environments (Galloway 2005).

Native plant species have been subject to a long process of natural selection and adaptation to local environmental variation and have potential value as genetic resources for ecosystem restoration and plant breeding (Blanco et al. 2005; Flowers and Flowers 2005; Colmer et al. 2006; Quiroga et al. 2009). Species of the genus *Trichloris* (Chloridoideae, Poaceae) studied here are important forage sources of Argentinian and North American (USA) rangelands (Lloyd-Reilley 2011; Lloyd-Reilley and Kadin 2011; Rúgolo and Molina 2012). The genus comprises two species of C4 perennial grasses (Rúgolo and Molina 2012), tetraploid *Trichloris crinita* (Lag.) Parodi ($2n = 4x = 40$) and hexaploid *Trichloris pluriflora* E. Fourn ($2n = 6x = 60$) (Fedorov 1969), which grow in Argentina in ecogeographically diverse environments widely spread across the central and northern part of the country (Marinoni et al. 2015). *Trichloris crinita* is a typical grass of arid, semiarid and saline Argentinian rangelands, though also occurs in humid environments (Ragonese, 1956; Covas, 1978; Cabrera, 1994), whereas *T. pluriflora* only occurs in semiarid to humid, non-saline rangelands (Morello and Saravia Toledo 1959; Bordón 1981).

We have previously reported a collecting strategy for *Trichloris* (Marinoni et al. 2015) using Ecogeographical Land Characterization maps (ELC), which has lead to a significant improvement in ecogeographical representation of the *Trichloris* genus in the genebank "Ing. Agr. José Mario Alonso" belonging to the Facultad de Ciencias Agrarias of the Universidad Nacional del Litoral, Argentina (FCA-UNL, WIEWS instcode ARG1188; http://www.fao.org/wiews-archive/institute.htm). However, little is known about the diversity of traits associated with this collection and the ecogeographical variation represented. The hypotheses tested in this study are (a) that there is inter- and intraspecific variation in seed weight within the *Trichloris* genus across its distribution range in Argentina; (b) that such variation is due to local adaptation (i.e. predictable genetic responses along ecogeographical gradients); and (c) that this adaptation includes the capacity to improve germination under stressful conditions (here represented by moderate salt stress).

Materials and methods

Seed source

Species seed records were obtained from the *Trichloris* germplasm collection held at genebank "Ing. Agr. José Mario Alonso" belonging to FCA-UNL. At the time, this contained 49 accessions of *T. crinita* and 25 accessions of *T. pluriflora* corresponding to georeferenced populations and genotypes within populations, and representative of each species' distribution in Argentina. According to the ELC map for the genus published by (Marinoni et al. 2015), accessions also represented different ecogeographical origins. Seed of accessions was stored immediately after collecting and has remained stored at 5% (w/w) seed moisture content and at

2–4 °C to insure long-term viability (Bank Curator personal communication). Seed storage behaviour of *Trichloris* species (http://data.kew.org/sid/SidServlet?ID=23355&Num=r0h) is described as orthodox.

Seed weight analysis

Original habitat. Seed weight of seed samples collected from the original habitats was obtained for 74 *Trichloris* accessions from their passport data. Each seed sample represented a bulk sample collected from a number of plants at a location, which hereafter is considered to represent a sample from the population at that site.

Common garden. A subset of populations from across the distribution of each species was selected for cultivation in a common-garden experiment conducted at the FCA-UNL campus in Esperanza city (31.4424°S latitude, 60.9410°W longitude). Environmental characteristics of the common garden are listed in Supplementary Table 1. A total of 21 and 20 populations were examined for *T. crinita* and *T. pluriflora*, respectively (Figure 1a), with approximately 20 plants raised from seed of each population sample.

Seeds of each population were sown in 5-l pots filled with a mix of two-thirds of soil (see soil class in Supplementary Table 1) and one-third of sand and kept in a greenhouse with controlled irrigation. After germination, seedlings were thinned to one per pot when they had 3–4 completely expanded leaves, before transferring pots to the field and arranging them in a completely randomised design. Transfer of pots took place in November 2012 (Southern Hemisphere spring) and seeds were harvested from plants that flowered at different times during February and March 2013 (end of summer). Both species are highly autogamous (Gutiérrez et al. 2016), and thus, outcrossing was considered to be negligible (Kozub et al. 2017). Spikelets were dehulled and 3 samples of 100 naked caryopses (i.e. seeds) were weighed per plant. Seed weight was determined using a balance with an accuracy of 0.1 mg and expressed as grams per 1000 seeds.

Ecogeographical evaluation of seed weight variation

To determine if seed weight variation of populations expressed in the common garden experiment was related to the local environmental conditions of original sites in the wild, we extracted ecogeographical information for each georeferenced collecting site (Parra-Quijano et al. 2014) from CAPFITOGEN tools (http://www.capfitogen.net/es) using the ECOGEO tool. To reduce the level of redundant information, we selected the following key variables: (a) bioclimatic variables: annual average temperature, minimum temperature for the coldest month, maximum temperature for the warmest month, annual rainfall, rainfall during the wettest month and rainfall during the driest month, (b) edaphic variables: clay and silt content in surface soil, organic carbon content and cation exchange capacity in
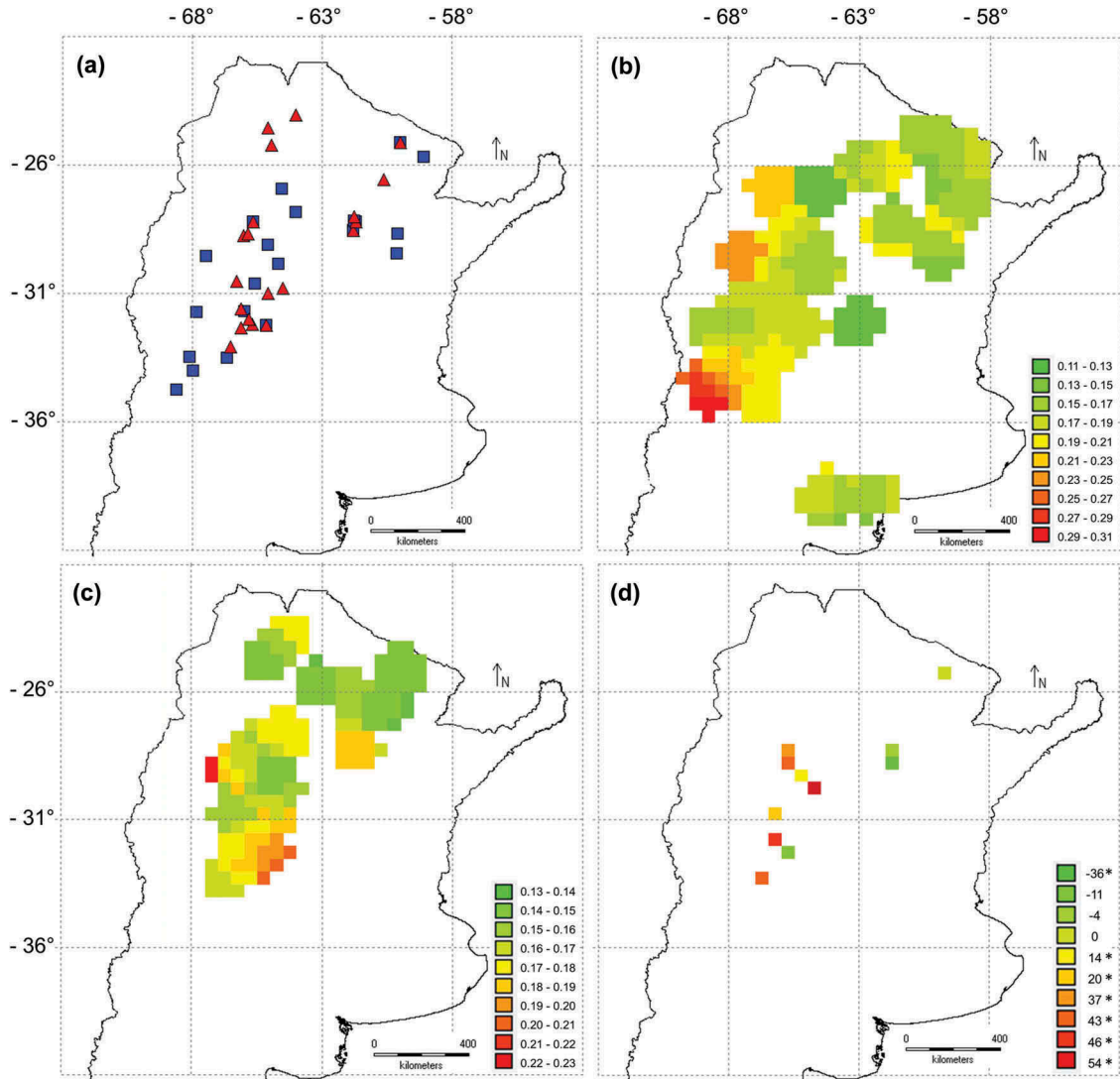
Figure 1. Spatial distribution of (a) selected populations of *Trichloris crinita* (blue squares) and *T. pluriflora* (red triangles) for common-garden assay; (b–c) seed weight (SW) (g/1000 seeds) from original habitat in (b) *T. crinita* and (c) *T. pluriflora*; (d) SW differences (mg) between sympatric populations at collecting site (*indicate statistically significant differences, *P* < 0.05). Different colours indicate SW range in (b) and (c), and SW differences between sympatric populations in (d) (SW difference = *T. crinita* SW − *T. pluriflora* SW).

surface soil, (c) geophysical variables: latitude, longitude and elevation. Variables have a cell resolution of 2.5 arc min (~5 × 5 km at the equator) (See supplementary Table 2) and were sourced from the WorldClim database (http://www.worldclim.org), Digital Elevation Model of Shuttle Radar Topography Mission (http://www.srtm.csi.cgiar.org) and the Harmonized World Soil Database (http://webarc hive.iiasa.ac.at/Research/LUC/External-World-soil-data base/HTML/).

Germination assay of seeds produced under common garden conditions

Germination of seed produced by plants raised in the common garden was tested under osmotic stress to mimic the conditions under which they would germinate in the wild on saline soils and conditions of drought.

Osmotic stress was generated by the addition of sodium chloride (NaCl) to a half-strength Hoagland solution (Hoagland and Arnon 1950), achieving an osmotic potential of −0.5 MPa (6.5 g of NaCl per litre or 120 mM). Munns et al. (2010) suggested that NaCl concentrations of ca. 100 mM do not have short-term toxic effects and may be preferable to produce osmotic stress rather than compounds such as a polyethylene glycol which interfere with oxygen circulation to roots. This is particularly relevant for small-seeded species such as *Trichloris* species. Half-strength Hoagland solution was used for controls.

Seeds were disinfected before sowing by placing them for 2 min in a hypochlorite solution (0.6% chlorine) followed by rinsing with distilled water. For each accession, 30 seeds were placed in 8 × 3 × 3 cm deep containers filled with perlite and covered with a thin layer of ground perlite before placing them in a highly humid

atmosphere to prevent desiccation. Four replicates were used per accession by randomly placing one container per accession in each of 4 large trays (total of 41 accessions × 4 trays = 164 containers). Trays were placed in a growth chamber set at 28 °C with continuous illumination provided by fluorescent lamps (100 μmol m^{-2} s^{-1}). Emergence of seedlings (germination) was recorded at 7 and 21 days after sowing, and seedlings were harvested for fresh (FSB) and dry seedling biomass (DSB) on the 21st day. Seed viability was evaluated in samples of 50 seeds for each accession by means of a tetrazolium test (according to Cabeza et al. 1999). Initial emergence percentage (IEP) of seedlings after 7 days and final emergence percentage (FEP) after 21 days were calculated based on viable seeds only. Seedling biomass was recorded for a subset of seedlings that emerged and survived after 7 days and was averaged for each replicate.

Data analyses

Weight of seeds collected from the wild. A nested Analysis of Variance (ANOVA) was performed to evaluate variation in seed weight among species and among populations within species for seeds collected from the wild. Mean seed weight by population was used as the response variable. Species was considered as a fixed factor and populations within species as a random factor because populations were sampled randomly within each species' distribution (Marinoni et al. 2015). Spatial distribution maps of mean seed weight were constructed with DIVAGIS software (http://www.diva-gis.org) to examine the distribution patterns of the trait in both species. For each species, a vectorial (point) shapefile was constructed from collection georeferenced records and seed weight data (average weight of 1000 seed) for each population. Maps were created with a 50 × 50-km cell resolution, assigning a circular neighbourhood of 200 km using the "point to grid/Statistics/mean" tool. Resolution and circular neighbourhood values were chosen according to the wide distribution of both species (2000 and 1200 km between the farthest populations of *T. crinita* and *T. pluriflora*, respectively).

Additionally, seed weight of seeds collected from the wild was compared between sympatric populations of both species sampled from the same site in the same year. Such comparisons were made at 11 sites and in each case, an *F*-test for equality of variances was performed to ensure that variances were equal and met assumptions of ANOVA. Seed weight differences between species were then calculated for each site and mapped onto a 50 × 50-km cell map of the region using DIVAGIS software and the "point to grid/Statistics/mean" tool.

Weight of seeds collected from common garden grown plants. A nested ANOVA on seed weight was performed on seed produced by plants raised in the common garden experiment in the same way as performed on seed collected from the wild. Pearson's correlation coefficient was calculated across populations to examine the association between weights of seed collected from the wild and seed produced in the common garden environment. Variation in seed weight in the common garden experiment is likely to reflect genetic differences, assuming that maternal environmental effects carried over from the previous generation are negligible, whereas variation for seed collected from the wild will reflect genetic and environmental differences. Thus, the correlation between both datasets provides an indication of the genetic component of seed weight variation expressed in the wild.

Pearson's correlation coefficients were also calculated between seed weight of plants raised in the common garden and several ecogeographical variables (some log-transformed), after discarding those variables that were highly correlated with other ones (correlation coefficient $r \geq 0.9$). A multiple linear regression analysis was conducted on this subset of variables, using the backward elimination procedure. The Bayesian Information Criterion (BIC) and variance inflation factor (VIF) were calculated to assess the relative contribution of each retained variable to the model and collinearity assumptions, respectively. Regression models were chosen by minimising values of BIC. Values of VIF from 1 to 10 reveal an absence of linear dependence or collinearity between variables when regressed against each other (Kleinbaum et al. 1988; Hair et al. 1999).

Germination and seedling biomass

IEP, FEP, FSB and DSB data were analysed by nested ANOVA, and Pearson's correlation coefficients were calculated between seed weight, germination and seedling biomass of common garden grown plants, to evaluate the influence of seed weight on stress tolerance. In all cases, normality and homogeneity of variances were verified, and statistical analyses were performed using the InfoStat statistical package (Di Rienzo et al. 2011).

Results

Seed weight analysis

The weight of seeds collected from the wild differed significantly among populations within species ($P < 0.0001$) (Supplementary Table 3), varying on average by a factor of 2.6 (0.113 minimum to 0.297 maximum g/1000 seeds) within *T. crinita* and a factor of 1.7 (0.136 minimum to 0.234 maximum g/1000 seeds) within *T. pluriflora* (Figure 1b and c). Although the overall difference in seed weight between species was not significant ($P = 0.18$), differences between species occurring in sympatry were significant ($P < 0.05$) (Supplementary Table 4) in 8 out of the 11 sites examined (Figure 1d). Seed of *T. crinita* were heavier than those of *T. pluriflora* at seven of these sites and in general were heaviest towards the west of the species' distribution (Figure 1b).

Similarly, for seeds produced in the common garden, there was a highly significant difference in seed weight
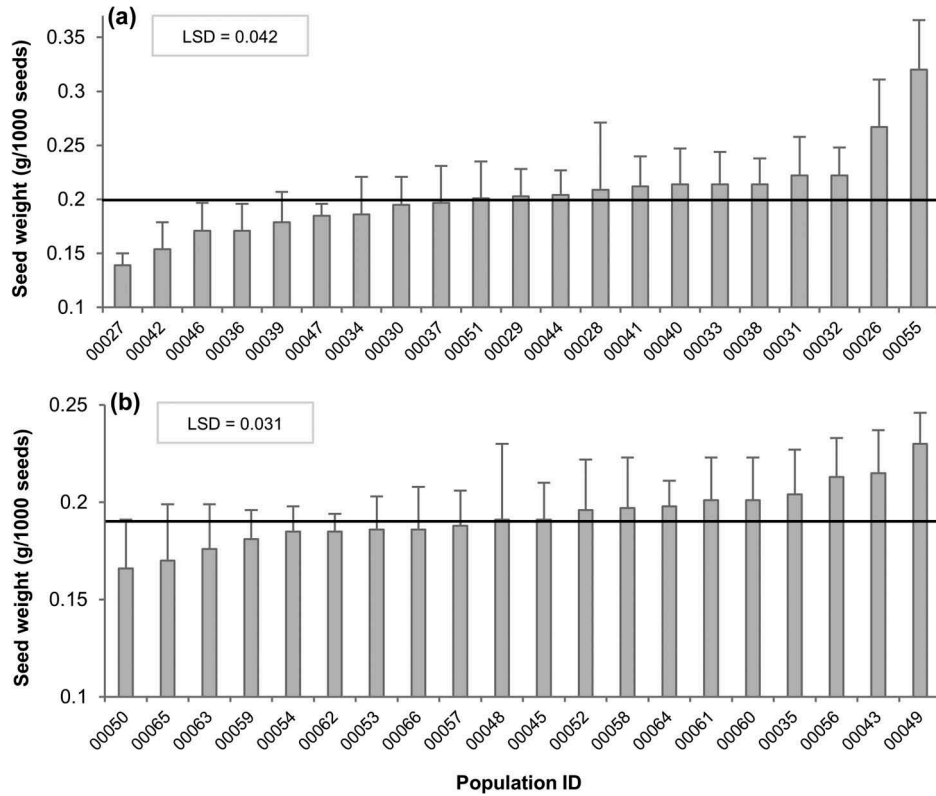
Figure 2. Seed weight (mean plus standard deviation) of selected populations from common garden: (a) *T. crinita* and (b) *T. pluriflora*. (ID population corresponds to the accession number in the *Trichloris* collection stored in the FCA-UNL germplasm bank.) The horizontal line shows the average SW of for each species. LSD: least significant difference.

among populations within species ($P < 0.0001$) but not between species ($P = 0.28$; Figure 2) (Supplementary Table 5). However, the correlation between seed weight of populations for seed collected from the wild and produced in the common garden experiment was highly significant in only *T. crinita* ($r = 0.86$; $P < 0.0001$), but not for *T. pluriflora* ($P = 0.33$) (Figure 3).

For *T. crinita*, correlation coefficients between seed weight and ecogeographical variables were significant ($P < 0.05$) and positive for latitude ($r = 0.67$) and altitude ($r = 0.47$), and significant and negative for annual mean temperature ($r = -0.74$), minimum temperature for the coldest month ($r = -0.6$), maximum temperature for the warmest month ($r = -0.7$), rainfall during the wettest month ($r = -0.53$) and longitude ($r = -0.44$). However, the correlations between seed weight and longitude, and also minimum temperature of the coldest month, were deemed non-informative because longitude is highly correlated with altitude ($r = -0.9$), and minimum temperature for the coldest month is highly correlated with annual average temperature ($r = 0.94$). Interestingly, there was no correlation between seed weight of populations and any edaphic variable examined.

Fewer significant correlations were evident between seed weight and ecological variables in *T. pluriflora*. In this species, seed weight was significantly and positively correlated with altitude ($r = 0.46$) and negatively correlated

with annual average temperature ($r = -0.47$) and maximum temperature for the warmest month ($r = -0.57$) (Figure 4).

In the multiple linear regression analysis of ecogeographical variables on seed weight conducted on *T. crinita*, the model retained 3 variables with a coefficient of multiple determination (R^2) of 0.74. The regression equation predicting seed weight was

$$SW = 0.5 - 0.00084MTWM - 0.00076RWM + 0.06Log_{10}RDM$$

where SW is the weight of 1000 seeds (g), MTWM is maximum temperature for the warmest month (°C), RWM is rainfall during the wettest month (mm) and RDM rainfall during the driest month (mm).

For *T. pluriflora*, three variables were also retained by the model with $R^2 = 0.57$ and the regression equation predicting seed weight was

$$SW = -0.13 - 0.00056MTCM - 0.0018RDM + 0.13Log_{10}AR$$

where MTCM is minimum temperature for the coldest month (°C), RDM is rainfall during the driest month (mm) and AR annual rainfall (mm). For both species, R^2 values reveal a good fit and the VIF for each variable was smaller than 3, indicating non-collinearity (Kleinbaum et al. 1988; Hair et al. 1999).
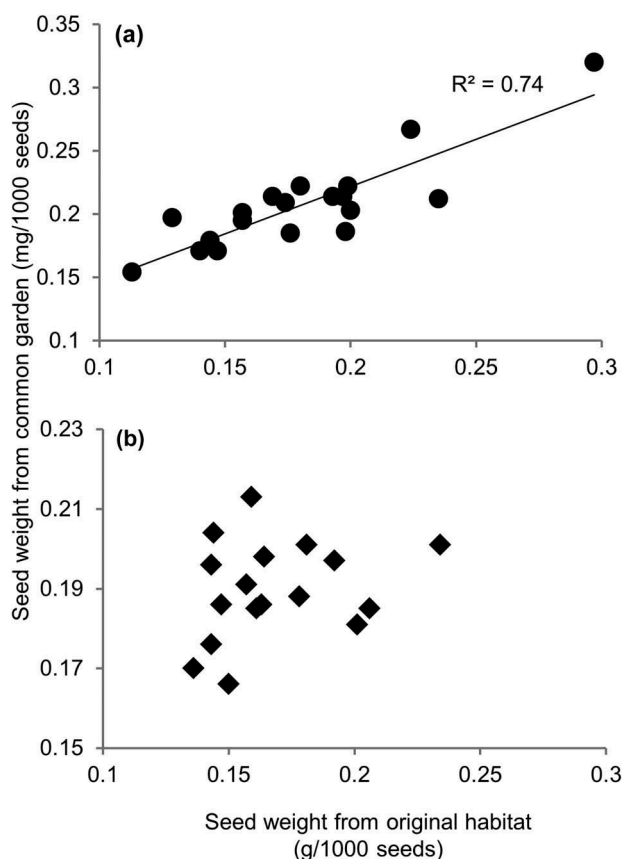
Figure 3. Relationships between seed weight from original versus common environment in (a) *T. crinita* and (b) *T. pluriflora*. The trend line and coefficient of determination (R^2) were only statistically significant for *T. crinita*.

Germination assay

IEP and FEP differed significantly among populations within species ($P < 0.0001$) and treatments ($P = 0.0003$) (Supplementary Table 6). In all cases, emergence percentage was lower in the osmotic treatment than in the control. Differences in germination among populations were not associated with seed weight. FSB and DSB differed between species ($P = 0.001$; $P = 0.0018$, respectively) and treatments ($P < 0.0001$), and the interaction between species and treatment was significant for both variables ($P = 0.0013$ and $P < 0.0001$, respectively). Seedling biomass was affected by salt stress to a much greater extent in *T. pluriflora* (2.42 and 1.27 mg DSB in control and osmotic stress, respectively) than in *T. crinita* (1.51 and 1.40 mg DSB under control and osmotic stress conditions). Seed weight was positively correlated with FSB and DSB regardless of the effect of stress in *T. crinita* (Figure 5), but not in *T. pluriflora*. IEP and FEP were highly correlated with each other ($r > 0.9$), as were FSB and DSB.

Discussion

Much of the seed weight variation found in the two species of *Trichloris* was attributed to intraspecific rather than interspecific variation. In *T. crinita*, differences among populations in seed weight for seeds produced in a common garden were highly correlated with that for seeds collected from the wild. This indicates that in this species, there is a large genetic component to the variation in seed weight between populations expressed in the wild, assuming that maternal effects carried over from plants in the wild are negligible. However, the same was not evident in *T. pluriflora* where variation for the two types of seed weight was not correlated. Thus, in this species, genetic variation between populations for seed weight in the common garden was not correlated with variation expressed for the trait in the wild.

The possibility that variation in seed weight among populations of *T. crinita* is adaptive and a product of natural selection is supported by the significant correlations recorded for this species between seed weight and several ecogeographical variables. Thus, inherited seed weight variation was positively correlated with latitude and altitude and negatively correlated with annual average temperature, maximum temperature for the warmest month and rainfall during the wettest month. These correlations might indicate that large seeds are favoured at sites where rainfall is more limited during the growing season and where overall aridity is greater as in sites at more southern latitudes in the region studied. Similar associations have been reported for other grassland species elsewhere (Pluess et al. 2005; Dainese and Sitzia 2013) and are in accordance with studies that have detected a relationship between seed weight and latitude and drought for grassland species in different communities around the world (e.g. Baker 1972; Moles et al. 2007; Pakeman et al. 2008; but see Parra-Quijano et al. 2012). It is possible, therefore, that larger seed weight associated with reduced temperature and rainfall during the growing season, and increased altitude and latitude, provides an important resource for breeding programmes aimed at poor-quality environments.

Interestingly, in *T. pluriflora*, seed weight was also positively correlated with altitude and negatively correlated with annual average temperature and maximum temperature for the warmest month, which might indicate that larger seed of this species is favoured at sites where temperatures are lower. However, in the absence of a correlation between the seed weight of populations expressed in the common garden and that expressed in the wild, there is unlikely to be a genetic basis to the correlations recorded between seed weight and environmental variables in *T. pluriflora*. Thus, in this species, the variation in seed weight expressed in the wild, though possibly adaptive, may be mainly environmentally induced and not shaped by natural selection.

Seed weight prediction from ecogeographical variables provides valuable information not only about the association of seed weight to environmental parameters of the original habitat but further represents an advance in developing collecting strategies focused on germplasm
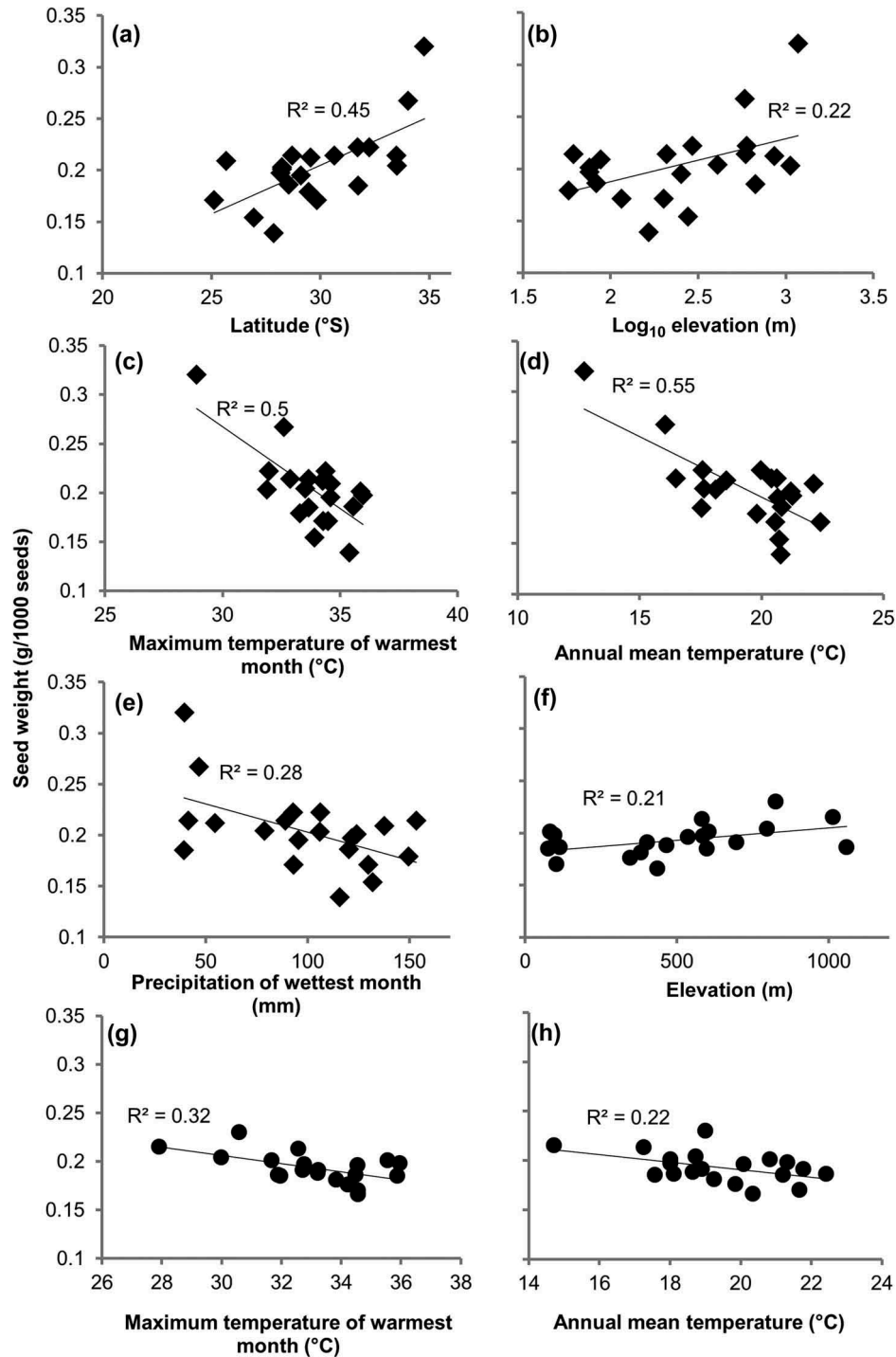
Figure 4. Relationships between seed weight from common garden and ecogeographical variables from collecting site of populations. For *T. crinita*: (a) latitude, (b) altitude, (c) maximum temperature for the warmest month, (d) annual average temperature and (e) rainfall during the wettest month. For *T. pluriflora*: (f) altitude, (g) maximum temperature for the warmest month and (h) annual average temperature. Trend lines and coefficients of determination (R^2) are shown for statistically significant correlations only.

with a desired seed weight. Several studies have used multivariate statistics to predict an organisms' responses to different habitat parameters (Gaudet and Keddy 1988; Hector et al. 1999; Conti and Díaz, 2013; Adler et al. 2014). In this study, the R^2 values for each analysis reveal a good fit (better for *T. crinita* than for *T. pluriflora*),

suggesting a high prediction capacity that can be validated for both species through future germplasm collections.

The possible advantage of the production of large seeds in more stressful sites, and the larger seedlings they produce, may be related to greater carbohydrate reserves contained in the endosperm and embryo
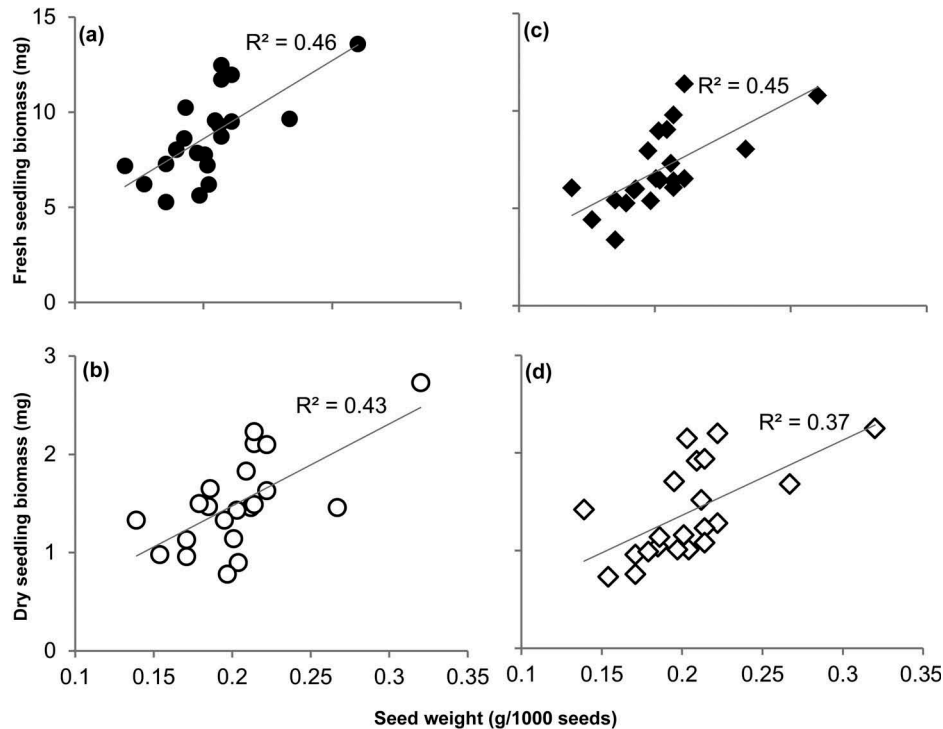
Figure 5.   Correlation between seed weight from common garden and seedling biomass in *T. crinita*, for control, no-stress treatment [closed symbols]: (a) fresh seedling biomass (FSB), (b) dry seedling biomass (DSB); and under osmotic stress (120 mM NaCl solution) [open symbols]: (c) FSB, (d) DSB. The trend line and coefficient of determination (R^2) are given for each scatterplot. Only statistically significant correlations are shown.

(Kidson and Westoby 2000), especially in grasses that have a lower embryo/endosperm ratio (Martin 1946). It has been reported that heavier seeded populations of *T. crinita* from stressful environments exhibited better germination behaviour under suboptimal temperatures (Zabala et al. 2011). There is also consistent evidence that larger seeded populations have an advantage during germination stages under abiotic stress conditions (Leishman et al. 2000; Westoby et al. 2002). In the present study, we confirmed that larger seeds improved germination ability through seedling initial growth under both stressful and non-stressful conditions for *T. crinita* but found no correlation between seed weight and germination ability in *T. pluriflora* in either set of conditions.

An increase of seed weight due to artificial selection has been shown to be important often in crop and forage species domestication (Doganlar et al. 2000; Hancock 2005; Orsi and Tanksley 2009; Adler et al. 2014) and plant breeding programmes (Vogel 2000; Casler and Van Santen 2010). A requirement for this is the presence of genetic variation for seed weight in the starting population. In the present study, we have laid the groundwork for future *Trichloris* breeding programmes, through the identification of genetic variation for seed weight in *T. crinita* which may be useful for restoration and cultivation purposes. In this regard, it is worth noting that local adaptation is not a property of a single population but of a group of populations located in different

environments (Lascoux et al., 2016). In this study, a common-garden experiment helped us identify differentiation among populations in seed weight, a fitness-related trait that in *T. crinita* showed a pattern of local adaptation. However, we do not know the consequences of adaptive traits for fitness in other habitats and whether local germplasm performs well in both its original site and other sites. Experiments using a reciprocal-transplant design can help elucidate specific patterns, such as costs and trade-offs in local performance, and how they relate to the ecological history of the seed source (Wettberg et al., 2016). Such experiments conducted in contrasting environments are already under way for our species.

Supplementary material

Supplemental data for this article can be accessed here.

Notes on contributors

Lorena Marinoni is a lecturer in plant and animal breeding and uses GIS tools in studies on native plant genetic resources and improvement of native forage species.

Juan Zabala lectures on genetics and plant and animal breeding and is the curator of the germplasm bank of UNL. His research focuses on native grasses and legumes with potential forage value.

Mauricio Parra-Quijano applies ecogeographical principles in collecting, conserving and using agrobiodiversity.

Roberto Fernandez lectures on ecology, biodiversity and environment and his research is centred on the effects drought and grazing on plants and ecosystems, desertification and human impacts on carbon and water cycling.

José Pensiero is a specialist in agrostology.

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