

Inter- and Intraspecific Variation in the Germination Response to Light Quality and Scarification in Grasses Growing in Two-phase Mosaics of the Chihuahuan Desert

FABIANA PEZZANI and CARLOS MONTAÑA*

Departamento de Biología Evolutiva, Instituto de Ecología A.C., Antigua Carretera a Coatepec km 2.5, Xalapa 91070, Veracruz, México

Received: 13 July 2005 Returned for revision: 2 December 2005 Accepted: 30 January 2006

• **Background and Aims** In many locations, plants are faced with adjacent, contrasting environments, and the between-species differential evolution of life history traits can be interpreted as an evolutionary response to this environmental heterogeneity. However, there has been little research on the intraspecific variability in these attributes as a possible evolutionary response of plants.

• **Methods** In the two-phase mosaic of the Chihuahuan Desert (adjacent patches with contrasting resource availability), analyses were carried out of the germination response to the scarification and light quality to which grass seeds growing on these patches are exposed (open and closed habitats).

• **Key Results** Species that grow in open habitats exhibited a higher germination success than those from closed habitats after scarification. At both the inter- and intraspecific level, there were differences in the germination percentage and in the germination speed in response to light quality. Intraspecific variation in the species from the closed habitat (*Pleuraphis mutica* and *Trichloris crinita*) and in *Chloris virgata* (which grows in both habitats) was due to genetic variation (the family factor was significant), but there was no genetic variation in phenotypic plasticity (non-significant interaction between family and light quality). In contrast, for the species that grows only in the open habitat (*Dasyochloa pulchella*), the family did not have a significant effect, but there was genetic variation in the phenotypic plasticity (significant interaction between family and light quality).

• **Conclusions** In *C. virgata*, *P. mutica* and *T. crinita*, natural selection could be favouring those genotypes that responded better in each light environment, but it is not possible that the natural selection resulted in different optimal phenotypes in each habitat. On the contrary, in *D. pulchella*, selection could have reduced the genetic variation, but there is the possibility of the evolution of reaction norms, resulting in the selection of alternative phenotypes for each habitat.

Key words: Arid zones, *Chloris virgata*, *Dasyochloa pulchella*, environmental heterogeneity, germination, inter- and intraspecific variation, light quality, phenotypic plasticity, *Pleuraphis mutica*, reaction norm, scarification, *Trichloris crinita*.

INTRODUCTION

In many sites [e.g. gaps in forests and vegetation bands (VBs) in arid zones], plants are faced with the presence of adjacent, contrasting environments, and the between-species differential evolution of life history traits could be interpreted as an evolutionary response to this environmental heterogeneity (Stearns, 1992; Grime, 1994; Bazzaz, 1996). However, the intraspecific variability in these attributes, as another possible evolutionary response in plants that could give them a certain degree of specialization at the micro-environmental level or as a local adaptation, has not been explored in much detail (Stewart and Schoen, 1987; Argyres and Schmitt, 1991). Although several studies have reported interspecific differences in sensitivity to light during the germination stage (Silvertown, 1980; Ballaré, 1994; Olf *et al.*, 1994; Vázquez-Yanes and Orozco-Segovia, 1994; Bazzaz, 1996), little is known about intraspecific differences in response to light, though such differences have been found in the germination response to water availability (Clauss and Venable, 2000) and to scarification and seed age (Gasque and García-Fayos, 2003). Variation in seed germination in relation to light can be considered an

evolutionary response of plants to the shade produced by vegetation (Smith, 1982), i.e. a set of adaptive traits that allow them to germinate under different light conditions. Among these adaptive traits, the seed coats and other seed structures can influence the quantity and quality of light that is absorbed, so removing these structures (scarification) will affect seed germination (Tian *et al.*, 2002; Gasque and García-Fayos, 2003).

When the light conditions are not favourable for germination – either because the seeds are too deeply buried or because they are shaded by vegetation – phytochromes may induce dormancy in the seeds (Fenner, 1985; Orozco-Segovia and Vázquez-Yanes, 1992). Pons (1992) asserts that the ultimate effect of light on seeds depends on the genotype and on the environmental conditions during seed maturation and dormancy.

In some arid and semi-arid zones of the world, the vegetation cover is discontinuous and forms a two-phase mosaic in which there are patches of vegetation scattered throughout a matrix of scant or no cover at all (Valentin *et al.*, 1999; Tongway *et al.*, 2001). In the Southern Chihuahuan Desert, Mexico, part of the vegetation of the Mapimí Biosphere Reserve forms parallel bands that alternate with open areas. These VBs are perpendicular to the slope and are

* For correspondence. E-mail montana@ecologia.edu.mx

the result of the combination of climatic, geomorphological and soil factors, although the structuring factor for these communities is the process of rainwater redistribution by runoff (Cornet *et al.*, 1992). Structural changes in the vegetation towards the interior of the VB have been interpreted as the result of a successional process where early successional stages occur in the upslope fringe while the mature stages occur in the central zone (Montaña, 1992; Vega and Montaña, 2004).

The VB represent patches with dense vegetation cover and more resources (water and nutrients) than the adjacent habitat where there is little or no plant cover. This difference in vegetation cover brings with it differences in the quality of the light reaching the soil: inside the VB (closed habitat), the light that reaches the soil is rich in far-red wavelength owing to the filtering that occurs when the light passes through the vegetation canopy, in contrast to the light that reaches the virtually bare soil of the zone between the VBs and in the upslope fringe of the VB (open habitat). Several species of grasses grow in this two-phase mosaic, some of which are exclusive to the upslope fringe (open habitat; *Dasyochloa pulchella*), or can be found in both habitats (*Chloris virgata*), while others appear almost exclusively in the interior of the VB (closed habitat; *Trichloris crinita* and *Pleuraphis mutica*). This spatial segregation contradicts the inference that the relative immobility of plants and the widespread environmental heterogeneity would allow for the evolution of sufficient plasticity to grow satisfactorily in a wide range of conditions (Bradshaw, 1965; Bell and Lechowicz, 1994).

The objective of this study is to analyse the inter- and intraspecific variation of the germination of grasses from open and closed habitats in response to scarification and light quality. An attempt was made to answer the following questions. (a) Does light quality matter to germination of grasses of open and closed habitats, and if so, is the effect important at the inter- or intra-specific level? (b) Are there differences due to seed coats that influence germination of grasses from open and closed habitats? A first experiment was carried out to evaluate whether there is variability among species in their germination response to light quality and to scarification. Based on the results of this first experiment, which confirmed the benefit of scarification and a differential response of the species to light, a second experiment (using only scarified seeds) assessed if there was intraspecific variability in germination as a response to light quality, and if that eventual variability was due to genotypic variation or the expression of alternative phenotypes better adapted to each type of habitat. In other words, we explored the possibility of the evolution of the reaction norms (Via, 1994) in germination variables under environmental heterogeneity – a topic that is poorly understood (Mathias and Kisdi, 2002).

MATERIALS AND METHODS

Species used in this study

The species selected for this study were: *D. pulchella* (Kunth) Willd ex Rybd. (representative of the open habitat,

colonizer), *C. virgata* Sw. (it can grow in both habitats but grows preferentially in the open habitat), and *P. mutica* Buckley and *T. crinita* (Lag.) Parodi (characteristic of the closed habitat, late-successional species). All four belong to the Poaceae family, Chloridoideae sub-family (Clayton and Renvoize, 1986). The sub-family Chloridoideae is characterized by well developed Kranz (C₄) anatomy, an advantage in hot, dry climates. *Chloris virgata* and *D. pulchella* (open habitat) are stoloniferous, with an erect growth habit, while *P. mutica* and *T. crinita* (closed habitat) have rhizomes with very short internodes, resulting in a tufted growth habit. With respect to life cycle, *C. virgata* is an annual, while *D. pulchella*, *P. mutica* and *T. crinita* are perennial. These species flower and fruit towards the last 2 months of the rainy season which starts in June and ends in September. The four species evaluated have bisexual plants, with bisexual spikelets with hermaphrodite florets, and they can produce seeds both by outbreeding and by inbreeding (Watson and Dallwitz, 1992).

Seed collection

The seeds of the four species were collected in September 2001 in the Mapimí Biosphere Reserve [26°N, 103°W; 1100 m above sea level; 264 mm annual rainfall, 80.2 % of which falls between June and October; 20.8 °C mean annual temperature; Cornet (1988)].

Mature inflorescences were collected from ten plants of each of the four species in ten randomly selected VBs. The seeds from a given plant are considered to belong to the same genetic family because they are at least half-siblings given that they have the same mother (we do not know the paternity of these seeds as they can be produced by inbreeding or outbreeding). All seeds were stored in a dry environment at 4 °C.

Germination conditions

The seeds were sown in Petri dishes using 2 % agar as a substrate, in a growth chamber (Lab-Line Biotronette, Melrose Park, IL, USA). The germination conditions were 14 h of light at 33 °C and 10 h of darkness at 27 °C. Micostatin (Nistatina 500 000 U, 1 g L⁻¹) and chloramphenicol (2 mL L⁻¹) were added to the agar to prevent contamination by fungus and bacteria. Seeds were sown under sterile conditions in a flow chamber. All seeds used were from the same harvest and were stored for 8 months for the first experiment and 9 months for the second.

Interspecific variation of germination response to scarification and light quality

The effect of light quality and seed scarification on germination was evaluated for four species of grasses. Three levels of light were used: white light to simulate the conditions of exposed seeds in bare soil (open habitat), light rich in far-red light to simulate the conditions beneath a herbaceous layer characteristic of the interior of the VB (closed habitat), and complete darkness to simulate the light environment of deeply buried seeds. The Petri dishes

were lined with blue and red Lee filters (numbers 071 and 182, respectively) to create light rich in the far-red frequency. The proportion of red/far-red was 0.08 and the photosynthetic photon flux density in the 400–750 nm range was $0.51 \mu\text{mol m}^{-2} \text{s}^{-1}$ in the far-red light treatment. The proportion of red/far-red light (655–665 nm/725–735 nm) created by the filters was estimated using a SKR-100 radiometer (Skye Instruments, Llandrindod Wells, UK). To achieve complete darkness, Petri dishes were covered with two layers of aluminium foil.

The effect of scarification was evaluated by sowing complete caryopses (seeds covered by the lemma and the palea, without scarification) and seeds that had been mechanically scarified with sandpaper. This resulted in six treatments per species (three light conditions and two scarification treatments), with five replicates per treatment. There were a total of 50 seeds per treatment per species (five Petri dishes with ten seeds each). For each species, a mixture of seeds from ten different mother plants was used. Germination percentage (G) was recorded 20 d after sowing.

Intraspecific variation of germination in response to light quality

Intraspecific variation in germination was evaluated under the light conditions found in the two zones of the VB: white light (open habitat) and far-red light (closed habitat). For each of the four species, previously scarified seeds from ten mother plants (ten genetic families) were sown. There were five replicates for each of the two light treatments (Petri dishes with ten seeds each) for a total of 50 seeds per light treatment per genetic family and 500 seeds per light treatment per species. The number of seeds that had germinated (foliar or radical structure visible) was counted each day for the 20 d following sowing. At the end of the experiment, we calculated G and the germination speed (M , seeds d^{-1} ; Maguire, 1962). The latter was calculated as: $M = \Sigma (n_i/t)$, where n_i is the number of seeds that have germinated on the i th day, and t is the time elapsed from the sowing until the germination of the last seed.

Data analysis

Germination percentages were arcsine transformed and M values were transformed by the Box-Cox method using the JMP software package to normalize data distribution (SAS Institute Inc., 1995, Version 3.1).

For the first experiment, the variability of G as a function of species (four levels), scarification (scarified and not scarified) and light condition (white light, far-red light and darkness) was analysed using a three-way analysis of variance (ANOVA) with the software program JMP 3.1 (SAS Institute Inc., 1995). Tukey’s multiple comparison of means was carried out to identify significant differences between means for species and light treatments.

In the second experiment, we analysed the variability of G and M as a function of species (four levels), genetic family (nested within species, with ten levels) and light conditions (white light and far-red light), using a nested two-way ANOVA (for each response variable separately) run on JMP 3.1 (SAS Institute Inc., 1995). For each response

TABLE 1. Analysis of variance of the germination percentage as a function of species (*C. virgata*, *D. pulchella*, *P. mutica* and *T. crinita*), scarification treatment (scarified vs. non-scarified control) and light conditions (white light, far-red light and darkness)

Source of variation	d.f.	Sum of squares	Explained variance (%)	<i>F</i>	<i>P</i>
Light quality (L)	2	0.509	2.1	8.559	0.0004
Scarification (Sc)	1	11.682	48.8	393.117	<0.0001
Species (S)	3	6.085	25.4	68.256	<0.0001
Sc × S	3	1.771	7.4	19.861	<0.0001
L × S	6	0.457	1.9	2.561	0.0241
L × Sc	2	0.169	0.7	2.845	0.0630
L × Sc × S	6	0.390	1.8	2.187	0.0508
Error	96	2.853	11.9		
Total	119	23.914			
Full model	23	21.062	88.07	30.816	0.0001

variable, reaction norms were then analysed graphically for each of the ten genetic families. A two-way ANOVA with the factors genetic family and light was carried out to evaluate genetic variation in phenotypic plasticity (given by the genotype × environment interaction; Via and Lande, 1985; Via, 1994) for each response variable and for each species. Finally, in order to estimate the degree of dependence of character states on environmental variability, the genetic correlation (r_G ; Falconer, 1952; Via and Lande, 1985; Via, 1994) for each species and for each of the two response variables (G and M) was calculated.

Given that r_G depends on the heritability of the character and on the effect of the environment (Schlichting and Pigliucci, 1998), estimated heritability (H^2) for G and for M for each species was estimated as $H^2 = 2t$ (modified from Roff, 1997). In this equation, t is the intra-class correlation coefficient, and we assumed that H^2 is an estimation of heritability in a wide sense since seeds that were collected in the field were used and no crossbreeding experiments were carried out to control the paternity of the seeds. The standard deviation of H^2 was calculated following Robertson’s (1959).

RESULTS

Interspecific variation of germination response to scarification and light quality

The three factors analysed (i.e. species, scarification and light quality) had a significant effect on G , scarification and species being the most important factors explaining the total observed variation (Table 1). Only $28.25 \pm 3.51\%$ s.e. of the non-scarified seeds germinated, while $75.17 \pm 2.18\%$ s.e. of the scarified seeds did so. The scarification × species interaction was significant and, as seen in Fig. 1, there was a marked increase in the germination of the open habitat species, *D. pulchella* and *C. virgata* when their seeds had been scarified as compared with those that had not been scarified. In the species common to the closed habitat, *T. crinita* and *P. mutica*, germination also increased,

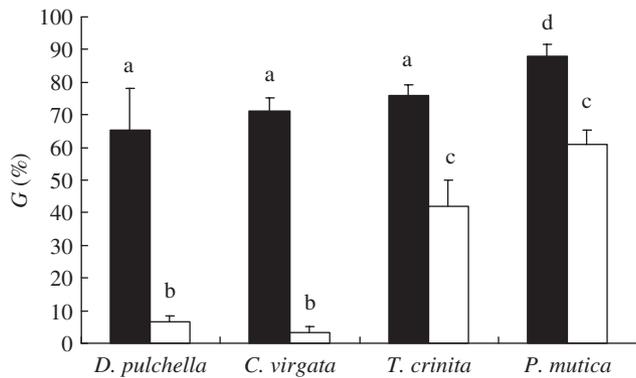


FIG. 1. Germination percentage (G , mean \pm s.e.) of scarified (closed bars) and non-scarified (open bars) caryopses of four grass species from the Chihuahuan Desert, Mexico. Different letters indicate significant differences ($P < 0.05$, Tukey test).

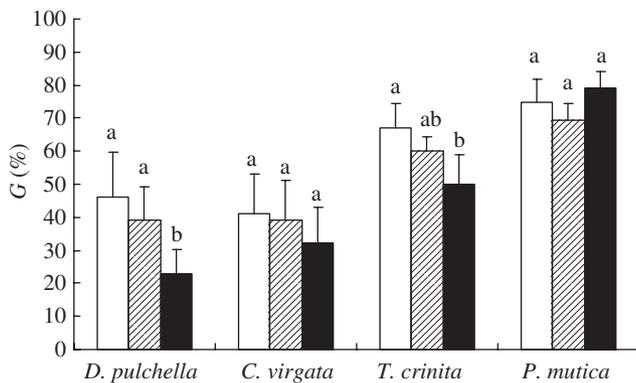


FIG. 2. Germination percentage (G , mean \pm s.e.) of four grass species (scarified caryopses) from the Chihuahuan Desert, México, under three light treatments: white light (open bars), far-red light (slashed bars) and complete darkness (closed bars). Different letters indicate significant differences between treatments for a given species ($P < 0.05$, Tukey test).

but to a lesser extent, when their seeds had been scarified, compared with the germination of intact seeds.

Even though the differences in germination success resulting from the three light treatments were significant, they only explained 2.1% of total variation. Seed germination was $57.25 \pm 5.48\%$ s.e. under white light, $51.88 \pm 4.64\%$ s.e. under far-red light and $46.00 \pm 5.28\%$ s.e. in darkness. The light \times species interaction was also significant: *D. pulchella* (open habitat) showed the greatest decrease in germination percentage in the dark and under far-red light (84.78 and 50% less, respectively, compared with germination in white light, Fig. 2). Given the statistical significance of the light \times species interaction, a one-way ANOVA was completed for each species and there were significant differences for the light treatments in *D. pulchella* ($F = 7.33$, d.f. = 2, 149, $P < 0.0001$) and in *T. crinita* ($F = 3.937$, d.f. = 2, 149, $P < 0.0001$). Tukey test showed that *D. pulchella* germination under white light and far-red light differed significantly from germination in the dark ($P = 0.004$ and $P = 0.02$, respectively), but there was no difference between germination under white light and far-red light treatments.

TABLE 2. Analysis of variance of the germination percentage as a function of species (*C. virgata*, *D. pulchella*, *P. mutica* and *T. crinita*), genetic family (ten families nested within each species) and light conditions (white light and far-red light)

Source of variation	d.f.	Sum of squares	Explained variance (%)	F	P
Species (S)	3	5.816	23.2	51.149	0.0001
Family [species] (F[S])	36	4.544	18.1	3.330	0.0001
Light quality (L)	1	0.412	1.7	10.881	0.0011
S \times L	3	0.179	0.7	1.576	0.1950
F[S] \times L	36	1.969	7.9	1.443	0.0537
Error	320	12.129	48.4		
Total	399	25.050			
Full model	79	12.921	51.58	4.315	0.0001

TABLE 3. Analysis of variance of the germination speed as a function of species (*C. virgata*, *D. pulchella*, *P. mutica* and *T. crinita*), genetic family (ten families nested within each species) and light conditions (white light and far-red light)

Source of variation	d.f.	Sum of squares	Explained variance (%)	F	P
Species (S)	3	5.795	13.3	26.783	<0.0001
Family [species] (F[S])	36	9.865	22.7	3.799	<0.0001
Light quality (L)	1	0.814	1.9	11.281	0.0009
S \times L	3	0.660	1.5	3.051	0.0288
F[S] \times L	36	3.338	7.7	1.286	0.1339
Error	320	23.079	52.9		
Total	399	43.549			
Full model	79	20.471	47.0	3.593	0.0001

For *T. crinita*, the Tukey test only revealed differences between germination under white light and germination in the dark ($P = 0.03$).

Intraspecific variation of germination in response to light quality

Species, genetic family and light quality all had a significant effect on G (Table 2). In this experiment, differences between species explained 23.2% of the total recorded variation while differences between families of the same species represented 18.1% of the variation. As in the first experiment, light quality had a significant effect but only explained a low proportion of the total variability (1.7%). Under white light, mean germination was $85.35 \pm 1.45\%$ s.e., while under far-red light germination was $81.1 \pm 1.93\%$ s.e.

There were significant differences between species in M , representing 13.3% of the observed variation (Table 3). Differences in this variable were observed not only at the species level, but also at the family level, and these differences accounted for 22.7% of the total variation. Light quality had a significant effect on M and accounted for 1.9% of the total observed variation.

Figures 3 and 4 show differences in the reaction norms in G and M , respectively, in response to the two light conditions. The slopes of the lines indicate the degree of

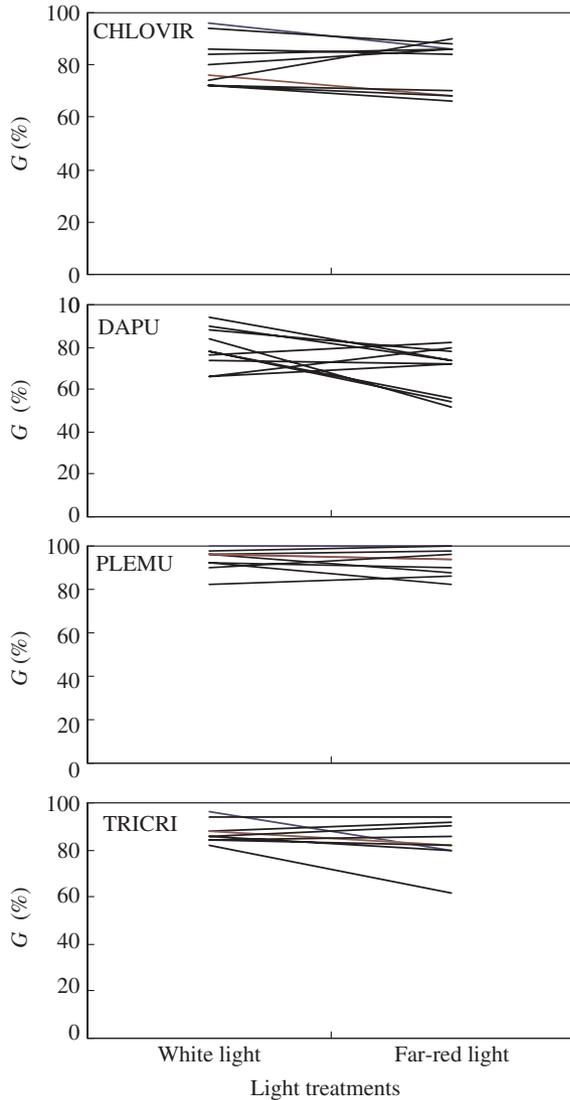


FIG. 3. Reaction norms of germination percentage (G) in response to two light treatments for the progeny of ten maternal families of *C. virgata* (CHLOVIR), *D. pulchella* (DAPU), *P. mutica* (PLEMU) and *T. crinita* (TRICRI).

phenotypic plasticity for each genotype (genetic family). Genotypes of open habitat grasses show both positive and negative responses to the environment (although the genotype \times environment interaction in *C. virgata* was not significant, see below). In contrast, most genotypes of grasses from closed habitats show similar responses to both light environments. The ANOVAs done on G for each species show that for *C. virgata*, *P. mutica* and *T. crinita*, the family factor was significant ($F = 5.477$, d.f. = 9, 99, $P < 0.001$; $F = 4.111$, d.f. = 9, 99, $P = 0.0002$; $F = 2.558$, d.f. = 9, 99, $P = 0.0123$, respectively), while the family \times light interaction was not. This means that there is genetic variation within each species and that there is a high correspondence between the phenotypic values (i.e. genotypes that have less germination success under white light are also less successful under far-red light), and this indicates that there is no genetic variation in the phenotypic plasticity (Via, 1994). In contrast, in

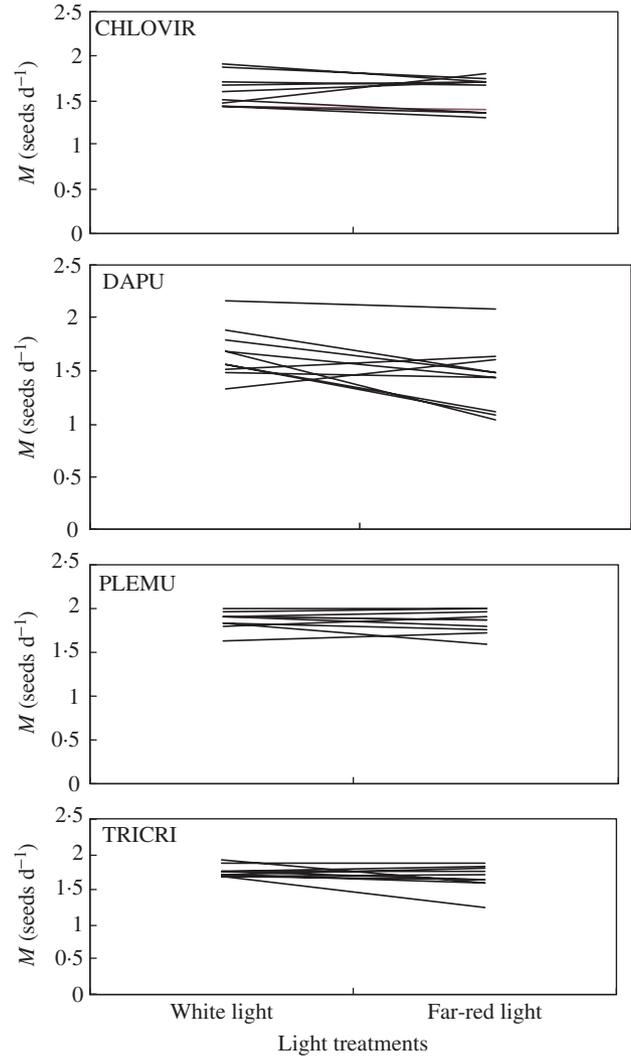


FIG. 4. Reaction norms of germination speed (M) under two light treatments for the progeny of ten maternal families of *C. virgata* (CHLOVIR), *D. pulchella* (DAPU), *P. mutica* (PLEMU) and *T. crinita* (TRICRI).

D. pulchella, one of the open habitat species, the family factor was not significant. However, for this species, light quality ($F = 10.7850$, d.f. = 1, 99, $P = 0.0015$) and the family \times light interaction ($F = 2.7729$, d.f. = 9, 99, $P = 0.0071$) were significant. Therefore, in *D. pulchella*, there is genetic variation in phenotypic plasticity (Via, 1994).

The values for the genetic correlations between the character states of G in each habitat in part support the results obtained with the ANOVA. In *D. pulchella*, this correlation was not significantly different from zero ($r_G = -0.047$, $P = 0.898$). This supports the existence of genetic independence and, as such, the possibility of the evolution of the reaction norm for this character under the two light environments considered (Via and Lande, 1985; Via, 1994). These correlations were different from zero ($r_G = 0.623$, $P = 0.044$ and $r_G = 0.742$, $P = 0.014$, respectively) in *C. virgata* and in *P. mutica*, confirming for these species the genetic dependence of these character states. In *T. crinita*, the correlation was not different from zero

TABLE 4. Heritability (\pm s.d.) of the germination percentage and of the germination speed of four grasses from the Chihuahuan Desert, México

Species	G	M
<i>Chloris virgata</i>	0.70 \pm 0.12	0.72 \pm 0.12
<i>Dasyochloa pulchella</i>	0.25 \pm 0.06	0.49 \pm 0.09
<i>Pleuraphis mutica</i>	0.59 \pm 0.11	0.47 \pm 0.09
<i>Trichloris crinita</i>	0.41 \pm 0.10	0.39 \pm 0.08

($r_G = 0.378$, $P = 0.282$) and, in contrast to the lack of statistical significance in the family \times light interaction for this species, this could indicate a certain genetic independence of these character states.

Heritability of G of *D. pulchella* is low (0.25 ± 0.06) and lower than in the other three species (Table 4). The H^2 values in the other three species are intermediate (*T. crinita* = 0.41 ± 0.10) or high (*P. mutica* = 0.59 ± 0.11 and *C. virgata* = 0.70 ± 0.12). These heritability estimations are in accordance with genetic correlation measures.

The ANOVAs for M in each species revealed that in each of the four species, the family factor was significant (*D. pulchella* $F = 3.777$, d.f. = 9, 99, $P = 0.0005$; *C. virgata* $F = 5.636$, d.f. = 9, 99, $P < 0.0001$; *P. mutica* $F = 2.956$, d.f. = 9, 99, $P = 0.004$; *T. crinita* $F = 2.536$, d.f. = 9, 99, $P = 0.013$), while the family \times light interaction was not significant for any species. Light was only significant in *D. pulchella* ($F = 9.366$, d.f. = 1, 99, $P = 0.003$).

The values of the genetic correlations between M in both habitats showed the same tendencies as the correlations between the germination percentages. Thus, in *D. pulchella* and in *T. crinita*, the correlation was not significantly different from zero ($r_G = 0.281$, $P = 0.432$ and $r_G = 0.356$, $P = 0.312$, respectively). In *C. virgata* and *P. mutica*, the genetic correlation between germination speed under white light and under far-red light was different from zero ($r_G = 0.695$, $P = 0.026$ and $r_G = 0.634$, $P = 0.049$, respectively). For all species, values for the H^2 of M were intermediate and high (Table 4).

DISCUSSION

The results of this study demonstrate that there are differences in the germination percentage in response to scarification among grasses from different habitats of a two-phase mosaic, and that there are both inter- and intraspecific differences in the germination percentage and in the germination speed in response to light quality. In addition, germination percentage and germination speed are plastic attributes regarding light quality in the germination environment. Responses to scarification were far more important than those to light, particularly in grasses from open habitat. These results would mean that dormancy or germination mechanisms that are responsive to environmental cues are almost non-existent in closed system grasses (*P. mutica* and *T. crinita*) whereas grasses from open areas (*C. virgata* and *D. pulchella*) have strong responses to environmental cues through a mechanism of

scarification in both species, and through different responses to light variation only in one of the species (*D. pulchella*). Apparently, the discrimination between germination conditions does matter more for grasses that grow in arid open areas than for grasses that grow in closed (shaded) systems, richer in nutrients and water which creates a more stable and less variable niche for germination (Donohue, 2005; Donohue *et al.*, 2005).

Interspecific variability of germination in response to scarification and light quality

Scarification increased germination in all species, but that increment varied between species of open and closed habitats (i.e. both species main factor and the interaction species \times scarification were significant). Light quality also influenced germination percentages (significant effect of the species factor), and the response to light treatment varied between species (significant light \times species interaction).

Under natural conditions, light is a factor that varies over time, and interacts with other factors that affect germination, and vary temporally, such as temperature and water availability (Pons, 1992; Vázquez-Yanes and Orozco-Segovia, 1994). In our experiments, light quantity, water and temperature were held constant and the possible effects of interactions among them were not evaluated.

The positive effect of removing the seed coats of the caryopses on the germination of *P. mutica* and *T. crinita* coincides with results of other studies on grasses with similar characteristics (tufted perennials; Simpson, 1990; Tian *et al.*, 2002; Gasque and García-Fayos, 2003). The greater response to scarification that occurred in the seeds of the species from the open habitat (*D. pulchella* and *C. virgata* had $<10\%$ germination when sown with complete caryopses) has been reported previously for the species of a grassland successional system where, in the more open, less productive sites, species germinated more slowly and responded better to scarification (Olf *et al.*, 1994). Gutterman (2000) proposed that in short-lived species that inhabit deserts, it is possible to find a set of plastic strategies in the germination phase that can extend the germination period. The dormancy that is induced by the seed coats of *C. virgata* and *D. pulchella* could be considered such a strategy.

According to our results, it is conceivable that there is some natural mechanism that scarifies *D. pulchella* and *C. virgata* caryopses. A possible explanation lies in the fluctuation of temperature, which is greater in the upslope of the VBs than in their interior owing to the greater proportion of bare soil in the former. This was also observed for other grass species in the Negev Desert (Israel) by Gutterman (2000), who suggests that the primary dormancy of seeds of *Hordeum spontaneum* and *Schismus arabicus* (Poaceae), that are found on the soil surface or in the soil seed bank, could be reduced by exposure to high temperatures during the hot, dry period. Pons and Schröder (1986) found that the combined action of thermal and light fluctuations can break dormancy in the seeds of *Fimbristylis littoralis* (Poaceae). Jurado and Westoby (1992) worked

with 105 species (32 of which were grasses) from the arid zone of central Australia and found that all species with low germination success tended to germinate much better once they had been scarified. They suggest that under natural conditions, the seed coats are broken in response to changes in temperature and humidity, or from friction as they move over the surface of the soil. In the VBs of Mapimí, both temperature fluctuations and the dragging of propagules over the soil by the runoff process could scarify the caryopses.

Far-red light slowed germination in the species of the open habitat particularly in the case of *D. pulchella* in which germination speed decreased 13.8% under this light condition. The germination timing could have important implications for the later development of the plants (González-Astorga and Núñez-Farfán, 2000; Adondakis and Venable, 2004; Donohue *et al.*, 2005). Jurado and Westoby (1992) proposed that desert plants in particular must germinate more quickly than those of humid zones because in arid zones water is not only scarce, it is also available for only very short periods. Therefore, germinating at the right time and quickly are critical factors for later survival and development of the seedlings (Shimono and Kudo, 2003) given that in the Chihuahuan Desert rain events are short, unpredictable and infrequent.

Intraspecific variability and phenotypic plasticity in germination attributes

The intraspecific variability in response to light treatments has different origins in the studied species. In *C. virgata*, *P. mutica* and *T. crinita*, the significance of the family factor indicates that there is genetic variation in the measured attributes. In contrast, in *D. pulchella*, this variability is due to phenotypic plasticity as indicated by the significant interaction between family and light and the lack of genetic correlation between the character states.

In *C. virgata*, *P. mutica* and *T. crinita*, natural selection in each light environment (open and closed habitats) could be favouring those genotypes with a greater germination percentage and/or those that germinate more quickly, taking into account the genetic variation within each of the species. Another possibility is that in these three species, natural selection is acting to maintain the high genetic variability observed, and this could represent an adaptation to the environmental heterogeneity in which these populations live. The limited genetic independence of the character states for the germination parameters evaluated suggests that it is unlikely for the reaction norms to have evolved in these species. In contrast, in *D. pulchella*, one of the colonizing species of the open habitat, the family factor was not significant, and this could indicate that natural selection has reduced the genetic variation in this species. Even so, for *D. pulchella*, there is the possibility of the evolution of the reaction norm for the capacity to germinate under different light conditions. The evolution of the reaction norms implies that phenotypic plasticity should be considered as a mechanism for the populations to produce alternative phenotypes fitted to the different environments. Assuming a stabilizing selection for some quantitative

characteristics, each phenotype could be selected towards an optimal mean value within each environment (Via, 1993). Therefore, there could be genotypes in *D. pulchella* that produce phenotypes for each of the light environments that are more specialized than those of the other three species. The heritability values support this interpretation of the results. Germination percentage in *D. pulchella* has a relatively low heritability (0.25) (cf. Mousseau and Roff, 1987; Roff, 1997), a value that is lower than that of the other three species. This supports the interpretation that in this species this attribute is a main fitness component, and has been under natural selection pressures. Similarly, in *Dicerandra linearifolia*, an annual, Winn (2004) found that attributes associated with germination (time to emergence, among others) have relatively low heritability values, indicating that this attribute has been a target for selection. In contrast, for the other three species, heritability for this character was high, suggesting that there is still sufficient genetic variance for natural selection to act upon, or that this attribute is neutral (Endler, 1986).

Heritability of the germination speed was intermediate to high, indicating that there is still genetic variance for natural selection to act upon. As such, we propose that the interspecific comparison of these values supports the results of the genetic correlations of the characters. The results on the differential effect of natural selection, both inter- and intraspecific, allow the construction of hypotheses about the action of selection in heterogeneous environments (García-Dorado, 1986).

We were not able to separate maternal effects in H^2 estimations, although we know that environmental maternal effects may provide phenotypic adaptation to local environmental conditions (Galloway, 2005). However, in any case, even using such a wide estimation of heritability, clear differences between species heritabilities could be detected.

As indicated by the 'bet-hedging' theory (Philippi and Seger, 1989; Philippi, 1993; Clauss and Venable, 2000) and in accordance with the results reported by Adondakis and Venable (2004) for annual species of the Sonoran Desert, the colonizer species in the present study had a low germination percentage. According to the 'bet-hedging' theory, viable seeds that do not germinate could represent a strategy to lengthen the germination period, thus increasing the probability that the seedlings encounter better conditions for their survival and development, considering the high variability and unpredictability of precipitation in arid zones (Philippi, 1993). An alternative explanation for the low germination in colonizer species (especially *D. pulchella*) is that those genotypes whose seeds are limited in their capacity to germinate under a vegetation canopy could represent a life history trait that favours dispersal and, as such, colonization, since the seeds need to be transported far from the mother plant and this would allow them to colonize new sites.

The intraspecific variability found in the germination percentage and in the germination speed shows that there are genotypes and/or phenotypes that can deal with the differences resulting from the environmental heterogeneity that occurs in the two-phase mosaic of the Chihuahuan

Desert. Dispersal plays a still unknown role because it may occur both between VBs and between zones within a VB, and most probably the dispersal capacity differs between species. Species with greater dispersal capacities may be more likely to consist of plastic individuals rather than locally specialized ecotypic populations (Sultan and Spencer, 2002).

ACKNOWLEDGEMENTS

We thank the Herrera family in the Mapimí Biosphere Reserve for facilitating our fieldwork. We thank L. E. R. Martínez for his assistance in the laboratory work. Jorge González-Astorga, Juan Núñez-Farfán and Joel Flores Rivas provided valuable comments on the manuscript. F.P. is indebted to the Universidad de la República, Uruguay for their logistic support and Red Latinoamericana de Botánica for the granted stipend (RLB-01-D1; Tyler Prize 2004). This work was partially funded by RLB-01-D1 (F.P.) and by CONACYT-36642-V (C.M.).

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