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STRONG PHENOTYPIC VARIATION IN FLORAL DESIGN AND DISPLAY TRAITS OF AN ANNUAL TARWEED IN RELATION TO SMALL-SCALE TOPOGRAPHIC HETEROGENEITY IN SEMIARID CHILE

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Floral trait expression in wild populations varies in relation to environmental gradients. Variation can be observed among and within plant populations. We investigated the changes in floral phenotype within populations and the probability of plant pollinator visits in relation to small-scale variation in soil moisture and plant cover in a semiarid ecosystem. We measured the variability of floral phenotypes of three wild populations of *Madia chilensis* along a gradient constituted by three topographic positions (south-facing slope, north-facing slope, and ravine). Changes in soil moisture, plant density, leaf water content, and internode elongation were measured for one population over two study years. Pollinator visit probabilities were also estimated. Floral phenotypes were strongly segregated among topographic positions but less segregated among populations. Plants with the lowest water contents and the smallest or least-conspicuous flower heads grew in the drier north-facing slope, while plants with the highest water contents and showier flower design and display grew in the wetter ravine. Probabilities of pollinator visits of *M. chilensis* were strongly connected to topographic variations at small spatial scale. Topographic heterogeneity at small spatial scale could thus have important consequences for floral trait evolution and pollination ecology.

Keywords: Madia chilensis, Asteraceae, Mediterranean ecosystems, floral traits variation, small-scale heterogeneity, drought.

Online enhancement: appendix.

Introduction

Floral features in angiosperms show wide variation among species and populations (Galen 1999; Herrera 2005, 2009; Pérez Barrales et al. 2009; Schlumpberger et al. 2009). Variation of pollinator-related floral traits of plants can be observed at the flower level, as corolla size, shape, color, and symmetry (hereafter "design" traits), and at the whole-plant level, as number of simultaneously open flowers per plant, inflorescence architecture, and total plant height at flowering (hereafter "display" traits). Because of their role as attractive cues (Stanton et al. 1986; Johnson and Dafni 1998; Ohashi and Yahara 1998; Suárez et al. 2009), floral traits have been considered as key factors influencing pollinator visits in animalpollinated plants (Eckhart 1991; Dafni and Kevan 1997; Pellmyr 2002). Variation of floral traits within populations is the raw material on which pollinator-mediated natural selection can operate, promoting adaptive evolution (Endler 1986).

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Floral trait expression can be affected by many environmental factors, both physical and biological (Frazee and Marquis 1994; Vogler et al. 1999; Elle and Hare 2002; Herrera 2005; Burkle and Irwin 2009; Suárez et al. 2009). Soil water availability is a particularly strong regulator of the floral phenotype in wild populations (Galen 1999). During bud development and bloom, cell expansion and turgor are indispensable and require constant movement of water from vegetative to reproductive parts of the plant (Mohan Ram and Rao 1984). Accordingly, soil moisture levels should influence plant size and flower traits via plant water status (Carroll et al. 2001). When experimentally subjected to water shortage, plants often produce smaller flowers and less conspicuous floral displays compared with well-watered ones (e.g., Carroll et al. 2001; Elle and Hare 2002; Caruso 2006). Floral phenotypes can also be affected by plant density. Steets et al. (2006) reported that as the neighborhood becomes more crowded, each individual plant should produce smaller and fewer flowers (but see Cresswell et al. 2001). Despite water requirements, the maintenance of specific floral design and display during flowering time may be critical (Galen 1999; Galen et al. 1999), and water loss via flower transpiration is a major constraint on reproduction in water-limited environments (Nobel 1977; Whiley et al. 1998). However, field

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studies addressing the ecology of floral trait expression have overlooked this connection and its ecological consequences. To our knowledge, relationships between plant water status and crowding on floral expression have not been assessed in field studies. Grime (1979) proposed that sparse plant populations tend to be more affected by interactions with other organisms and/or their abiotic environment than crowded populations, where plant-plant interactions should be more influential. Under this view, it can be expected that water status should be a stronger driver than plant density for floral trait expression, particularly in semiarid environments.

Spatial variation in the biotic (e.g., plant-plant interactions, plant-animal interactions) and abiotic (e.g., soil moisture, light) environments surrounding individual plants will promote strong spatial variation of the floral phenotype (Galen 1999; Caruso et al. 2003; Schlumpberger et al. 2009). Concomitant effects on pollination ecology may result from such differences, as indirect consequences of floral variation on visitor identity and behavior, or separately, as environment direct effects on pollinator activity. It has been reported that pollinator identity and behavior may vary even at small spatial scales, for instance, within plant populations or at nearby sites (Herrera 1995, 1997; Rozzi et al. 1997; Kilkenny and Galloway 2008), thus locally shaping small-scale differences of plantpollinator relationship and selective regimes. Despite this, most studies addressing variation in pollinator-related floral design and display traits have assessed differences over large geographic scales, usually comparing plant populations separated by several kilometers (Herrera 2005; Bull-Hereñú and Arroyo 2009; Herrera 2009; Pérez-Barrales et al. 2009; Schlumpberger et al. 2009). In contrast, small-scale spatial patterns of floral variation and pollination ecology (within or between populations) are still insufficiently documented but growing (Herrera 1995; Rozzi et al. 1997; Petit and Thompson 1998; Galen 1999; Franke et al. 2006; Hansen and Totland 2006; Kilkenny and Galloway 2008).

In semiarid ecosystems, one of the main environmental features (Breshears et al. 1998; Aguiar and Sala 1999) is related to physical heterogeneity at spatial scales of few meters (e.g., Monson et al. 1992). Mediterranean-type ecosystems in central Chile show pronounced differences in soil water storage associated with marked contrasts of sunlight incidence and mean temperature between slopes of opposite aspect (Parsons 1973; Armesto and Martínez 1978; Rozzi et al. 1989). In addition, strong temporal variation in water availability for plant growth also occurs among years due to the El Niño Southern Oscillation (ENSO), which can cause periodical increases in precipitation of at least four times the annual average (Allan et al. 1996). Both temporal and spatial variation in semiarid environments can strongly affect the distribution and abundance of organisms in the community and in populations, even at the scale of meters (Armesto and Martínez 1978; Rozzi et al. 1989, 1997; Gutiérrez et al. 2000). It is well known (Gutiérrez et al. 2000) that annuals and geophytes are present at higher ground cover and greater densities on south-facing slopes than on north-facing ones, where incident radiation is much higher and lasts longer during daylight. Moreover, this spatial difference can be enhanced in dry years. It is likely that floral variation and evolution are being affected by such spatial heterogeneity, particularly regarding soil humidity and plant cover in semiarid ecosystems.

Madia chilensis (Asteraceae) is a Chilean native annual tarweed that shows remarkable levels of variability in both vegetative and floral traits among and within populations in the wild. This species has broad habitat requirements and hence is one of the few that inhabits both south- and north-facing slopes as well as more sheltered ravines in semiarid central Chile. To assess how the floral phenotype of M. chilensis varies across the small-scale spatial gradient constituted by the south-facing slope, the north-facing slope, and the adjacent ravine that separates them, we sampled floral trait variation across the entire range of topographic positions within three independent populations (separated from each other by 1-1.5 km) in semiarid Chile. To assess the possible mechanisms behind the observed patterns of floral variation across topographic positions, we focused on one of the three populations. For this population, we compared measurements of soil water availability, plant density, plant water status, and plant crowding index among the three topographic positions over two study years. In addition, we examined the association of floral trait variation with individual plant water status and a crowding index measured for all topographic positions in both years. Finally, to explore possible consequences of this small-scale topographic heterogeneity for M. chilensis pollinators, we estimated plant visitation probabilities within one population in each slope and the adjacent ravine bottom. Specifically, we asked the following questions: (1) Does M. chilensis floral phenotype differ among topographic positions and between successive years within populations? (2) Does individual water status of plants and population crowding index vary according to soil moisture and plant density changes across topographic positions and between years? (3) Do floral traits-plant water status and floral traits-crowding index correlations differ in magnitude and/or sign among topographic positions? And finally, (4) Do pollinator visit probabilities differ among topographic positions? We expect to find marked differentiation of floral phenotypes amongtopographic positions, with plants displaying few and smaller flowers in north-facing slopes, which should be the most xeric condition (lowest soil moisture). We also expect that the relationship between plant water status and floral expression will be stronger on the more xeric north-facing slopes at the same time that the relationships between crowding index of plants and floral expression will be stronger in the more crowded habitats (south-facing slopes and/or ravine bottoms). Finally, probabilities of pollinator visits should be higher in the south-facing slopes and/or ravine bottoms, where plant density should be higher than on the more xeric north-facing slopes.

Material and Methods

Study Site and Species

The study site was located in Las Chinchillas National Reserve (31°29'S, 71°08'W, 600–700 m elevation), in the locality of Aucó, 17 km from Illapel, in semiarid Chile. Rainfall is sporadic, falling exclusively in the austral winter because of the Mediterranean climate influence. The 90-yr average of annual rainfall is 189 mm, with high interannual variability (Jaksic 2001). During the past 30 yr, a minimum of 20.3 mm yr⁻¹ (1999) and a maximum of 505 mm yr⁻¹ (1987) have been recorded. In wet years, annual rainfall can be up to 300% of the long-term average due to the strong effects of El Niño Southern Oscillation on the local climate (Jaksic 2001). Annual rainfall did not show significant variation between the two years of this study. Total annual rainfall was 109.8 mm in 2008 (monthly distribution, 4 mo) and 115.8 mm in 2009 (distributed over 5 mo; data from Aucó weather station).

Madia chilensis Mol. (Asteraceae) is an annual tarweed that is endemic to Chile and is distributed between 29°54'S and 45°34'S. This species is a common herb that inhabits mostly open landscapes of Chilean matorral and grazed anthropogenic prairies in Mediterranean-type ecosystems (Matthei 1995; Hoffmann 1998). Its leaves and stems are covered with biseriate glandular and uniseriate nonglandular trichomes. It has a yellow flower head (i.e., the reproductive unity in the Asteraceae that resembles the typical flower of other families; fig. 1A), with hermaphroditic florets forming a disk at the center and female ray florets arranged along the perimeter (Hoffmann 1998). The species shows remarkable levels of variability in floral traits (fig. 1B). In the study area, M. chilensis grows during the late austral winter (late Augustearly September) and disperses its seeds during spring (early November), for a total life span of about 2 mo. The end of its life cycle extends into the beginning of the dry period under the Mediterranean climate (Mooney and Dunn 1970; Di Castri and Hajek 1976). Although the focal species is selfcompatible and potentially autogamous, its flowers are visited by a diverse array of pollinators whose activities enhance seed set per plant by up to 70% (L. H. Suárez, unpublished data). In the study area, populations of M. chilensis were present on both south-facing (polar) and north-facing (equatorial) slopes (hereafter, SFS and NFS, respectively) and also occurred at the bottom of adjacent ravines, resulting in a fairly continuous patch over the three topographic positions.

Variation of Floral Design and Display Traits

We evaluated the effect of topographic position on floral design and display traits in three different populations (S1, S2, and S3) located at similar elevations but separated from each other by about 2 km, within Las Chinchillas National Reserve, Chile. The three populations were located on slopes with similar orientations, covering NFSs and SFSs and the bottoms of adjacent ravines (fig. 2). During the growing season of 2009, a total of 480 juvenile plants were randomly marked (S1, 300; S2, 90; S3, 90). Sample sizes within each population were evenly distributed over a similar area on the three topographic positions (SFS, NFS, and an adjacent ravine). At the peak flowering season, we photographed M. chilensis flower heads, using a scale metric reference to assess differences in floral design and display traits of marked plants. To assess flower design characters, at least three flower heads per plant were photographed. We measured total plant height at peak flowering and counted the total number of flower heads per plant as characters related to floral display. Traits representing floral design were measured in the lab from flower digital images, using SIGMASCAN software. These included total diameter of each flower head, diameter of the central disk, number of ray florets, width and length of one to three ray florets per flower head, and the number of disk florets. We evaluated the variability of floral design and display traits from a larger sample of plants from the three topographic positions over the two study years within one of the three populations studied (S1), following the protocol described above. For this purpose, we used a database of 660 randomly marked plants sampled in two subsequent years (2008: N = 360, 2009: N = 300). The longest distance between Madia individuals in population S1 was about 30-40 m (separating plants on SFSs from plants on NFSs). Because of mortality during early development of marked plants, extremely short floral longevity in some plants, and logistic problems, the effective sample sizes for statistical analyses of population S1 were 322 plants in 2008 and 235 in 2009.

To assess the effect of topographic position (SFS, NFS, and ravine bottom) on the selected floral design and display traits for the three populations studied (S1, S2, and S3), a multivariate ANOVA was performed, using topographic position (TP) and population (S) as factors. Because MANOVA showed significant effects, a series of univariate ANCOVAs was performed to identify individual responses of each floral design or display trait to the main factors TP and S. To assess spatial (TP effect; TP = NFS, SFS, R) and temporal (Y effect; Y = 2008, 2009 variation of floral traits within population S1, ANCOVAs were performed, using TP and Y as main factors and the averages for each floral design trait and the unique values of each display trait per plant as response variables. We controlled for plant-size effects by using total plant height at flowering (H) as a covariable for all floral traits considered. A square root transformation was applied to number of ray florets, number of disk florets, and number of flower heads per plant to correct for nonnormality (Gotelli and Ellison 2004).

Soil Water Potential, Plant Water Status, Plant Density, and Crowding Index

To assess the possible mechanisms behind small-scale spatial differences in floral traits, we focused on the relationship between floral phenotypes and local environmental variables for the S1 population over two study years. During the growing seasons of 2008 and 2009, we measured soil moisture availability and plant density in all topographic positions. Three Watermark 253-L Soil Matric Potential Sensors located at root depth (\sim 40 cm) were randomly placed in each topographic position. More negative values of soil water tension recorded by the sensor indicated drier soil up to -0.2 M Pa, which is the lower range limit of the sensor, when soil becomes completely dry. Soil water tension measurements were taken on three consecutive days, in three periods during the development of the plants: at the beginning of the growing season (late August), at the time of bud development and/or flower head opening (late September), and at the time of fruit ripening and/or seed maturation (late October). Plant population density of M. chlensis and all ephemeral plant species (annuals and geophytes) at the peak of the growing season (September) was estimated within a total of nine 1-m² plots

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Fig. 1 *A*, Flower head of *Madia chilensis* and floral design traits measured in this study. *B*, Natural variation of flower head diameter and number of ray florets of *M. chilensis*. Samples taken from different but adjacent topographic positions in semiarid Chile (drawn from photos of real flower heads collected in the field).



Fig. 2 Map of the study site in Aucó, semiarid Chile. Rectangles represent the three populations, S1, S2, and S3. Within each population, south-facing slopes are represented by triangles, ravine bottoms are represented by squares, and north-facing slopes are represented by circles.

(three per topographic position) placed at sites immediately adjacent to soil water potential sensors.

During late bud development and early flowering, we collected one leaf of each marked plant in the population S1 to estimate fresh and dry mass required for a posteriori calculation of relative water content (RWC). Fresh and fully expanded leaves located at about the midpoint of plant height were collected from all marked individuals. Relative water content is a good indicator of plant water status and represents the actual leaf water content relative to a potential maximum (Koide et al. 1989). Leaves were weighed in the field immediately after collection to obtain fresh mass and then placed inside a plastic petri dish, where the petiole was fully covered with water-saturated cotton. Each individual leaf was weighed again 24 and 48 h later until a constant (saturation) weight was attained. Finally, leaves were placed between two dry sheets of paper and dried at room temperature (24°C) to obtain dry mass, using a 0.0001-g precision scale. A posteriori estimates of RWC were obtained from equation (1), where W_f is the leaf fresh mass, W_d is the leaf dry mass, and W_s is the saturation weight:

$$RWC = \frac{(W_f - W_d) \times 100}{W_s - W_d}.$$
(1)

When the first flower head opened, three internode lengths were measured per plant (located at one-half total plant stature) with a digital calliper. The nearly universal elongation response of plants to shading (Smith et al. 1990), known as the plastic phytochrome-mediated response (Schmitt et al. 1995, 1999), was considered a suitable plant-crowding index. A posteriori estimation of plant crowding index as the ratio of internode length to total plant height was obtained from equation (2), where CI is the crowding index, $D_{\rm I}$ is the average of one to three internode lengths per plant, and *H* is total plant height at flowering:

$$CI = \frac{D_I}{H}.$$
 (2)

We divided internode lengths by total plant height to remove effects associated purely with size or growth functions.

To estimate differences in soil moisture availability between opposite slope aspects, to assess whether slope soils differed from the ravine, and to compare these values between years (2008 vs. 2009), a univariate repeated-measures ANOVA was performed, with TP and Y as main factors and soil moisture measurement dates (late August, late September, and late October) as within-subject factors or repeated measures. To test for differences in total plant density, *M. chilensis* population density, plant water status (RWC), and crowding index (CI) among topographic positions for univariate ANOVAs were performed, with TP and Y as main factors. The analysis was repeated for both study years.

Floral Traits in Relation to Plant Water Status and Crowding Index

To examine the magnitude and/or sign of the relationships between floral traits and plant water status as well as between floral traits and crowding index in the different topographic positions, we used correlation matrices (Pearson analysis) for RWC and CI versus all floral design and display traits in each of the two study years. RWC and CI data were arcsine–square root transformed to correct for nonnormality (Gotelli and Ellison 2004).

Pollinator Visits

Observations of pollinator visits to flowers were conducted within population S1 to determine the effect of topographic position. Data were taken at the peak of the flowering season of 2008 by two observers simultaneously, between 9:00 a.m. (onset of pollinator foraging activity) and 12:00 p.m. (flower head closure), on two consecutive days, for a total of 720 min of observation. The sampling period was defined after preliminary field observations of pollinator activity and flower closure patterns. We recorded the identity of pollinators and estimated the percentage of visited plants per topographic position in a total of 422 plants (NFS, 44; SFS, 168; R, 210). Significant differences in the proportion of plants visited by insects among topographic positions were assessed by a χ^2 test.

Results

Variation of Floral Design and Display Traits

The floral phenotype of Madia chilensis differed among topographic positions (MANOVA; Wilk's $\lambda = 0.42$; $F_{16, 1473} =$ 26.99; P < 0.00001) and among the three populations (Wilk's $\lambda = 0.66; F_{16, 1473} = 11.68; P < 0.00001$). Differences among plants from contrasting topographic positions (a few meters apart) within each population were greater in magnitude than differences among populations (1-1.5 km apart; fig. 3). All floral traits were affected by topographic position (ANCOVAs; TP effect; $F_{2,405} > 3.94$; P < 0.02; table A1 in the online edition of the International Journal of Plant Sciences), except for the number of disk florets per flower head and the number of flower heads per plant, which did not differ $(F_{2,405} < 0.51; P > 0.59;$ table A1). Significant spatial variation was documented in the length and width of ray florets, the number of ray florets, and the diameter of flower heads and central disks. These differences were partly but not entirely due to plant height variation (*H* effect; $F_{1,405} > 6.82$; *P* < 0.009; table A1).

Populations (*S* effect) differed in the length of ray florets, the number of disk florets, the number and diameter of flower heads, and plant height ($F_{2,405} > 3.10$; P < 0.04; table A1). Plants from population S3 were generally larger and had more conspicuous flower heads than plants from S2 and S1. Nevertheless, floral phenotype variation of *M. chilensis* followed the same pattern across topographic positions (SFS, NFS, ravine) in the three populations studied (fig. 3; no significant TP × S interactions for most traits: $F_{4,405} < 2.06$; P > 0.09; table A1). According to our expectations, plants growing on the NFS, where incident radiation is higher, were shorter, had smaller flower heads, and had fewer and smaller ray florets than plants from the SFS and the ravine bottom. Plants from the ravine were taller and had the largest flower heads and ray florets, with the highest numbers of ray florets per flower head and flower heads

per plant. Floral traits of plants from the SFS showed intermediate values between the drier NFS and the wetter ravine (fig. 3).

In population S1, all floral design and display traits of *M. chilensis* showed significant natural variation (table 1). Such variation in the floral phenotype within the S1 population is explained not only by topographic position but also by interannual differences (Y effect; MANOVA: Wilk's $\lambda = 0.74$; $F_{8,532} = 23.87$; P < 0.01). Despite the small difference in rainfall, plants in 2009 were shorter, with smaller ray florets per flower head and fewer flower heads per plant, than plants in 2008. Nevertheless, the spatial pattern related to topographic positions described above was repeated in the two study years (Wilk's $\lambda = 0.74$; $F_{8,532} = 23.87$; P < 0.01; see table 2 and fig. 3 for trait-specific details).

Soil Water Potential, Plant Water Status, Plant Density, and Crowding Index

Striking differences in soil water potential and plant water status were found among topographic positions (TP effect) for population S1 over the two study years (table 3; figs. 4, 5). The NFS was the drier habitat for annual plants, with soil water potentials that were about two times lower (more negative) than those of the opposite-facing slope and nearly three times lower than those of the ravine. Differences in soil water potentials between the SFS and the ravine were much smaller (fig. 4). Differences across topographic positions were maintained in both years (no significant $TP \times Y$ interactions; table 3; fig. 4). However, in 2009, the soil was significantly drier (22% on average for population S1, all sites pooled) than in 2008 (table 3). In addition, soil moisture levels were strongly dependent on the sampling date (table 3; fig. 5). The transition from winter to a drier spring was documented by the progressive soil drying at the three topographic locations (fig. 5). Drought levels for late October marked the end of the growing season, and desiccation was more rapid in 2009 than in 2008 (significant $Y \times T$ interaction; table 3; fig. 5). In both 2008 and 2009, soils remained drier on the NFS than on the SFS and were much drier than in the ravine throughout the growing season. Maximum soil dryness on the NFS was recorded in late October, during fruit ripening and seed dispersal, while soils still maintained some moisture on the SFS and in the ravine (fig. 5). As expected, leaf RWC varied significantly among topographic positions following trends in soil water potential (table 3; fig. 4). RWC of plants growing on the drier NFS was 16% lower than that of plants from the SFS and the ravine. Although a small difference in soil water potential was detected between the SFS and the ravine, plants from these sites did not differ in RWC.

Plant density also differed among topographic positions (table 3; fig. 6) as well as between years (table 3; fig. 6). In both years, the SFS showed the highest plant density (average ± 1 SE = 842 ± 121 plants m⁻² in 2008, 713 ± 94 plants m⁻² in 2009). Although the ravine had slightly higher plant density than the NFS in 2008 (350 \pm 50 vs. 302 ± 46 plants m⁻²), and two times the density in 2009 (525 ± 167 vs. 240 ± 18 plants m⁻²), the difference between these sites was not statistically significant in 2009 (least significant difference [LSD]



Fig. 3 Variation of floral traits in three populations (S1, S2, and S3) of *Madia chilensis* from plants occurring on a south-facing slope (SFS), on a north-facing slope (NFS), and in an adjacent ravine bottom (R). Data were collected during the growing seasons of 2008 (only S1; gray line) and 2009 (populations S1, S2, and S3; black lines). Vertical bars represent ± 1 SE of the mean.

test; P = 0.059; fig. 6). Overall, topographic position had a significant effect on *M. chilensis* density (ANOVA; TP; $F_{2, 12} = 6.70$; P < 0.05; fig. 6). The NFS always presented the sparser population. Despite the fact that densities in the ravine were slightly greater than in the SFS, the differences

between them were not statistically significant (LSD test; P > 0.37). Overall density of *M. chilensis* differed between 2008 and 2009 (ANOVA; $F_{1,12} = 10.34$; P < 0.01; fig. 6), with a five times greater density in 2009, which was a drier year, than in 2008. Despite these interannual differences in

Tabl	e 1
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Natural Variation in Floral Design and Display Traits in a Wild Population of *Madia chilensis* in Semiarid North-Central Chile

Floral traits	Mean \pm 1 SE	Range	CV
Design:			
Longitude of ray floret (mm)	$4.97 \pm .07$.24-9.73	31.59
Width of ray floret (mm)	$3.13 \pm .04$.25-5.68	26.11
No. ray florets	7.32 ± .12	0-13	37.53
No. disk florets	$10.00 \pm .27$	0-36	62.66
Diameter of flower head (mm)	$14.00 \pm .20$	2.50-43.00	33.95
Diameter of central disk (mm)	$4.42 \pm .08$.18-13.87	41.76
Display:			
Total plant flowering height (cm)	$24.14 \pm .45$	3.0-63.5	43.98
No. flower heads per plant	$4.08 \pm .12$	1-18	69.58

Note. All samples from two years (2008 and 2009) and three topographic positions are pooled. N = 557 plants. Mean, range, and coefficient of variation (CV; %) are shown.

plant density, topographic variation was similar in both years (no significant TP × Y effect; table 3; for *M. chilensis* density: $F_{2, 12} = 2.61$; P = 0.11).

As expected, crowding index varied according to topographic position following the same trends of plant density (table 3; fig. 6). Plants from the more crowded SFS showed the greatest internode length relative to plants from the NFS and the ravine. The difference in total plant density between the ravine and the NFS was marginally significant during 2009 (see above), but plants from these locations showed a significant difference in internode length (fig. 6). Although there was no overall effect of study year on internode elongation, plants from the ravine had longer internodes in 2009 than in 2008, following the changes in density of *M. chilensis* (significant TP \times Y effect; table 3).

Floral Traits in Relation to Plant Water Status and Crowding Index

As expected, relationships between floral traits, plant water status, and crowding index differed among topographic positions (table 4). Leaf RWC was more strongly related to floral traits on the drier NFS, but this effect was evident only during 2009, the drier year. While during 2009 RWC was positively correlated with five floral traits for plants growing on the NFS (table 4), only one significant correlation was documented for plants from the ravine and the SFS. On the other hand, in the wetter and more crowded sites (ravine and SFS), plant CI was strongly related to floral traits, in agreement with expectations, and such a trend was stronger in the year 2008, when crowding was greater. During 2008, plant CI was negatively correlated with eight floral traits on the SFS and six traits in the ravine (table 4). In contrast, only one (in 2008) and two (in 2009) significant correlations between CI and *M. chilensis* floral traits were detected on the NFS.

Pollinator Visits

Over all sites, a total of 15 insect species were recorded visiting the flowers of *M. chilensis* in population S1. Only

ropulation of madia entensis from Semiaria ente						
Floral traits	Н	ТР	Y	$TP \times Y$		
Design:						
Longitude of ray floret	101.63***	25.22***	16.16***	.62		
Width of ray floret	13.25***	14.42***	.02	.2		
No. ray florets	125.88***	12.45**	100.50***	33.61***		
No. disk florets	215.67***	2.88*	39.29***	12.00***		
Diameter of flower head	122.90***	20.59***	19.20***	6.25**		
Diameter of central disk	156.74***	5.88**	54.35***	8.15***		
Display:						
Total plant height at flowering ^a		136.67***	28.80***	.35		
No. flower heads per plant	551.53***	20.63***	.56	8.55***		

ANCOVAs (F Values) for Floral Design and Display Traits in a Wild Population of *Madia chilensis* from Semiarid Chile

Table 2

Note. Main effects are topographic position (TP) and year (Y); covariate is the total plant height at flowering (H).

^a A covariate was not included in this ANOVA.

* P < 0.05.

** P < 0.001.

*** P < 0.0001.

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ANOVAs (F Values) for Soil Water Potential (SWP), Total Plant Density (D_t; All Ephemeral Plant Species), Relative Water Content (RWC), and Plant Crowding Index (CI; Ratio of Internode Length to Plant Height) for a Population of Madia chilensis

Effect	SWP	RWC	$D_{\rm t}$	CI		
TP	64.01***	100.56***	14.16***	40.09***		
Y	6.11*	.09	.005	.26		
$TP \times Y$	2.53	.08	1.37	5.49**		
Т	45.79***					
$TP \times T$.39					
$Y \times T$	3.85*					
$TP \times Y \times T$.53					

Note. Main factors are topographic position (TP) and year (Y). Soil water potential was measured three times each year (withinsubject factor or repeated measures; T).

* P < 0.05.

** P < 0.001.

*** *P* < 0.0001.

one visitor was shared among the all three topographic positions, *Arthrobrachus flavipennis* (Coleoptera : Melyridae), which was responsible for 23% of the total number of visits (N = 82 visits). In addition, five species were exclusive to the ravine, three were exclusive to the NFS, and one was exclusive to the SFS. The proportion of plants visited by insect pollinators differed among topographic positions ($\chi^2 = 15.80$; df = 2; P < 0.001), as more plants received pollinator visits in the NFS (29.5% of the total sample) and in the ravine (23.3%) than in the SFS (9.5%).

Discussion

Madia chilensis, an annual plant with broad habitat requirements in Chilean semiarid ecosystems, showed considerable variation in traits related to floral design and display within short distances across a small-scale topographic gradient. Flowers had a similar pattern of differentiation across the topographic gradient for three different wild populations located under similar climates and at the same elevation. Notably, flower variation at a small spatial scale of meters within each population was greater than variation among populations located 1-2 km apart. The three wild populations of M. chilensis showed strong and similar segregation of floral phenotypes among topographic positions. Such variation was also conserved over two subsequent growing seasons in population S1, which was sampled in 2008 and 2009. Plants with smaller and less conspicuous flower heads grew on the NFS, taller plants with larger flower heads and florets grew in the ravine bottom adjacent to the slopes, and intermediate phenotypes occurred on the SFS. Differences in floral display traits among topographic positions remained even after removing the lineal effect of plant size.

Spatial differentiation of floral traits seems to be closely related to fine-scale variation of soil water availability and plant density across topographic sites. Analyses within population S1 indicated that plants with smaller and less conspicuous flower heads occurred on the slopes, where soils were drier throughout the year and plant leaves had the lowest water content. On the opposite end, taller plants with larger flower heads and more florets grew in the adjacent ravine, where soil conditions were the wettest and the relative water content of plants was high. Accordingly, it has been reported that flower size and display traits (size or number of flowers) decrease as aridity increases at regional geographic scales (Sapir et al. 2002; Bull-Hereñú and Arroyo 2009). Likewise, plants that are experimentally subjected to water shortage frequently have smaller flowers and floral displays than wellwatered ones (e.g., Carroll et al. 2001; Elle and Hare 2002; Caruso 2006). However, even though the plants from the ravine and the SFS do not show significant differences in their RWCs, most of the floral design and display traits were evidently more conspicuous in the ravine than in the SFS (length



Fig. 4 Variation of soil water potential (SWP; average for the entire growing season) and relative water status (RWC) for *Madia chilensis* plants occurring on a south-facing slope (SFS), on a north-facing slope (NFS), and in a ravine (R) in the years 2008 (top) and 2009 (bottom) in semiarid Chile. Different uppercase (for SWP) and lowercase (for RWC) letters indicate statistically significant differences (P < 0.05) after planned comparisons among topographic positions within years (least significant difference a posteriori test). Vertical bars represent ±1 SE.



Fig. 5 Seasonal variation of soil moisture availability on a southfacing slope (SFS; triangles), on a north-facing slope (NFS; circles), and in an adjacent ravine bottom (R; squares) in semiarid Chile. Data were collected in late austral winter (late August [A]), early spring (late September [S]), and midspring (late October) during the growing seasons of 2008 (top) and 2009 (bottom). Different letters indicate statistically significant differences (P < 0.05) after planned comparisons among topographic positions and measurement dates (least significant difference a posteriori test). Vertical bars represent \pm 1 SE.

of ray florets, number of disk florets, diameter of central disk, total plant flowering height, and number of flower heads per plant). A nonlinear relationship between RWC and floral expression is a possible but unlikely explanation. Small differences in RWC between the SFS and the ravine may trigger strong differences in size or number of the above traits. Alternatively, concomitant differences in plant density observed among the SFS and the ravine sites may explain such effects. Total plant density recorded on the SFS was due mainly to the high local abundance of grasses (~500 stems vs. 30 of M. chilensis and 260 of other ephemerals, on average, within 1-m² plots; data from 2008), which represented about 60% of the total plant density. Grasses have dense, shallow root systems that enhance water and nutrient uptake and hence may negatively affect the growth of other herbaceous species and woody seedlings (D'Antonio and Vitousek 1992 and references therein). Higher levels of interspecific crowding on the SFS may have enhanced internode length compared with in the ravine, where densities were lower (142 grass stems vs. 70 of M. chilensis and 110 of other ephemerals within 1-m² plots), thus limiting the development of floral traits in M. chilensis.

Spatial and temporal correlation analyses of plant water status and crowding index versus floral design and display traits also suggest that water availability and crowding are important local factors shaping the floral phenotype of *M. chilensis*. In our study, greater plant elongation under crowded conditions was negatively related to floral traits on the SFS than on the NFS, especially during the wetter year 2008, as characterized by higher total cover values. Conversely, plant water status was more strongly related to floral design and display traits on the NFS than in all other sites, and this was more evident during the drier year 2009. Thus, when drought becomes severe, larger and more conspicuous flower heads can be produced and/or maintained only by plants with a higher RWC. To our knowledge, this is the first time that an association between a plant's relative water content, inter-



Fig. 6 Variation in population density for *Madia chilensis* and for other ephemeral plant species and crowding index (CI) for *M. chilensis* estimated within $1-m^2$ plots on a south-facing slope (SFS), on a north-facing slope (NFS), and in a ravine bottom (R) in the years 2008 (top) and 2009 (bottom). Different uppercase (for plant density) and lowercase (for CI) letters indicate statistically significant differences (P < 0.05) after planned comparisons among topographic positions within years (least significant difference a posteriori test). Vertical bars represent ± 1 SE.

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Table 4

Pearson's Correlation Coefficients (r_p) of Plant Water Status (Relative Water Content [RWC]) and Crowding Index (CI) versus Floral Design and Display Traits for *Madia chilensis* Plants Occurring in Different Topographic Positions (North-Facing Slope, South-Facing Slope, and Ravine) in Semiarid Chile

	North-facing slope		South-facing slope		Ravine	
Floral traits	CI	RWC	CI	RWC	CI	RWC
2008:						
Design:						
Longitude of ray floret	0007	.17	20*	.17	.03	13
Width of ray floret	.07	.21*	21*	.19*	01	14
No. ray florets	19	.09	40***	.19*	58***	.12
No. disk florets	20	.09	43***	.11	54***	.12
Flower head diameter	04	.18	23*	.12	16	11
Diameter of central disk	17	.17	30**	.13	36***	03
Display:						
Total plant height at flowering	40***	.22*	61***	.12	56***	.22**
No. flower heads per plant	19	.19	33***	.12	40***	.20*
2009:						
Design:						
Longitude of ray floret	.27*	.24*	.09	.17	.07	.09
Width of ray floret	.20	.11	.07	.01	04	.02
No. ray florets	.17	.21	14	.01	45***	03
No. disk florets	.12	.31**	23*	.02	43***	05
Flower head diameter	.31**	.33**	06	.17	16	00
Diameter of central disk	.19	.28*	21	.18	36**	07
Display:						
Total plant height at flowering	12	.19	45***	.13	33**	.14
No. flower heads per plant	.10	.24*	34**	.22*	13	.31**

^{*} *P* < 0.05.

** P < 0.001.

*** P < 0.0001.

node elongation, and floral traits has been documented under field conditions. Our results agree with Grime's (1979) thesis that, in harsher environments, biotic or physical constraints on plant growth tend to dominate over plant-plant interactions. In contrast, under crowded conditions, interactions among plants would acquire greater relevance and, in our case, influence floral phenotype. Recently it has been proposed that not only water availability but also plant-plant facilitation interactions and density-dependence factors are important regulators of plant populations in arid zones (Butterfield et al. 2010). Because in the study area M. chilensis grows strictly in open areas (outside existing canopies), and because of the low population density on the north-facing slopes (maximum of seven plants per square meter), facilitation interactions and density-dependence factors are unlikely to affect Madia populations. Although crowding may limit flower development in *M. chilensis* on wetter sites and years, limited soil moisture availability appears to be a major constraint on flower development in drier sites and years. Even though our results suggest that spatial differentiation of floral traits was strongly related to differences in soil water availability and density among topographic positions, we cannot entirely rule out other possible covarying physical factors associated with slope aspect. Differences in soil fertility could also have an effect on floral trait expression (Burkle and Irwin 2009) and account for topographic differences. Nevertheless, a preliminary analysis of nutritional status of the soils from different topographic positions where population S1 occurred revealed no differences (MANOVA; Wilk's $\lambda = 0.029$; $F_{2,12} = 0.82$; P = 0.67; dependent variables were nitrate, available and exchangeable K, Olsen P, organic matter, and pH).

From our data, we cannot determine whether floral trait variation in *M. chilensis* is mainly an expression of phenotypic plasticity (e.g., Vogler et al. 1999; Mal and Lovett-Doust 2005; Caruso 2006), a fine-scale adaptation of individual plants to local variation in soil moisture (e.g., Monson et al. 1992; Heschel et al. 2002; Knight and Miller 2004), or both. Most traits (perhaps all) lie between plasticity and strictly genetic determination, two extremes of a continuum (Pfennig et al. 2010). Phenotypic plasticity has often been reported as a mechanism that can contribute to explain floral variation in wild populations (Vogler et al. 1999; Weinig 2002; Mal and Lovett-Doust 2005; Caruso 2006; Suárez et al. 2009). Between-year variability of M. chilensis floral phenotypes suggests that some traits have some level of plasticity that could contribute to either constrain or spur population differentiation, depending on the genetic architecture of involved traits, selective pressures, and gene flow (Pfennig et al. 2010). Multiple reproductive barriers are probably limiting gene flow among topographic positions. The identities of pollinators seem to be as differentiated across the topographic gradient as the floral traits are. While only one species of visitor was shared among all three topographic positions, several

pollinators were restricted to just one topographic position. ments but also to physiological, phenotypic, behavioral, and In addition, we have found considerable displacement in the genetic divergence among populations (Korol et al. 2006; flowering periods of M. chilensis plants growing in different Nevo 2009). Furthermore, in the case of Drosophila flies but adjacent topographic positions (Lorena H. Suárez, unfrom opposite slopes in the Evolution Canyon of Israel, inpublished data). Low pollinator and flowering overlap cipient sympatric speciation has been reported (Korol et al. among plants in different topographic positions may strongly 2006). Despite the great importance of such small-scale topoconstrain gene flow (e.g., Grant 1981; Rozzi et al. 1997), graphic heterogeneity on the selective regime of plant populathus promoting fine-scale genetic structure (e.g., Hirao and tions, studies addressing these effects on pollination ecology Kudo 2008). In addition, plants on the NFS received more and evolution of floral traits are still wanting. insect visits than the other sites. Plants in the ravine and in the SFS presumably represented more attractive patches in

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terms of density, with larger flower heads and florets. However, even though the ravine experienced the highest absolute

number of insect visits (followed by the SFS and the NFS;

data not shown), this difference did not reverse the effect of

trends in plant density, which resulted in a rather similar per-

plant probability of pollinator visits between the ravine and the sparser NFS but one that was much higher than that of

the SFS. Because pollinators enhance seed set of M. chilensis

plants, differential visitation may lead to a difference in out-

crossing rates, which could restrict gene flow between slopes

(e.g., Martin and Willis 2007). Work in progress using genetic

markers should reveal whether this process is occurring in M.

erogeneous at fine spatial scales, which is in agreement with

the work of Parsons (1973) and Armesto and Martínez

(1978), who showed that topographic position accounts for much of the spatial variation in plant populations and com-

munities in Chilean semiarid Mediterranean-climate ecosys-

tems. Moreover, we suggest that such spatial heterogeneity can be a generalized cause of phenotypic and genetic differ-

entiation among plants from semiarid environments. Factors

such as local differences in soil water availability and plant

density can locally shape plant-pollinator interactions and,

hence, selective regimes over short distances at the study site.

It is known that microclimatic divergence among opposite

slopes may lead not only to ecologically contrasting environ-

We show that semiarid environments can be extremely het-

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