

# Genetic variation in the reduction of attractive floral traits of an annual tarweed in response to drought and apical damage

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## Abstract

### Aims

Foliar herbivory and water stress may affect floral traits attractive to pollinators. Plant genotypes may differ in their responses to the interplay between these factors, and evolution of phenotypic plasticity could be expected, particularly in heterogeneous environments. We aimed at evaluating the effects of simulated herbivory and experimental drought on floral traits attractive to pollinators in genetic families of the annual tarweed *Madia sativa*, which inhabits heterogeneous environments in terms of water availability, herbivore abundance and pollinator abundance.

### Methods

In a greenhouse experiment with 15 inbred lines from a *M. sativa* population located in central Chile (Mediterranean-type climate), we measured the effects of apical bud damage and reduced water availability on: number of ray florets per flower head, length of ray florets, flower head diameter, number of open flower heads per plant, flowering plant height and flowering time.

### Important Findings

Apical damage and water shortage reduced phenotypic expression of floral traits attractive to pollinators via additive and non-additive effects. Plants in low water showed decreased height and had

fewer and shorter ray florets, and fewer and smaller flower heads. Damaged plants showed delayed flowering, were less tall, and showed shorter ray florets and smaller flower heads. The number of ray florets was reduced by damage only in the low water treatment. Plant height, flowering time and number of flower heads showed among-family variation. These traits also showed genetic variation for plasticity to water availability. Ray floret length, flower head size and time to flowering showed genetic variation for plastic responses to apical damage. Plasticity in flowering time may allow *M. sativa* to adjust to the increased aridity foreseen for its habitat. Because genetic variation for plastic responses was detected, conditions are given for evolutionary responses to selective forces acting on plastic traits. We suggest that the evolution of adaptive floral plasticity in *M. sativa* in this ecological scenario (heterogeneous environments) would result from selective forces that include not only pollinators but also resource availability and herbivore damage.

**Keywords:** flower, genetic variation, herbivory, phenotypic plasticity, water availability

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## INTRODUCTION

Plant populations frequently show variation in floral display (e.g. number of open flowers, inflorescence architecture, plant height) and floral design (e.g. corolla size, colour

and symmetry, nectar guides), which can be explained by a genetic component (Galen 1996; Young *et al.* 1994) and/or plastic responses to environmental factors (Carroll *et al.* 2001; Wolfe and Mazer 2005). Water availability and herbivores are central factors of the plant environment that may affect

floral traits. Following leaf damage plants show smaller corollas and petals (Lehtilä and Strauss 1997; Mothershead and Marquis 2000; Parra-Tabla and Herrera 2010; Suárez *et al.* 2009) and fewer open flowers (Elle and Hare 2002; Juenger and Bergelson 2000; Mothershead and Marquis 2000; Sharaf and Price 2004; Suárez *et al.* 2009). Cell expansion and turgor maintenance are essential processes during bud development and flowering, and require constant water transport within the plant (Ram and Rao 1984). Consequently, plants subjected to drought show smaller flowers and less conspicuous displays than well-watered ones (Carroll *et al.* 2001; Caruso 2006; Elle and Hare 2002). However, plant responses to the interplay between biotic and abiotic factors may not be predictable from responses to isolated factors (Gianoli *et al.* 2007; Valladares *et al.* 2007). The direct and indirect effects of foliar herbivory on floral traits of the shrub *Cnidioscolus aconitifolius* (Euphorbiaceae) vary with the environment (Parra-Tabla and Herrera 2010). Halpern *et al.* (2010) showed that drought and foliar damage had additive and non-additive effects on attractive and defensive traits of *Nicotiana quadrivalvis* (Solanaceae). In the perennial herb *Convolvulus demissus* (Convolvulaceae) from semiarid Chile, plastic responses of vegetative traits to drought are constrained by defoliation (Gianoli *et al.* 2009; Quezada and Gianoli 2010).

At the population level, plant genotypes may differ in their responses to environmental variation (G × E interactions; Pigliucci 2001). This variation in plasticity among genotypes may determine the evolutionary potential of such responses (Godoy *et al.* 2011; Pigliucci and Byrd 1998; Schlichting and Pigliucci 1995); it is the raw material on which natural selection can act, likely resulting in adaptive evolution of phenotypic plasticity (Gianoli and Valladares 2012; van Kleunen and Fischer 2005). Lack of genetic variation and genetic correlations (expressing tradeoffs or fitness costs) are limiting factors for evolutionary change (Blows and Hoffmann 2005; Lande and Shannon 1996). Very few studies addressing the interactive effects of biotic and abiotic factors on floral traits have included the genotypic component in the analysis (but see Elle and Hare 2002; Halpern *et al.* 2010). The study in *Nicotiana quadrivalvis* described above (Halpern *et al.* 2010) included maternal families, but all of them originated from a single USDA accession. Therefore, little genetic variation could be detected and its existence in natural plant populations remains to be evaluated. A study addressing the effects of insect herbivory and irrigation on floral traits in *Datura wrightii* (Solanaceae) (Elle and Hare 2002) included families expressing one of two trichome types, but the family × environment interaction was not evaluated.

In the present study, we evaluated the effect of apical bud damage and water availability on phenotypic expression and evolutionary potential of floral traits attractive to pollinators in 15 genetic families of the annual tarweed *Madia sativa* (Asteraceae). *M. sativa* is distributed in the Mediterranean-climate zone of central Chile, where plants often experience drought during the dry season (spring–summer) (Di Castri

and Hajek 1976; Mooney and Dunn 1970). This region shows high spatial heterogeneity in soil moisture partly due to large differences in sunlight incidence on a mountainous landscape (Parsons 1973). A sympatric congeneric species, *M. chilensis*, showed significant variation in floral design and display traits with topographic heterogeneity (Suárez *et al.* 2011). Previous work has shown that experimental drought limits the ability to compensate for apical bud damage in *M. sativa*, which is commonly attacked by caterpillars that produce this type of damage (González *et al.* 2008a). *M. sativa* shows significant variation in floral design and display traits proven to be attractive to pollinators, which show significant spatial and temporal changes in abundance between years (Celedón-Neghme *et al.* 2007). We expected that apical damage and experimental drought would show additive (and negative) effects on floral design and display traits of *M. sativa* because of reduced resource availability. We also expected to find significant genetic variation for the responses of the floral phenotype to the experimental factors (G × E interactions). This because *M. sativa* populations are located in heterogeneous habitats in terms of water availability (Suárez *et al.* 2011) and pollinator (Celedón-Neghme *et al.* 2007) and herbivore (González *et al.* 2008a) abundance, and in view of the association between phenotypic plasticity and environmental heterogeneity (Baythavong 2011; Gianoli 2004; Gianoli and González-Teuber 2005). We calculated genetic correlations among traits across environments to test for possible constraints to the evolutionary response to selection of traits showing genetic variation (e.g. Etterson and Shaw 2001; Gómez-González *et al.* 2011).

## MATERIAL AND METHODS

### Study species

The Chilean tarweed *Madia sativa* (Asteraceae) is an annual selfing plant species (Arroyo and Uslar 1993) that grows in open habitats, disturbed fields and sunny slopes along Chile (Matthei 1995). The main stems are 20–120 cm tall, and glandular and non-glandular trichomes are found on stems, leaves and involucre (González *et al.* 2008a, 2008b). The basal leaves form a rosette and upper leaves are opposite along the branches. Flower heads (1.5–2 cm diameter) are apical and have yellow female ray florets and hermaphroditic disk florets (Hoffmann 1998). Although *M. sativa* is self-compatible, pollinators contribute to enhance seed production (Celedón-Neghme *et al.* 2007). The most frequent visitors are dipterans (Bombyliidae and Syrphidae) and hymenopterans (*Bombus* and Colletidae), and they show preference for taller plants with more flower heads and ray florets (Celedón-Neghme *et al.* 2007).

Plants of *M. sativa* in populations from central Chile complete their life cycle during the dry season (spring–summer) of this region with Mediterranean-type climate, when drought may be a limiting factor for plant development (Di Castri and Hajek 1976; Mooney and Dunn 1970). In the seed-source

population (Farellones; 33°21'S, 70°17'W; 1970 m a.s.l.) we observed plants frequently attacked by specialist native aphids (Delfino and González 2005) and lepidopteran larvae (González et al. 2008a; LH Suárez, personal observations). These caterpillars feed on reproductive buds and flower heads, and the damage mostly occurs on apical buds of the main stem.

### Plant material and genetic families

We collected seeds from 70 randomly chosen, widely spaced plants growing in Farellones. We produced 15 experimental inbred lines (maternal families) in the greenhouse. Flower heads were bagged before pollination to avoid exogenous pollen contamination. During flowering, plant florets of maternal families were manually pollinated using pollen from the same plant to generate inbred lines. In order to minimize differences among plant families driven by their original environment, we propagated each inbred line for two generations before the set-up of our experiment.

Seeds obtained from the 15 inbred lines (30–40 seeds per family) were scarified with 98% sulphuric acid for 2 min and placed on moistened paper at room temperature ( $T^{\circ} \sim 25^{\circ}\text{C}$ ) and darkness to allow germination. When cotyledons were completely unfolded, seedlings were transferred individually to 3 L pots filled with potting soil and placed in a greenhouse at the campus of Universidad de Concepción (PAR at noon  $\approx 1000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ; 24°C and 12°C mean maximum and minimum temperatures, respectively; watering every 4 days). Experimental plants grew excluded from pollinators.

### Water and damage treatments

We evaluated the effect of apical damage and water availability on floral design and display traits in the 15 inbred lines. Plants were randomly assigned to control and low-watering (drought) treatments within families. Within each watering regime, plants were randomly assigned to undamaged and apical damage treatments. The watering treatment started 70–72 days after plants were transferred to the greenhouse. Control and drought plants were watered to soil capacity every 4 and 8 days, respectively. We applied the apical damage 14 days after the onset of watering treatments, when plants showed at least one reproductive bud on the main stem. Apical damage by caterpillars was simulated with scissors, making 6–8 cuts on the reproductive buds. We initially had five plants per family per group (total  $N = 300$ ), but some plants died during the experiment due to unidentified causes. A total of 288 plants from the 15 inbred lines were finally analyzed. To avoid microenvironmental (random) effects on potted plants associated with the greenhouse setting, experimental plants were randomly redistributed every 5–7 days throughout the experiment.

### Traits measured and data analysis

We measured the following floral design traits on three flower heads per plant: number of ray florets per flower head, length of ray florets and flower head diameter (determined with a

digital calliper, 0.01 mm resolution). The display traits measured were the number of open flower heads per plant and flowering plant height. Flowering time (time from germination to the opening of first flower head) was recorded as a phenological trait relevant for the synchrony of plant–pollinator interactions and indicated the time to reproductive maturity.

In order to assess the effects of genetic families and environment factors on the expression of floral traits, we used a mixed-model ANOVA. The full ANOVA model included the effects of apical damage (D), water availability (W) and seed family (F); D and W were fixed factors and F and all interactions involving F were random factors. When testing for the fixed factors D and W, we used as error term (denominator) for the  $F$ -ratio the Mean Squares (MS) of  $F \times D$  and  $F \times W$ , respectively; and when testing for the  $D \times W$  interaction (Table 1) we used as error term the MS of the  $D \times W \times F$  interaction. Particular contrasts between groups were done using Fisher's least significant difference (LSD) tests. We used Pearson product–moment correlations to estimate genetic correlations among traits across environments. All analyses were conducted with Statistica (Statsoft). The among-family variance detected in the analyses represents total rather than additive genetic variance (Mitchell-Olds and Rutledge 1986). Estimates of total genetic variance are appropriate because *M. sativa* is a self-compatible species and natural selection does not act exclusively on additive genetic variation, as in out-crossing species, but on total genetic variation (see Mauricio and Rausher 1997).

## RESULTS

We found that both water availability (W) and apical damage (D) affected almost all studied traits (significant effects of W and D, Table 1). Overall, the attractiveness of plants decreased in both low water and damage treatments. Plants grown in low water showed decreased height and had fewer and shorter ray florets and fewer and smaller flower heads (Fig. 1). Flowering time was delayed by apical damage but was not modified by water shortage. Damaged plants were less tall and showed shorter ray florets and smaller flower heads, but the number of flower heads was not affected. The number of ray florets was reduced by damage only in the low water treatment (significant  $D \times W$  interaction, Table 1; Fig 1).

Plant height, time to flowering and number of flower heads showed among-family variation (F effect, Table 1). The response of these traits to water availability also exhibited genetic variation ( $F \times W$  interaction; Table 1). Ray floret length, flower head size and time to flowering showed among-family variation for plastic responses to apical damage ( $F \times D$  interaction; Table 1).

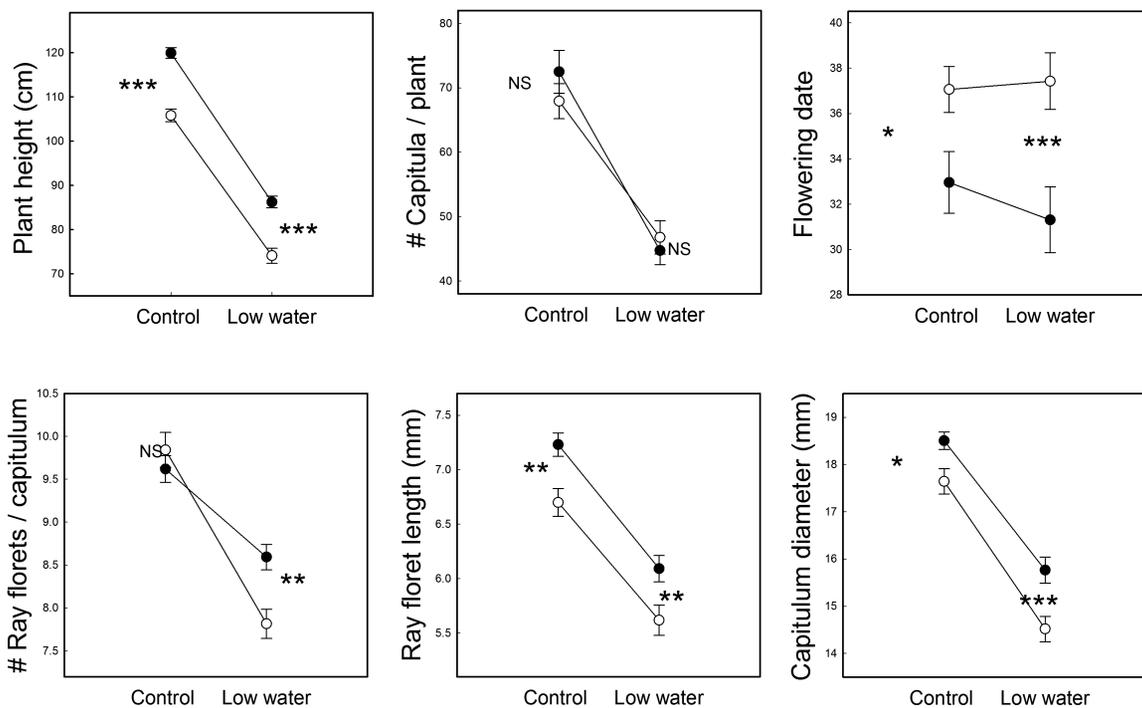
Almost all traits showed positive genetic correlations between them (Table 2). The exception was flowering time, which was only significantly (and negatively) correlated with the number of flower heads (Table 2).

**Table 1:** mixed-model ANOVA of floral design and plant display traits of *Madia sativa* considering the effects of damage (undamaged versus apical damage) and water availability (control versus low water) as fixed factors, and maternal family (inbred line) as random factor

Factor	df	Flowering plant height	Number of flower heads	Flowering time	Number of ray florets	Length of ray florets	Flower head diameter
Damage (D)	1	180.68***	0.90	22.17***	1.36	7.26*	5.95*
Water (W)	1	255.16***	36.98***	0.03	47.70***	58.40***	96.81***
D × W	1	0.24	2.60	1.69	5.43*	0.009	0.71
Family (F)	14	3.11*	2.85*	8.92***	1.25	2.19	1.03
F × W	14	3.28*	3.58*	3.11*	1.81	1.61	1.99
F × D	14	0.77	1.06	3.16*	1.55	2.56*	4.02**
F × W × D	14	0.94	1.07	0.85	1.19	1.26	0.90

F-ratios are shown. See Methods for the error terms used in each case.

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



**Figure 1:** effects of water availability ( $X$  axis) and apical damage (undamaged plants = filled circles; plants with apical damage = open circles) on floral design and plant display traits in *Madia sativa*. All traits showed significant differences between damage treatments within watering regimes (LSD test, \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ), with the exception of those indicated by 'NS' (LSD test,  $P > 0.05$ ). For each plant trait, means  $\pm$  SE are shown.

## DISCUSSION

Our results agree with earlier evidence showing that plants exposed to drought or folivory show smaller flowers, fewer open flowers and a shift in flowering phenology (Caruso 2006; Elle and Hare 2002; Juenger and Bergelson 2000; Lehtilä and Strauss 1997; Mal and Lovett-Doust 2005; Sharaf and Price 2004; Suárez *et al.* 2009; Strauss *et al.* 1996). The number of ray florets showed non-additive effects of the stressors: drought reduced trait expression, while damage caused detrimental effects only in the low water treatment.

Non-additive effects may occur because the balance between costs and benefits of plasticity can vary with resource availability and/or because simultaneous responses to components of the environment may limit particular plastic responses (Alpert and Simms 2002; Valladares *et al.* 2007). Apical damage often leads to the breakage of apical dominance, resulting in increased branching (Benner 1988; González-Teuber and Gianoli 2008; Huhta *et al.* 2000). It has been shown that the ability of *M. sativa* to enhance branching in response to apical damage is limited by drought (González *et al.* 2008a). Likewise, damage increased the density of glandular trichomes, a plant

**Table 2:** correlations among plant and floral traits of 15 families of the tarweed *M. sativa* across environments (water availability × apical damage treatments,  $N = 60$ )

	I	II	III	IV	V	VI
Plant height (I)		<b>0.58***</b>	<b>0.71***</b>	<b>0.77***</b>	0.01	<b>0.55***</b>
Number of ray florets (II)			<b>0.39**</b>	<b>0.66***</b>	-0.19	<b>0.33*</b>
Ray floret length (III)				<b>0.92***</b>	0.14	<b>0.32*</b>
Capitulum diameter (IV)					-0.01	<b>0.39**</b>
Flowering date (V)						<b>-0.29**</b>
Number of capitula (VI)						

\* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .

resistance trait, only under control watering (González et al. 2008b). Therefore, the simultaneous occurrence of water shortage and herbivory would significantly impair the plant mechanisms to cope with either factor separately.

Genetic variation for adaptive plastic traits is a key determinant of the population potential for evolutionary change under heterogeneous environments (Schlichting and Pigliucci 1995; van Kleunen and Fischer 2005). We detected genetic variation for plasticity to drought and/or damage in five of the six studied traits, namely length of ray florets, number and size of open flower heads, plant height and flowering time. The latter was the only trait showing genetic variation for plasticity to both water shortage and apical damage. Flowering time has shown rapid evolution in response to sustained drought in the annual *Brassica rapa* (Brassicaceae) (Franks et al. 2007) and it has been proposed that flowering time plasticity may facilitate niche shifts in plants (Levin 2009). Therefore, we expect that (plasticity in) flowering time will play a central role in the adaptation of this native tarweed to the increased aridity foreseen in central Chile (Fuenzalida et al. 2007), as has been shown for a native mustard in the Rocky Mountains facing climate change (Anderson et al. 2012).

Negative genetic correlations may constrain adaptive evolution in plant populations (Antonovics 1976; van Kleunen and Fischer 2005). This has been demonstrated for populations of the annual *Chamaecrista fasciculata* (Fabaceae) along an aridity gradient, where genetic correlations between traits that oppose the direction of selection cause slower evolutionary responses to increased aridity (Etterson and Shaw 2001). We did not find such genetic constraints in the study system, where most floral traits were positively correlated. Although flowering time and number of flower heads were negatively correlated in *M. sativa*, it does not necessarily imply a genetic constraint to adaptive evolution. There is evidence that early flowering is an adaptive response to drought in annuals (Franks 2011; Franks et al. 2007; Heschel and Riginos 2005; Sherrard and Maherali 2006; Wu et al. 2010) and short-lived perennials (Anderson et al. 2012). If this is the case in the study species, any shortening in flowering time in response to increased aridity would result in more, and not fewer, flower heads. In Andean populations of *M. sativa* the growing season is very short, and a little delay of flowering onset may have

serious consequences on pollination service by affecting the synchronicity between anthesis and pollinators' foraging time (Arroyo and Uslar 1993; Torres-Díaz et al. 2011).

Maintenance of conspicuous floral traits may involve fitness costs for plants (Andersson 2000, Andersson 2005), particularly in low-resource environments. Thus, the increase in corolla size in *Polemonium viscosum* (Polemoniaceae) entails a greater physiological cost in a population where flowering plants are water-stressed (Galen et al. 1999). The reduction of flower size in *Epilobium angustifolium* (Onagraceae) after experimental drought is associated with a decrease in leaf water potential (Carroll et al. 2001). Number of ray florets in *M. sativa* has shown to be costly in terms of seed mass and seed germination in a field experiment during dry summer (Celedón-Neghme et al. 2007). The evidence suggests that plants are able to adjust the floral phenotype to resource availability. In the same vein, we think that our results reflect 'active/true' plasticity rather than 'passive/apparent' plasticity (see Gedroc et al. 1996; Gianoli and Valladares 2012; Weiner 2004). Thus, most of the phenotypic changes observed in *M. sativa* are consistent with well-established functional responses of plants to reduced water availability and herbivore damage, and are unlikely to result merely from changes in plant size or ontogeny caused by the experimental treatments. Even if the latter were the case, it would not affect the ecological/evolutionary significance of such phenotypic changes (Gianoli and Valladares 2012).

We found that apical damage and water shortage had additive and non-additive (detrimental) effects on floral design and display of *M. sativa*, likely because of reduced resource availability. The observed plasticity in flowering time should be important for adaptation of *M. sativa* to the expected increased aridity in its habitat, as shown for other forb species (Anderson et al. 2012). Genetic variation for plastic responses was detected, thus conditions are given for evolutionary responses to selective forces acting on plastic traits. We suggest that the evolution of adaptive floral plasticity in this ecological scenario would result from selective forces that include not only pollinators but also resource availability and herbivores. To better understand the integrated phenotypic responses of this plant to a complex environment from an evolutionary perspective, further studies could explore the

phenotypic integration of physiological and morphological responses to herbivory and drought together with the display of attractive floral traits, and their impact on plant fitness. The integrated responses of plants to environmental challenges, and their genetic bases, are crucial research topics in the context of current climate change (Matesanz *et al.* 2010; Valladares *et al.* 2014).

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