

## Phenotypic plasticity in the holoparasitic mistletoe *Tristerix aphyllus* (Loranthaceae): consequences of trait variation for successful establishment

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**Abstract** The ability of a genotype to respond to changes in the environment through modifications in the phenotype is adaptive when the plastic genotypes attain a higher fitness than non-plastic genotypes. In this study we examine whether parasite traits involved in host infection exhibit adaptive phenotypic plasticity to the heterogeneous host microenvironment. We focused on a host-parasite relationship characterized by the holoparasitic mistletoe *Tristerix aphyllus* and the cactus host *Echinopsis chilensis*. Unlike most mistletoes, whose seeds are deposited on the host branches, seeds of *T. aphyllus* are often deposited on the spines of the cactus. The extremely long radicles of *T. aphyllus* have been suggested to represent a parasite adaptation to overcome the barriers to infection imposed by the spines of cacti. However, plastic rather than canalized phenotypes may represent a better strategy in changing environments. We evaluated whether *T. aphyllus* exhibits adaptive plasticity in radicle length through a sire half-sib genetic design under field conditions in two contrasting microenvironments (seeds deposited on spines 4 and 28 mm from the host surface). We used phenotypic and genotypic selection analyses to evaluate the relationship between radicle length and seed establishment. Our results revealed significant phenotypic plasticity for radicle length and family level variation among maternal but not paternal families.

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Short radicles and large seeds were favored in the short-distance environment, and long radicles were favored in the long-distance environment, suggesting that no single optimal phenotype exists for *T. aphyllus*. The observation that the heritability of radicle length and seed mass did not differ from zero was consistent with this finding. Overall, our results indicate that plastic rather than long radicles seem to be a better parasite strategy to overcome the microenvironmental heterogeneity imposed by host defensive traits.

**Keywords** Adaptive phenotypic plasticity · Heritability · Host-parasite relationship · Mistletoe · Quantitative genetics · Radicle elongation · Seed mass

## Introduction

Phenotypic plasticity is the ability of genotypes to produce different phenotypes in response to environmental heterogeneity (Bradshaw 1965; Lively 1986; Schlichting 1986; Pigliucci 2001). This ability is adaptive if plastic genotypes show a higher fitness than non-plastic genotypes in heterogeneous environments (Sultan 1987; Scheiner 1993; Via et al. 1995; Agrawal 1998; Schlichting and Pigliucci 1998; Pigliucci 2001). Most evidence on adaptive plasticity is indirect or restricted to laboratory conditions (e.g., Spitze and Sadler 1996; van Buskirk et al. 1997; Dorn et al. 2000; Agrawal et al. 2002; Relyea 2002; but see Dudley and Schmitt 1996; Agrawal 1998; Donohue et al. 2001). However, to test adaptive plasticity, it is necessary to demonstrate: (1) that natural selection on the focal trait differs among contrasting environments, and (2) that the plastic response induced by each environment corresponds to the phenotype that is favored by selection in that environment (Dudley 1996; Dudley and Schmitt 1996; van Buskirk et al. 1997; Dorn et al. 2000; Juenger and Bergelson 2000). These conditions, however, do not relate necessarily to the issue of whether plasticity can evolve (Schlichting and Pigliucci 1998; Pigliucci 2001). The evolution of phenotypic plasticity requires at least that the trait under study possesses heritable variation for plasticity, and that this variation influences Darwinian fitness. Several works have reported significant genetic variation for plasticity and significant potential for evolutionary change (Scheiner 1993; Schlichting and Pigliucci 1995; Scheiner and Berrigan 1998; Juenger and Bergelson 2000; Pigliucci 2001; Agrawal et al. 2002; Relyea 2002). However, the evolution of phenotypic plasticity may be constrained by costs or genetic correlations within or across environments (Via and Lande 1985; Gomulkiewicz and Kirkpatrick 1992; Roff 1997; DeWitt et al. 1998).

Several studies have evaluated adaptive plasticity in antagonistic ecological interactions such as competition in plants (Dudley and Schmitt 1996), induced plant defense against herbivore attack (Karban and Baldwin 1997; Agrawal 1998), morphological defensive responses against predators in invertebrate prey (Tollrian and Harvell 1999), and vertebrates (Van Buskirk et al. 1997; Relyea 2002). To our knowledge, no study has examined whether parasite traits involved in host attack exhibit adaptive plasticity and evolutionary potential in natural populations. Here, we report a test of the adaptive plasticity hypothesis for the radicle length of the holoparasitic mistletoe *Tristerix aphyllus* (Loranthaceae). The radicle represents a key trait in most parasitic plants because it represents the morphological and physiological bridge between host and parasite tissues that allows successful

infection (Kuijt 1969; Lamont 1983; Musselman and Press 1995). The radicle of *T. aphyllus*, which ranges from 14 to 90 mm (Kuijt 1969; Martínez del Río et al. 1995; Medel et al. 2002), is the longest in the family Loranthaceae (Kuijt 1969, 1988). Unlike other mistletoes where seed deposition occurs directly on the host branches, the seeds of *T. aphyllus* are often deposited on the spines of cacti. Seeds germinate and the radicle undergoes elongation until they eventually reach the host cuticle. The extremely long radicle shown by *T. aphyllus* has been suggested to represent an adaptation to overcome the barrier imposed by cactus spines (Martínez del Río et al. 1995). However, even in the most specialized species such as *T. aphyllus*, not every dispersed seed experiences the same microenvironmental conditions during the early stages of the life cycle. For example, variation in the distance between the seed and the host cuticle may be a major source of phenotypic variance that promotes plasticity in traits associated to parasite establishment. In this study, we evaluated: (1) the prevalence of phenotypic plasticity, in the radicle of *T. aphyllus*, (2) the functional relationship between radicle elongation and seed establishment in two experimental environments, and (3) the narrow-sense heritability of the mean and plasticity of radicle length and seed mass, two traits influencing seed establishment.

## Materials and methods

### Study system

This study was conducted from March 2002 to March 2003 at the Reserva Nacional Las Chinchillas (31°30'S, 71°06'W, Aucó, IV Región, Chile), located ~300 km northeast of Santiago de Chile. The climate is of a semiarid Mediterranean type with most rainfall concentrated in the winter season. Mean annual precipitation is 167 mm with ample between-year variation, and frequent droughts interspersed with unusual years of high precipitation that seemingly co-occur with the El Niño Southern Oscillation events (Jaksic 2001). Vegetation is thorny, with *Flourensia thurifera* (Asteraceae), *Bahia ambrosioides* (Asteraceae), and *Porlieria chilensis* (Zygophyllaceae) as the most common shrub species. Cactus species are represented by the columnar *Echinopsis chilensis* and *Eulychnia acida*, and the globular *Opuntia berteroniana* and *Eriosyce sandillon*. Additional information about the study area has been reported previously (Medel 2000, 2001; Medel et al. 2004).

*Tristerix aphyllus* (Miers ex DC.) Tiegh. ex Barlow et Wiens, is a self-compatible holoparasitic mistletoe (Smith-Ramirez 1999; Medel et al. 2002), that infects only cacti species in north and central Chile (Kuijt 1969, 1988). Unlike most Loranthaceae, this species has leaves reduced to minute scales, and the only portion that emerges from the stems of cacti is its red inflorescence. The vegetative portion exists as an endophyte within the host tissue where it taps the phloematic vessels of the cacti (Mauseth et al. 1984, 1985; Mauseth 1990). The flowering season occurs between March and August, with a peak from April to June (Medel et al. 2002). Flowers are exclusively pollinated by the hummingbird *Sephanoides sephaniodes*. During the pollination season, *S. sephaniodes* visits most of the *T. aphyllus* population, and often probes different flowers of the same inflorescence in successive bouts (Medel et al. 2002). Even though *T. aphyllus* is a

self-compatible species, selfing reduces significantly fruit production (González et al. 2006). The fruiting season occurs from April to November. Fruits are single-seeded berries containing 80% water that turn pale pink or translucent white when ripe. The Chilean mockingbird *Mimus thenca* Molina (Mimidae) is the only disperser of the mistletoe in the study site (Martínez del Río et al. 1995, 1996; Medel 2000; Medel et al. 2002, 2004). Once defecated by the bird, most seeds adhere to the cuticle and cactus spines by a sticky viscid layer. Seeds germinate the day after defecation, and the bright red radicle grows up to 8 weeks or until making contact with the cuticle of the cactus. After that, a haustorial plate develops, from which several filaments penetrate into the cactus tissues (Mauseth et al. 1984, 1985). The haustorium, rather than being a permanent, absorptive organ, is a temporary structure that gives rise to the endophytic vegetative body of the parasitic plant (Mauseth et al. 1985). The mistletoe exists as an endophyte for ~17 months before emerging from the cactus tissues as a red inflorescence (Mauseth et al. 1984, 1985; Botto-Mahan et al. 2000). The host *E. chilensis* [*Echinopsis chiloensis* (Colla) Friedrich et G. D. Rowley] is a columnar cactus that inhabits mainly north-facing slopes of central Chile (Rundel 1976). The spine length shows a wide variability both within and among populations (Rundel 1976; Hoffmann 1986). Areoles of *E. chilensis* show 1–2 long central spines and 8–12 short lateral spines that project in all directions. In the study area, the length of central spines is ~10 cm, the length of short lateral spines is ~2 cm, and the spine density is ~2.5 spines per cm<sup>2</sup> (Martínez del Río et al. 1995).

### Experimental procedures

To estimate the quantitative genetic parameters of the infection-related traits and plasticity of the radicle length, experimental plants of *T. aphyllus* were randomly chosen from different host individuals during April 2002, in an area of ~30 ha. Because *E. chilensis* is the most common host in the study site (Martínez del Río et al. 1995; Medel 2000; Medel et al. 2002, 2004), we performed all experiments on this host species. To avoid pollinator visits and fruit removal, every inflorescence was isolated using fine and transparent mesh from the bud stage until the end of the fruiting season. We set up a paternal half-sibling mating design (Roff 1997), by crossing 21 sire plants with three different dam plants each. The maximal distance between sire and dam plants was ~0.6 km. Flowers were previously emasculated to avoid autogamy, and manual pollination of ~200 flowers per experimental plant was performed according to the half-sib design during 40 days (mid April to end May). The ripe fruits were collected in September 2002. Mortality of some maternal plants unbalanced our design, resulting in 58 full-sibling and 21 paternal half-sibling families. Seeds were weighed in a microbalance with precision of 0.01 g ( $N = 2,531$  seeds). As with most mistletoes, seeds do not require host stimuli to germinate (Lamont 1983; Norton and Carpenter 1998); removal of the epicarp is sufficient (Mauseth et al. 1985; Kuijt 1988).

We evaluated the plasticity of radicle length by assigning seeds from each full-sib family to two distance treatments from the cactus surface: (1) short-distance (4 mm) and (2) long-distance (28 mm). Seeds were located on areolas (short-distance treatment) or along central spines (long-distance treatment). Only one seed was located per host branch in a complete random design ( $N = 10$  replicates/full-sib family/treatment). Criteria to choose experimental environments were

based on empirical measurements of seed deposition in the field (González 2004). About 15% of the seeds deposited on cacti are found in direct contact with the cactus cuticle and 50% are found attached to a spine, 0–30 mm from the cactus surface ( $N = 200$  seeds, González 2004). To avoid potential biased seedling mortality attributable to differences in sun exposure and seed desiccation (Bottomahan et al. 2000), all artificial infections were performed on southeast orientation (arc of  $130^\circ$  on the cactus perimeter). Only cases when the seed contact derived into a haustorial plate development were considered successful establishment. We confirmed the establishment status of the seedlings by checking the persistence of the haustorial plate for 5 months after experimental infection. The radicle length was measured from pictures using a scaled digital camera. All images were analyzed in UTHSCSA ImageTool for Windows, Version 2.0 (University of Texas Health Science Center, San Antonio, TX, USA).

## Statistical procedures

### *Quantitative genetics and fitness*

The heritability of radicle length and seed mass was estimated from variance components in a mixed model (PROC MIXED SAS, Cary, NC, USA). Because our design was unbalanced, we used restricted maximum-likelihood method (REML) (Littell et al. 1996). Genetic variances and heritability estimates within treatments included sire and dam nested within sire as random effects. The narrow-sense heritability ( $h^2$ ) for normally distributed traits at each environment was estimated from  $V_A/V_P$ , where,  $V_A$  is the 4 ( $V_{SIREs}$ ) and  $V_P$  is the phenotypic variance. The standard error of heritabilities was computed as  $4 [SE (V_{SIREs})]/V_P$  (Roff 1997). Our estimates of additive genetic variances and narrow-sense heritabilities assume that random mating underlies the reproduction of *T. aphyllus* (Roff 1997).

Radicle length plasticity was analyzed using a mixed model (PROC MIXED SAS). Sire and dam (nested within sire) effects were considered as random effects and treatments were considered as a fixed effect. Evidence for radicle length plasticity was attained from the level of significance of the seed deposition treatments. The significance of the sire half-sib family  $\times$  treatment interaction reveals additive genetic variation for radicle plasticity. The interaction dam full-sib family  $\times$  treatment involves additive and non-additive genetic effects. The significance of random effects was calculated directly from  $Z$ -values (variance component divided by standard error) (SAS Institute 1997). The significance of fixed effects was tested from type III estimable functions, with denominator degrees of freedom taken from Satterthwaite's approximation.

We estimated fitness at each treatment (4 and 28 mm from the cactus surface) from seed establishment success (successful seeds = 1, unsuccessful seeds = 0). Because our fitness variable is dichotomous, we used a generalized linear model (GLIM) approach with the GLIMMIX macro of PROC MIXED in SAS (SAS Institute 1997). GLIM is an extension of the traditional linear model (GLM) that is applicable to a wider range of data analysis and error structures (Crawley 1993). The GLIMMIX macro fits a GLM with random effects to the data by an iterative pseudolikelihood estimation of the model parameters. We modeled variation on fitness using a binomial error distribution and a logit link function. The

significance of random (sire and dam) and fixed (distance treatment) factors was tested as previously described for radicle length. Because the sire and dam variance components are potentially composed of both additive ( $V_A$ ) and specific dam ( $V_{ME}$ ) or sire ( $V_{PE}$ ) effects (Cockerham and Weir 1977), significant differences between estimates may reveal specific maternal or paternal environmental effects.

The effect of variance components on fitness was evaluated by comparing the fit of an unconstrained model (allowing different estimates of sire and dam variances) to a constrained model of equal variances in a likelihood-ratio test (Littell et al. 1996; Juenger and Bergelson 2000). Because we did not detect differences between models, only the simplest constrained model was considered. A significant paternal or maternal variance component in this model indicates significant additive genetic covariance between the states of fitness expressed in each treatment (Fry 1992; Via and Conner 1995; Juenger and Bergelson 2000).

### *Adaptive plasticity*

To evaluate the strength of selection on plastic radicle elongation, we used the multiple regression approach (Lande and Arnold 1983). We estimated the phenotypic selection coefficients for seed mass and radicle length within experimental treatments (4 and 28 mm) using establishment as a surrogate of fitness. We estimated the linear univariate ( $S_i$ ), and linear multivariate ( $\beta_i$ ) selection gradients (Lande and Arnold 1983). Selection gradients were calculated by regressing relative fitness ( $w_{\text{relative}} = w_{\text{individual}}/w_{\text{average}}$ ) on standardized traits (mean = 0, SD = 1). The differential of selection,  $S_i$ , is an estimate of the total selection acting upon the focal trait, including both direct and indirect selection through correlated characters. The linear gradient of selection,  $\beta_i$ , provides information on the direction and magnitude of change in the mean phenotype after selection (Lande and Arnold 1983). Because conventional significance tests do not apply to dichotomous fitness measures (Lande and Arnold 1983; Janzen and Stern 1998), we used non-parametric logistic regression analyses to evaluate statistical significance (Janzen and Stern 1998). To compensate for potential experimental type 1 error arising from multiple comparisons, a sequential Bonferroni adjustment with a tablewide  $\alpha$ -level = 0.05 was performed. Comparison of selection differentials between experimental treatments was performed by examining the significance level of the radicle  $\times$  treatment interaction in a GLIM, see above, using the standardized trait as a continuous independent variable, treatment as a classificatory variable, and establishment (fitness) as dependent variable. We modeled variation in fitness using a binomial error distribution and a logit link function. Finally, depending on whether plasticity was detected, the magnitude and sign of the selection coefficients was inspected. Overall, the plasticity of radicle length was considered adaptive when the response of the trait to the contrasting environments was in the same direction as the selection differentials for the trait within treatments.

In addition to phenotypic selection analyses, we applied a genotypic selection approach to each treatment (Rausher 1992). This analysis is complementary to the standard phenotypic selection analyses because it accounts for possible biases due to environmental correlations between traits and fitness (Rausher 1992). Because the dam component was often stronger than the sire component, we used dam means (rather than sire means) as raw data. We estimated  $S_i$  and  $\beta_i$  by regressing the mean standardized traits of maternal full-sib families on relative fitness. In addition, we

examined the relationship between radicle length plasticity and fitness across the experimental treatments. The level of plasticity of each dam full-sib family was calculated from the mean value of the radicle length in the long-distance treatment minus the short-distance treatment. The across-treatment fitness for each dam full-sib family was estimated as the average seed establishment between treatments. A significant positive selection differential for plasticity provides an additional line of support for the adaptive plasticity hypothesis. Notwithstanding, because a positive between-environment correlation may represent a potentially strong constraint on the evolution of plasticity (Via and Lande 1985), we examined this possibility by assessing the between-treatment correlation for radicle length.

## Results

### Heritability estimates and plasticity

Even though seed mass did not show a significant heritability ( $h^2$ ), we detected a significant dam effect for this trait (Table 1). The seed mass of full-sib families ranged from 0.125 to 0.259 g. The heritability of radicle length, however, was contingent to the treatment involved. While no additive genetic variance could be estimated in the short-distance treatment, significant dam effects were detected (Table 1). The radicle length of full-sib families ranged from 10.2 to 20.7 mm. In the long-distance treatment, heritability values were low and non-significant, and the radicles of sire families ranged from 12.4 to 32.9 mm. Significant dam effects for this trait were also detected and full-sib families had radicles that ranged from 10.9 to 40.1 mm. Radicles showed a significant plasticity as revealed by the significant treatment term in the mixed-model analysis (Table 2). Radicles in the short-distance treatment were shorter on the average than radicles in the long-distance treatment (Table 1). Even though sire and dam variances, as well as the sire  $\times$  treatment interaction did not show statistical significance, the interaction dam  $\times$  treatment was statistically significant (Table 2, Fig 1A).

The seeds assigned to the short-distance treatment showed a higher establishment on the average (48.6%,  $N = 527$ ) than seeds assigned to the long-distance treatment (9.9%,  $N = 435$ ) (Fig. 1B), which implies that the chance of successful establishment

**Table 1** Descriptive statistics, variance components, and heritability ( $\pm$ standard error) of traits of *Tristerix aphyllus*

Trait	Sire	Dam	<i>N</i> -value	Mean (mm) $\pm$ SE	$V_s$	$V_d$	$V_e$	$h^2_{\text{Sire}} \pm \text{SE}$
Seed mass (g)	21	58	2,531	0.175 $\pm$ 0.001	0 <sup>NS</sup>	0.0011***	0.0007***	0
Radicle length (SDT)	21	58	527	13.66 $\pm$ 0.023	0 <sup>NS</sup>	1.7897*	25.0541***	0
Radicle length (LDT)	21	58	435	21.16 $\pm$ 0.51	14.4429 <sup>NS</sup>	32.1279**	69.8049***	0.496 $\pm$ 0.318

The significance level for heritability values was calculated from  $z$ -tests

$V_s$  is the sire variance component,  $V_d$  is dam variance component, and  $V_e$  is the error variance component. Radicle length was calculated for each treatment [*SDT* short-distance treatment (4 mm), *LDT* long-distance treatment (28 mm)]

\*\*\*  $P < 0.001$ , \*\*  $P < 0.01$ , \*  $P < 0.05$ , <sup>NS</sup>  $P \geq 0.05$  ( $P$ -value, ANOVA test for each trait)

**Table 2** Summary of results of a linear mixed-model analysis of radicle length data using PROC MIXED

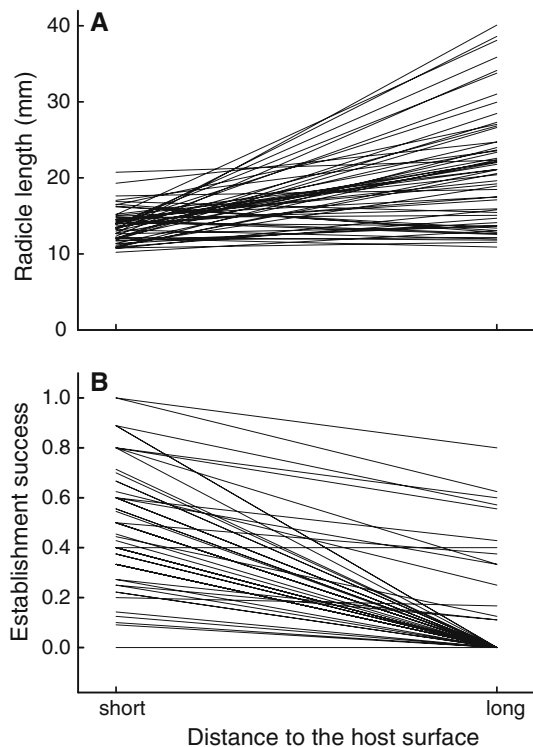
Source	Variance estimate	z-value	P-value
Sire	1.676 ± 3.389	0.49	0.6210
Dam (sire)	0	0	–
Sire × treatment	5.590 ± 4.432	1.26	0.2072
Dam(sire) × treatment	16.448 ± 3.758	4.75	0.0001
Residual	45.119 ± 2.201	20.51	0.0001
Fixed effect	<i>F</i>		<i>P</i>
Treatment (df = 1, 20)	41.14		0.0001

is contingent to the distance from the host surface where seed deposition occurs. Significant additive genetic variation for sire effects was detected for the seed establishment process (Table 3).

### Fitness-trait relationship

Both radicle length and seed mass were under direct selection in the short-distance treatment (Table 4). Short radicles and large seeds attained a higher chance of establishment. However, in spite of the significant effects, the multiple regression model accounted for a low (5%) variation in establishment success. Likewise, the univariate selection differentials revealed significant selection for increasing radicle length and seed mass in the long-distance treatment. However, only the radicle length remained significant after removing indirect effects. Unlike the short-distance

**Fig. 1** Reaction norms for radicle length (A) and establishment (B) of *Tristerix aphyllus* across two experimental treatments related with the distance of seed deposition to the host surface (short-distance and long-distance, 4 and 28 mm, respectively). Each line represents the reaction norm for one dam family ( $N = 58$  dam families)





**Table 3** Summary of results of a generalized linear mixed-model analysis of establishment using GLIMMIX macro

	Estimate	z-value	P-value
Sire	0.4386 ± 0.1667	2.63	0.0085
Dam (sire)	0.4386 ± 0.1667	2.63	0.0085
Sire × treatment	0.4368 ± 0.2786	1.57	0.1169
Dam(sire) × treatment	0.1357 ± 0.1833	0.74	0.4591
Fixed factor	<i>F</i>		<i>P</i>
Treatment ( <i>df</i> = 1.20)	82.63		0.0001

Standard errors (*SE*) were estimated from asymptotic theory following variance component estimation. *P*-values were obtained from *z*-values (variance component divided by standard error) in a maximum-likelihood ratio test

treatment, the multiple regression model accounted for a large (45%) fraction of variation in establishment success. The pattern of selection on radicle length differed between the distance treatments as indicated by significant radicle length × treatment interaction in GLIM analysis (Wald statistics = 47.98, *P* < 0.0001), and the plastic response induced by each environment was in the same direction of the phenotype favored by selection within treatments (Fig. 2).

In general, results from genetic and phenotypic analysis were consistent. In the short-distance treatment, short radicles (albeit non-significant,  $S_i \pm SE = <minus> 0.076 \pm 0.064$ , *P* = 0.239; and  $\beta_i \pm SE = <minus> 0.084 \pm 0.061$ , *P* = 0.177) and large seeds ( $S_i \pm SE = 0.145 \pm 0.061$ , *P* = 0.022; and  $\beta_i \pm SE = 0.149 \pm 0.061$ , *P* = 0.018) had a higher successful establishment. In the long-distance treatment, long radicles were favored ( $S_i = 0.162 \pm 0.019$ ; *P* < 0.0001; and  $\beta_i = 0.154 \pm 0.020$ , *P* < 0.0001) and seed mass was only indirectly selected ( $S_i = 0.069 \pm 0.027$ , *P* = 0.016; and  $\beta_i = 0.031 \pm 0.020$ , *P* = 0.119). The plasticity of radicle length showed a positive selection differential ( $S_i = 0.504 \pm 0.056$ ; *P* < 0.0001), indicating that plastic rather than canalized radicles are favored across environments. Finally, we did not find a significant between-treatment association for radicle length (Pearson’s product-moment correlation, *r* = 0.066, *P* = 0.623), which implies that between-environment correlations probably do not constraint the evolution of plasticity in this species.

**Table 4** Linear coefficients of phenotypic selection coefficients on radicle length and seed mass

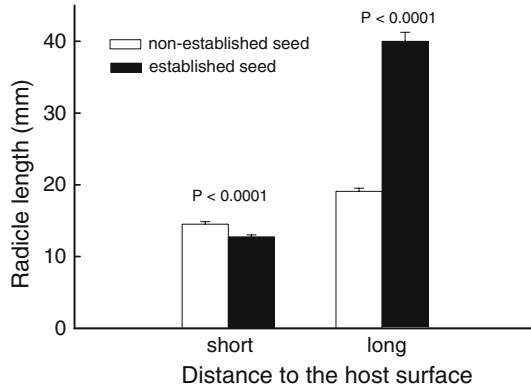
	$S_i \pm SE$	$\beta_i \pm SE$
Short-distance ( <i>N</i> = 512)		
Radicle length	-0.170 ± 0.043***	-0.173 ± 0.043**
Seed mass	0.113 ± 0.043*	0.114 ± 0.043*
<i>R</i> <sup>2</sup> = 5.0%		
Long-distance ( <i>N</i> = 421)		
Radicle length	1.767 ± 0.118***	1.73 ± 0.118***
Seed mass	0.512 ± 0.143**	0.314 ± 0.118
<i>R</i> <sup>2</sup> = 46.0%		

Coefficients were estimated in each treatment (short-distance and long-distance, 4 and 28 mm, respectively). *S<sub>i</sub>* is the standardized univariate differential of selection, and *β<sub>i</sub>* is the standardized linear gradient of selection

*SE* standard error

\*\*\* *P* < 0.0001; \*\* *P* < 0.001; \* *P* < 0.01; NS *P* ≥ 0.05 after Bonferroni sequential adjustment

**Fig. 2** Mean radicle length ( $\pm$ SE) in the two experimental treatments (short-distance and long-distance to the host surface, 4 and 28 mm, respectively) as a function of the status of establishment



## Discussion

We have presented different lines of evidence that support the hypothesis of phenotypic plasticity for radicle length, a critical trait of the holoparasitic mistletoe *T. aphyllus* that is involved in the first stages of the infection process. Our results not only describe that natural selection acting upon radicle length changes significantly across microenvironments but also that plasticity is consistent with the expected phenotypes that are favored by natural selection within environments. The observation that plasticity rather than optimizing selection promoted a single optimal parasite phenotype emphasizes the importance of considering flexible rather than canalized responses of parasites when faced with host defensive traits.

Recent works have documented that the expression of genetic variance (and covariance) is contingent on the environment in which it is measured (Houle 1991; Roff 1997; Hoffmann and Merilä 1999). Moreover, both the additive genetic variance ( $V_A$ ) and heritability ( $h^2$ ) tend to change between environments, resulting in environment-specific estimates (Merilä and Sheldon 2000; Conner et al. 2003; Messina and Fry 2003). In this particular case, the relative contribution of sire and dam effects to the overall variation increased with the distance to the host, which suggests that the evolutionary response to selection may change across experimental environments. Even though the heritability of radicle length and seed mass did not differ from zero in this study, we detected significant dam effects. Variation in the offspring phenotype due to effects of the maternal parent can result from (1) an additive, heritable fraction attributable to segregation of the nuclear genes expressed in maternal individuals, (2) a fraction attributable to organelle genes, which are often maternally inherited; and (3) a non-heritable fraction that include the maternal environment and gene interactions expressed in the maternal parent (Byers et al. 1997). Thus, non-nuclear genes, dominance, and maternal effects can all contribute to the variance in phenotypic traits (Roff 1997). Even though our breeding design does not permit to us distinguish the specific maternal source of variation, it is widely known that besides the direct expression of genes following their Mendelian transmission to progeny, many traits of juveniles (i.e., seed size) tend to be greatly influenced by maternal attributes (Roach and Wulff 1987; Rossiter 1996; Byers et al. 1997).

The shape and distribution of reaction norms has important implications for the evolution of plasticity because they characterize the potential of a population to respond to selection imposed by environmental heterogeneity. While there is ample evidence of genetic variation for plasticity (reviewed in Sultan 1987; Scheiner 1993; Karban and Baldwin 1997; Tollrian and Harvell 1999), evidence for additive genetic variance on trait plasticity is more limited (e.g., Juenger and Bergelson 2000; Relyea 2002, Agrawal et al. 2002). In this study we detected significant plasticity for radicle length across experimental environments. However, we did not detect additive genetic variance for plasticity among paternal half-sib families. It is possible that selection events in the past have reduced heritable variation on this trait. The evolutionary potential through maternal family variation for plasticity must be interpreted with caution because the maternal component may include environmental and non-additive genetic variation, which in turn may overestimate the additive genetic component (Roff 1997). On the other hand, the absence of between-environment correlation for radicle length permits us to reject this factor as a potential constraint on the evolution of adaptive plasticity in this trait (Via and Lande 1985).

Regarding seed establishment, our results revealed significant sire and dam main effects, and no family  $\times$  treatment interactions (Table 3). This indicates a significant additive genetic covariance between states of seed establishment across treatments. Some genotypes performed better in the two experimental environments, which raises the question of the mechanisms responsible for the existence of additive genetic covariance in the two experimental treatments. One possible answer lies in the fact that our fitness estimate is based on an early life-history stage that is critical for subsequent parasitic plant survival (Kuijt 1969; Musselman and Press 1995; Norton and Carpenter 1998). It is known that additive fitness variance quantified on early life-history stages tend to be higher than those estimated on later stages (Barrowclough and Rockwell 1993; Campbell 1997). It is likely that antagonistic pleiotropic effects with another fitness component maintain the additive genetic variation on seed establishment. Clearly, more work is needed to test this hypothesis.

Our results provide evidence that support, at least in part, the hypothesis of adaptive plasticity in the radicle of *T. aphyllus*. We have shown that (1) natural selection differed between experimental environments and (2) environmentally induced radicle plasticity is expressed toward the phenotype that is favored by selection within each environment (Dudley 1996; Dudley and Schmitt 1996; van Buskirk et al. 1997; Dorn et al. 2000; Juenger and Bergelson 2000). Seedlings from heavier and short radicle-lengthed seeds were favored in the environment close to the host epidermis where a small radicle elongation is required to contact the host. Because external sources of water and mineral nutrients, which are often available to most seedlings of autotrophic species, are unavailable to the mistletoe (Lamont 1983), seedlings of *T. aphyllus* may require a rapid contact and penetration to the host tissues in the face of external desiccation (Botto-Mahan et al. 2000) and large seeds provide immediate energy to reach the vascular traces within the host (Mauseth 1985, 1990). The situation in the long-distance treatment corroborates the somewhat expected result that long radicles were favored over short ones. This result may be related to the high amount of nutrients that large seeds often contain in the endosperm, which facilitate radicle growth and the chance of seedling establishment. This hypothesis is partially supported by the observation that

elongation rate correlates positively with seed size (Pearson's product-moment correlation,  $r = 0.11$ ,  $P < 0.0001$ ;  $N = 421$ ).

Although our evidence strongly suggest an adaptive role for radicle plasticity, it is necessary to explain the contradictory observation that the mean radicle length in the short-distance treatment (13.7 mm, Table 1) greatly exceeds the length necessary to reach the host surface (4 mm). It is possible that the short-distance treatment simulates a very rare situation that limits the potential for selection to shorten the radicle length under these circumstances. However, about 15% of seeds are deposited in direct contact with the cactus cuticle, which makes this supposition untenable. Alternatively, aspects related to the geometry of radicle elongation may account at least partially for this observation. Our study based on the assumption that radicle elongation proceeds in a straight line and perpendicular to the host surface. This is not necessarily true because radicles often require a minimal length to develop the haustorial plate, especially in cases where short secondary spines act as additional barriers to infection. Moreover, it is not infrequent to observe seeds that elongate radicles below the spine axes, probably due to gravity. Whether the geometry of radicle elongation provides a satisfactory explanation for our conflicting data needs to be evaluated in future studies.

Our findings have important consequences for a more complete description of the ecological and evolutionary forces molding the peculiar reciprocal adaptations in the cactus-mistletoe system. Previous experimental and correlative evidence indicate that long spines in Chilean cacti probably evolved as a way to prevent bird perching, seed deposition, and mistletoe infection (see Silva and Martínez del Río 1996; Medel 2000; Medel et al. 2002, 2004). On the contrary, the extremely long radicles shown by *T. aphyllus* have been suggested to be a counteradaptation that overcomes the long spines of cacti (Martínez del Río et al. 1995). Our data suggest that long radicles may be adaptive only under a restricted set of circumstances. Rather, plastic radicles may be a better evolutionary strategy to cope with the heterogeneous environment provided by the cactus morphological defense.

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## Reference

- Agrawal AA (1998) Induced response to herbivory and increased plant performance. *Science* 279:1201–1202
- Agrawal AA, Conner JK, Johnson MTJ, Wallsgrove R (2002) Ecological genetics of an induced plant defense against herbivores: additive genetic variance and costs of phenotypic plasticity. *Evolution* 56:2206–2213
- Barrowclough GF, Rockwell RF (1993) Variance of lifetime reproductive success estimation based on demographic data. *Am Nat* 141:281–295
- Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. *Adv Genet* 13:115–155
- Botto-Mahan C, Medel R, Ginocchio R, Montenegro G (2000) Factors affecting the circular distribution of the leafless mistletoe *Tristerix aphyllus* (Loranthaceae) on the cactus *Echinopsis chilensis*. *Rev Chil Hist Nat* 73:525–531
- Byers DL, Platenkamp GAJ, Shaw RG (1997) Variation in seed characters in *Nemophila menziesii*: evidence of a genetic basis for maternal effect. *Evolution* 51:1445–1456

- Campbell DR (1997) Genetic and environmental variation in life history traits of a monocarpic perennial: a decade-long field experiment. *Evolution* 51:373–382
- Cockerham CC, Weir BS (1977) Quadratic analyses of reciprocal crosses. *Biometrics* 33:187–203
- Conner JK, Franks R, Stewart C (2003) Expression of additive genetic variance and covariances for wild radish floral traits: comparison between field and greenhouse environments. *Evolution* 57:487–495
- Crawley MJ (1993) *GLIM for ecologists*. Blackwell Scientific Publications, Oxford, UK
- DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits to benefits as constraints on the evolution of phenotypic plasticity. *Trends Ecol Evol* 13:77–81
- Donohue K, Pyle EH, Messiqua D, Heschel MS, Schmitt J (2001) Adaptive divergence in plasticity in natural population of *Impatiens capensis* and its consequences for performance in novel habitats. *Evolution* 55:692–702
- Dorn LA, Pyle EH, Schmitt J (2000) Plasticity to light cues and resources in *Arabidopsis thaliana*: testing for adaptive value and costs. *Evolution* 54:1982–1994
- Dudley SA (1996) Differing selection on plant physiological traits in response to environmental water availability: a test of adaptive hypotheses. *Evolution* 50:92–102
- Dudley SA, Schmitt J (1996) Testing the adaptive plasticity hypothesis: density-dependent selection on manipulated stem length in *Impatiens capensis*. *Am Nat* 147:445–465
- Fry JD (1992) The mixed-model analysis of variance applied to quantitative genetics: biological interpretation of the parameters. *Evolution* 46:540–550
- Gomulkiewicz R, Kirkpatrick M (1992) Quantitative genetics and the evolution of reaction norms. *Evolution* 46:390–411
- González WL (2004) Valor adaptativo de la longitud de la radícula de la planta holoparásita *Tristerix aphyllus* (Loranthaceae): detectando causas de selección natural. Tesis de Doctorado. Facultad de Ciencias, Universidad de Chile
- González WL, Suárez LH, Medel R (2006) Outcrossing increases infection success in the holoparasitic mistletoe *Tristerix aphyllus* (Loranthaceae). *Evol Ecol* doi: 10.1007/s10682-006-0021-0
- Hoffmann A (1986) Cactáceas en la flora silvestre de Chile. Ediciones Fundación Claudio Gay, Santiago, Chile
- Hoffmann AA, Merilä J (1999) Heritable variation and evolution under favourable and unfavourable conditions. *Trends Ecol Evol* 14:96–101
- Houle D (1991) Genetic covariance of fitness correlates—what genetic correlations are made of and why it matters? *Evolution* 45:630–648
- Jaksic FM (2001) Ecological effects of El Niño in terrestrial ecosystems of western South America. *Ecography* 24:241–250
- Janzen FJ, Stern HS (1998) Logistic regression for empirical studies of multivariate selection. *Evolution* 52:1564–1571
- Juenger T, Bergelson J (2000) The evolution of compensation to herbivory in scarlet gilia, *Ipomopsis aggregata*: herbivore-imposed natural selection and the quantitative genetics of tolerance. *Evolution* 54:764–777
- Karban R, Baldwin IT (1997) *Induced responses to herbivory*. University of Chicago Press, Chicago
- Kuijt J (1969) *The biology of parasitic flowering plants*. University of California Press, Berkeley
- Kuijt J (1988) Revision of *Tristerix* (Loranthaceae). *Syst Bot Monogr* 19:1–61
- Lamont B (1983) Germination of mistletoes. In: Calder M, Bernhardt P (eds) *The biology of mistletoes*. Academic Press, Sidney, pp 129–143
- Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. *Evolution* 37:1210–1226
- Littell RC, Milliken GA, Stroup WW, Wolfinger RD (1996) *SAS System for mixed models*. SAS Institute, Cary, NC
- Lively CM (1986) Canalization versus developmental conversion in a spatially variable environment. *Am Nat* 128:561–572
- Martínez del Río C, Silva A, Medel R, Hourdequin M (1996) Seeds dispersers as disease vectors: bird transmission of mistletoe seeds to plant hosts. *Ecology* 77:912–921
- Martínez del Río C, Hourdequin M, Silva A, Medel R (1995) The influence of cactus size and previous infection on bird deposition of mistletoe seeds. *Aust J Ecol* 20:571–576
- Mauseth JD (1985) Relations between *Trichocereus chilensis* and the holoparasite *Tristerix aphyllus*. *Medio Ambiente* 7:39–44
- Mauseth JD (1990) Morphogenesis in a highly reduced plant: the endophyte of *Tristerix aphyllus* (Loranthaceae). *Bot Gaz* 151:348–353

- Mauseth JD, Montenegro G, Walckowiak AM (1984) Studies of the holoparasite *Tristerix aphyllus* (Loranthaceae) infecting *Trichocereus chilensis* (Cactaceae). *Can J Bot* 62:847–857
- Mauseth JD, Montenegro G, Walckowiak AM (1985) Host infection and flower formation by the parasite *Tristerix aphyllus* (Loranthaceae). *Can J Bot* 63:567–581
- Medel R (2000) Assessment of parasite-mediated selection in a host-parasite system in plants. *Ecology* 81:1554–1564
- Medel R (2001) Assessment of correlational selection on tolerance and resistance traits in a host plant-parasitic plant interaction. *Evol Ecol* 15:37–52
- Medel R, Botto-Mahan C, Smith-Ramirez C, Mendez MA, Ossa CG, Caputo L, González WL (2002) Historia natural cuantitativa de una relación parásito-hospedero: el sistema *Tristerix-cactáceas* en Chile semiárido *Revista Chilena de Historia. Natural* 75:127–140
- Medel R, Vergara E, Silva A, Kalin-Arroyo M (2004) Effects of vector behavior and host resistance on mistletoe aggregation. *Ecology* 85:120–126
- Merilä J, Sheldon BC (2000) Lifetime reproductive success and heritability in nature. *Am Nat* 155:301–310
- Messina FJ, Fry JD (2003) Environment dependent reversal of a life history trade-off in the seed beetle *Callosobruchus maculatus*. *J Evol Biol* 16:501–509
- Musselman LJ, Press MC (1995) Introduction to parasitic plants. In: Press MC, Graves JD (eds) *Parasitic plants*. Chapman and Hall, London, UK, pp 1–13
- Norton DA, Carpenter MA (1998) Mistletoes as parasites: host specificity and speciation. *Trends Ecol Evol* 13:101–105
- Pigliucci M (2001) *Phenotypic plasticity: beyond nature and nurture*. Johns Hopkins Press, Baltimore, MD
- Rausher MD (1992) The measurement of selection on quantitative traits: biases due to environmental covariances between traits and fitness. *Evolution* 46:616–626
- Relyea RA (2002) Cost of phenotypic plasticity. *Am Nat* 159:272–282
- Roach DA, Wulff RD (1987) Maternal effects in plants. *Annu Rev Ecol Syst* 18:209–235
- Roff DA (1997) *Evolutionary quantitative genetics*. Chapman and Hall, New York
- Rossiter MC (1996) Incidence and consequences of inherited environmental effects. *Annu Rev Ecol Syst* 27:451–476
- Rundel PW (1976) Population variability in the genus *Trichocereus* (Cactaceae) in central Chile. *Plant Syst Evol* 127:1–9
- SAS institute (1997) *SAS/STAT software: changes and enhancements through release 6.12*. SAS Institute, Cary, NC
- Scheiner SM (1993) Genetics and evolution of phenotypic plasticity. *Annu Rev Ecol Syst* 24:35–68
- Scheiner SM, Berrigan D (1998) The genetic of phenotypic plasticity. VIII. The cost of plasticity in *Daphnia pulex*. *Evolution* 52:368–378
- Schlichting CD (1986) The evolution of phenotypic plasticity in plants. *Annu Rev Ecol Syst* 17:667–693
- Schlichting CD, Pigliucci M (1995) Gene-regulation, quantitative genetics and the evolution of reaction norms. *Evol Ecol* 9:154–168
- Schlichting CD, Pigliucci M (1998) *Phenotypic evolution: a reaction norm perspective*. Sinauer Associates, Sunderland
- Silva A, Martínez del Río C (1996) Effects of the mistletoe *Tristerix aphyllus* (Loranthaceae) on the reproduction of its cactus host *Echinopsis chilensis*. *Oikos* 75:437–442
- Smith-Ramírez C (1999) Selección fenotípica secuencial sobre rasgos reproductivos del muérdago *Tristerix aphyllus*. Tesis de Doctorado. Facultad de Ciencias, Universidad de Chile
- Spitze K, Sadler TD (1996) Evolution of a generalist genotype: multiple analysis of the adaptiveness of phenotypic plasticity. *Am Nat* 148:S108–S123
- Sultan SE (1987) Evolutionary implications of phenotypic plasticity in plants. *Evol Biol* 21:127–178
- Tollrian R, Harvell CD (1999) *The ecology and evolution of inducible defenses*. Princeton University Press, Princeton, NJ
- Van Buskirk J, Mccollum SA, Werner EE (1997) Natural selection for environmentally induced phenotypes in tadpoles. *Evolution* 51:1983–1992
- Via S, Conner J (1995) Evolution in heterogeneous environments: genetic variability within and across different grains in *Tribolium castaneum*. *Heredity* 74:80–90
- Via S, Gomulkiewicz R, de Jong G, Scheiner SM, Schlichting CD, Van Tienderen PH (1995) Adaptive phenotypic plasticity—consensus and controversy. *Trends Ecol Evol* 10:212–217
- Via S, Lande R (1985) Genotype-environmental interaction and the evolution of phenotypic plasticity. *Evolution* 39:1317–1331