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Exogenous selection shapes germination behaviour and seedling traits of populations at different altitudes in a *Senecio* hybrid zone

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• *Background and Aims* The *Senecio* hybrid zone on Mt Etna, Sicily, is characterized by steep altitudinal clines in quantitative traits and genetic variation. Such clines are thought to be maintained by a combination of 'endogenous' selection arising from genetic incompatibilities and environment-dependent 'exogenous' selection leading to local adaptation. Here, the hypothesis was tested that local adaptation to the altitudinal temperature gradient contributes to maintaining divergence between the parental species, *S. chrysanthemifolius* and *S. aethnensis*.

• *Methods* Intra- and inter-population crosses were performed between five populations from across the hybrid zone and the germination and early seedling growth of the progeny were assessed.

• *Key Results* Seedlings from higher-altitude populations germinated better under low temperatures (9–13 °C) than those from lower altitude populations. Seedlings from higher-altitude populations had lower survival rates under warm conditions (25/15 °C) than those from lower altitude populations, but also attained greater biomass. There was no altitudinal variation in growth or survival under cold conditions (15/5 °C). Population-level plasticity increased with altitude. Germination, growth and survival of natural hybrids and experimentally generated F_1s generally exceeded the worse-performing parent.

• *Conclusions* Limited evidence was found for endogenous selection against hybrids but relatively clear evidence was found for divergence in seed and seedling traits, which is probably adaptive. The combination of low-temperature germination and faster growth in warm conditions might enable high-altitude *S. aethnensis* to maximize its growth during a shorter growing season, while the slower growth of *S. chrysanthemifolius* may be an adaptation to drought stress at low altitudes. This study indicates that temperature gradients are likely to be an important environmental factor generating and maintaining adaptive divergence across the *Senecio* hybrid zone on Mt Etna.

Key words: *Senecio aethnensis*, *S. chrysanthemifolius*, hybrid, germination, exogenous selection, early life history, temperature.

INTRODUCTION

Most hybrid zones are thought to be maintained in a balance between natural selection and the diffusion of genes by dispersal across the species boundary (Moore, 1977; Barton and Hewitt, 1985). But what is the nature of such selection, and on what traits does it act? On the one hand, selection might be 'exogenous', whereby fitness depends on the ecological context in which genes are expressed (G × E interactions) (Endler, 1977; Moore, 1977; Harrison and Rand, 1989; Barton and Gale, 1993). On the other hand, selection might be 'endogenous', with the fitness of genes depending on the genomic context in which they are expressed (G × G interactions). Here, genes are selected against when they are expressed in the background of a different species' genome (Dobzhansky, 1936, 1937; Muller, 1942; Mayr, 1963; Barton and Hewitt, 1985; Barton, 2001). In practice, these two modes of selection are not mutually exclusive and they result in very similar clines in allele frequencies across a hybrid zone (Kruuk *et al.*, 1999). A key question is therefore the relative extent to which endogenous vs. exogenous selection shapes the genetic and phenotypic clines we observe in a particular hybrid setting.

Recent studies in plants using reciprocal transplant experiments in hybrid zones across environmental gradients have found strong evidence for exogenous selection in terms of significant genotype by environment (G × E) interactions, but there is comparatively weak evidence for endogenous selection (including Wang *et al.*, 1997; Miglia *et al.*, 2005; Campbell *et al.*, 2008; Kameyama *et al.*, 2008; Kimball *et al.*, 2008; Taylor *et al.*, 2009). While it is accepted that environmental clines can contribute to the maintenance of hybrid zones, identifying the specific environmental variable responsible has been challenging (Campbell, 2004; Angert, 2006). In contrast to the number of studies considering the role of biotic variation, the extent to which abiotic gradients specifically play a role in shaping plant hybrid zones has been surprisingly little studied. Temperature is one of the primary determinants

of species distributions on a global scale and so may also determine species distributions across a smaller spatial scale such as a narrow hybrid zone. Altitudinal gradients potentially provide a fruitful context in which to investigate this effect as ambient temperature decreases by approx. 5·5 °C per 1000 m increase in altitude (Körner, 2007), and this temperature change affects a wide range of life-history traits, including growth rates and germination behaviour (Körner and Larcher, 1988; Baskin and Baskin, 1998).

Mortality at the seed and seedling stages tends to be high (Harper, 1977; Kitajima and Fenner, 2000), meaning that selection acting on early life-history traits might have a disproportionate effect on gene flow across a hybrid zone. For logistical reasons, field experiments measuring exogenous selection often start with established seedlings, yet the direction of selection at these early stages of development need not match that acting at later stages (Johnston *et al.*, 2003). Therefore field studies that start with seedlings may fail to find evidence for the role of exogenous selection in shaping a cline even when it is present (Gimenez-Benavides *et al.*, 2008). Common garden experiments with seeds and seedlings are a useful way to explore which environmental factors affect fitness-related traits, indicating how exogenous selection might act.

Here, we investigate possible divergence of early life-history traits between the groundsel species *Senecio aethnensis* and *S. chrysanthemifolius*, which hybridize across a steep altitudinal gradient on the slopes of Mt Etna in Sicily, Italy. Both species are diploid ($2n = 20$), herbaceous, self-incompatible, short-lived perennials (Crisp, 1972). The main hybrid zone between them, confirmed by RAPD and chloroplast haplotype analysis (James and Abbott, 2005), lies along a road on the southern slope of the volcano, between about 1300 and

1800 m a.s.l. *S. aethnensis* occurs between 2000 and 3500 m a.s.l. and *S. chrysanthemifolius* occurs from sea level up to about 1000 m (Fig. 1). Numerous phenotypic traits vary between the two species across the hybrid zone, including leaf dissection (see Fig. 1), capitulum size and achene length, and patterns of gene expression (Ronsisvalle, 1968; Crisp, 1972; Abbott *et al.*, 2000; Hegarty *et al.*, 2009). Given the steep environmental gradient across which the hybrid zone occurs, it seems likely that exogenous selection plays a role in maintaining species integrity, but the nature of this selection remains unknown. Recent clinal analysis suggests that endogenous selection against hybrids has probably also been important in shaping the hybrid zone (Brennan *et al.*, 2009), but there is as yet no direct evidence for reduced fitness in hybrid genotypes.

Our study tests the hypothesis that germination behaviour and early seedling growth is sensitive to endogenous selection by comparing the performance of F_1 hybrids and a natural hybrid population with that of the parental species across a range of temperatures. G × G interactions result in deviations from the expected result under an additive genetic architecture (Lynch and Walsh, 1998). We therefore expect that, if intergenomic or cytonuclear incompatibilities are weak or absent, the trait values of the F_1 hybrid will equal the pooled average of the parents, while negative G × G interactions would be manifested by poorer F_1 performance than predicted from the mid-parent average (Rhode and Cruzan, 2005). Alternatively, hybridization may lead to heterosis, whereby F_1 trait values deviate in a positive direction from the mid-parental average (Falconer, 1981). The most common cause of heterosis is probably dominance or overdominance at loci where, as a result of inbreeding or genetic drift, inferior alleles have become fixed in the parental species (Whitlock *et al.*, 2000; Burke and Arnold, 2001). Interestingly, microarray studies in the *Senecio* species complex studied here have also identified non-additive changes in gene expression in early-generation hybrids (Hegarty *et al.*, 2009); such changes may also result in heterosis if they translate into observable changes in phenotype. As the level of heterozygosity decreases each generation after hybridization, we expect the natural hybrid population not to show heterosis (Rhode and Cruzan, 2005). Non-additive negative performance in the natural hybrid combined with non-additive positive performance in the F_1 indicates both heterosis and hybrid breakdown. We also investigate whether populations of the two parental species differ in their response to different temperatures in terms of their seed germination behaviour, seedling growth and seedling survival, which gives an indication whether exogenous selection might be involved in structuring the hybrid zone.

MATERIALS AND METHODS

Sampling and seed material

We collected seeds of *Senecio aethnensis* and *S. chrysanthemifolius* by sampling at least 30 individuals from each of five populations on the southern slope of Mt Etna, including the parental species and three hybrid populations from intermediate altitudes and with intermediate morphology (Table 1 and Fig. 1). To minimize possible maternal effects on germination and seedling growth,
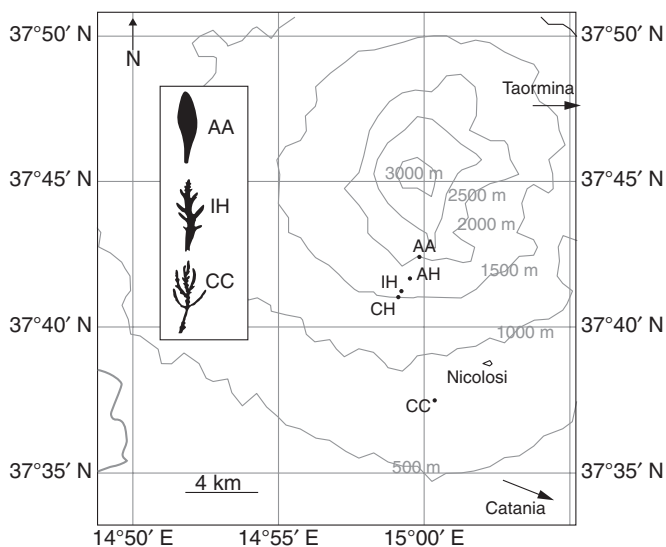

FIG. 1. Sample populations of *S. chrysanthemifolius*, *S. aethnensis* and their hybrids on Mt Etna, Sicily. *Senecio chrysanthemifolius* is found up to 1000 m, the hybrid zone lies between 1000 and 2000 m, and *S. aethnensis* is found above 2000 m. The insert shows the variation in leaf morphology between the parental species and their hybrids. See Table 1 for an explanation of population codes.

we grew plants from each population to maturity in a common glasshouse at the Department of Plant Sciences, University of Oxford, and crossed them to produce seed for our experiment. Genetic background had previously been established at population level by genetic assays (James and Abbott, 2005; Brennan *et al.*, 2009). Leaf morphology of glasshouse-grown plants supported the designation of populations as either hybrid or parental.

Plants were established as single individuals from each of ten half-sib families per population in 5-inch pots containing a 3 : 1 mixture of peat-based compost and grit. Ten blocks were set up, each containing one replicate plant from each population. The glasshouse was maintained on a 16-h photoperiod with a constant temperature of approx. 22 °C, and watering was performed as necessary. Crosses were performed by rubbing ripe inflorescences together, as described in Hiscock (2000). The parental and natural hybrid populations were replicated through crosses among individuals within each population (intra-population crosses). The first generation seed produced from these crosses was used in the subsequent experiments to reduce maternal effects. Between seven and 15 intra-population crosses per natural population were performed. A similar number of inter-population crosses between the two parental populations were performed to generate the F_1 hybrid cross-type. The F_1 cross-type and the progeny of intra-population crosses are collectively referred to below as 'genetic classes'.

Seed germination experiment

We established bulked seed samples of 180 seeds for each genetic class. Seeds were stratified by sowing on moist filter paper and placing in the dark at 4 °C for 1 week. A linear thermal gradient was set up between 7 °C and 35 °C using a one-metre temperature gradient bar and monitored at 17-cm intervals with a thermocouple array (Berti and Johnson, 2008). Temperatures were fixed without oscillation. Two lanes were randomly allocated to each genetic class and 90 seeds sown at equal spacing along each lane. Seeds were watered daily and lighting provided by fluorescent strip lights on a 12-h photoperiod. Germination was recorded daily as the time at which cotyledons were fully expanded. The experiment was run for 35 d, at which point almost all the seeds in the central part of the bar had germinated.

Seedling growth experiment

Seedlings were collected from the temperature bar once they had germinated, transplanted to 3-inch pots containing peat-based compost and randomly allocated to warm (25 °C/15 °C) or cold (15 °C/5 °C) growth cabinets (SANYO-Biomedical Europe MLR plant growth chambers) over a period of 16 d. Although it would have been preferable to use only individuals that germinated at the same temperature, the temperature at which individuals germinated will not have influenced the results because individuals were randomly allocated to the seedling growth treatments. Between 26 and 41 seedlings (mean = 35) were transplanted for each treatment × genetic class combination. The temperatures used for the warm and cold treatments were based on weather station data in the habitat of *S. aethnensis* (cold) and *S. chrysanthemifolius* (warm) during spring (available from Meteo Sicilia). All cabinets were set to 75 % humidity and a 12-h photoperiod. Pots were randomized within cabinets daily. As there may be other non-measured differences between cabinets, cabinets were allocated to different temperature regimes each week (leaving other conditions the same) and pots were moved between cabinets so that they maintained the same treatment. Pots were watered daily by irrigating the capillary matting beneath them.

Seedlings were harvested after 83 d (stratified by potting date). At harvest, the above-ground height was measured and chlorophyll content was estimated using a Minolta SPAD-502 meter (Kariya *et al.*, 1982) on a living mid-cauline leaf, which was then air-dried and weighed using a Sartorius 1212 balance. The dry above-ground biomass was also weighed. Seedlings that died before harvesting were also recorded to determine the mortality rate. Mortality rate was only assessed on seedlings that had survived to the first true leaf stage, to exclude death due to damage incurred on transplantation as this was assumed to be independent of seed source.

Data analysis

All statistical analyses were implemented in the statistical software package, R, versions 2·12·1 to 2·14·1 (R Development Core Team, 2010–2011). The final germination proportion was compared between the two parental genetic classes (AA and CC) in a generalized linear model (GLM) with binomial errors. Linear orthogonal treatment contrasts

TABLE 1. *Locality information for the sample populations used to generate seed material for the experiments*

Population	Description	Altitude (m)	Latitude (N)	Longitude (E)
CC	*S. chrysanthemifolius*	749	37°36·861′	15°00·961′
CH	*S. chrysanthemifolius*-like hybrid	1532	37°40·783′	14°58·901′
IH	Intermediate hybrid	1624	37°41·128′	14°59·215′
AH	*S. aethnensis*-like hybrid	1820	37°41·669′	14°59·505′
AA	*S. aethnensis*	2097	37°42·409′	14°59·830′

Genetic assays confirmed these populations to be either largely parental or hybrid genotypes (James and Abbott, 2005; Brennan *et al.*, 2009). Populations were collected from the road side or on recent lava flows, growing in fine volcanic soil and generally unshaded. A map of the region is shown in Fig. 1.

were fitted to compare the F_1 and intermediate altitude natural hybrid (IH) to the expected mid-parent value to test whether the traits being investigated conformed to an additive genetic structure (Campbell and Waser, 2001). Throughout the Results section, only the intermediate altitude hybrid is statistically compared with the F_1 in this manner. Final germination proportion was analysed in a GLM with quasibinomial errors. Final biomass, height, leaf mass and SPAD-value were analysed using ANOVA. Final biomass, leaf mass and SPAD-value were transformed to help meet the assumptions of the Normal model, as suggested by the boxcox function in R. Seedling survival of the F_1 and IH was compared against the combined survival of the parental genetic classes using a proportion test.

To investigate altitudinal variation in life-history traits, trait values were regressed on the collection altitude of the field-sampled populations that were used as the parents of the experimental generation (not including the F_1 cross-type). Low temperature seed germination responses were investigated by stratifying the data into 2 °C temperature intervals from 7 to 19 °C and regressing the germination proportion for each interval on altitude of seed source. To account for non-independence of seeds sampled from the same population, a single value for the germination proportion was calculated for each population and temperature interval combination, logit transformed [log $y/(1 - y)$] (Warton and Hui, 2011) and regressed on altitude, rather than using a GLM with binomial errors. Pearson's product moment correlation was also calculated.

Seedling traits were analysed by ANCOVA, with altitude of seed source and temperature treatment as the independent variables. Where interaction terms were significant, the data for each treatment were independently regressed on altitude. To account for non-independence, we used a mixed-effects model, with population fitted as a random effect and altitude and temperature fitted as fixed effects. We used the *lme* package in R for linear mixed-model analyses and the *MCMCglmm* package to analyse seedling survival rates (Hadfield, 2010). Significance of fixed effects for *lme* models was tested by comparing the maximum likelihood fits of full and reduced models using a likelihood ratio test. In addition, the mean values for each population were regressed on the altitude of the source populations.

Phenotypic plasticity was quantified for each genetic class using the Relative Distances Plasticity Index (RDPI), which gives a value between 0 (no plasticity) and 1 (maximal plasticity) (Valladares *et al.*, 2006):

$$\text{RDPI} = \sum \left(d_{ij} \to i'j'/x_{i'j'} + x_{ij} \right)/n$$

where d_{ij} is the absolute value of the distance between trait values (x) for all pairs of individuals (j) within a genetic class grown under different temperature regimes (i) and n is the number of distances. Genetic-class-level RDPI was compared between traits using an ANOVA and compared between populations by linear regression.

RESULTS

Intrinsic genetic effects

Of the 42 inter-population crosses performed to generate the F_1, 37 crosses were successful in at least one direction. Crosses seemed to be equally successful independent of the species of the maternal and paternal parent, i.e. the direction of crossing did not appear to affect success rates. *S. chrysanthemifolius* was the maternal parent for 11 of the 25 families used in the experiment. Thus there is no clear evidence for asymmetric pre-zygotic incompatibility.

For most seed and seedling traits, trait values of both natural and experimental F_1 hybrid genotypes were intermediate between the parental values, with the exception of seedling growth and survival under cold conditions (Tables 2 and 3). *Senecio chrysanthemifolius* germinated significantly less well than *S. aethnensis* across the range of experimental temperatures ($t = -3.26$, $P = 0.02$; Fig. 2). Germination proportion of F_1 and natural hybrid seeds was equal to the mid-parent value (F_1: $t = -0.65$, $P = 0.53$; IH: $t = 0.48$, $P = 0.64$). Average time to germination was similar for all the genetic classes (ranging between 7 and 10 d) and the F_1 and natural hybrid values were between the parental values. F_1 and natural hybrid class means were not different from the expected mid-parent value for seedling biomass, height, leaf mass, chlorophyll content (SPAD value) and survival under warm conditions (Table 3). Under cold conditions, the mean biomass, height, and leaf mass of the F_1 class exceeded the mid-parent average ($P < 0.04$ for all traits). The natural hybrid class mean was lower than the mid-parent average for height ($P = 0.04$) and survival rate ($P < 0.01$). F_1 and natural hybrid class means were not different from the expected mid-parent value for chlorophyll content.

Temperature effects on seed germination

Seeds from high-altitude populations were better able to germinate at low temperatures than seeds from low-altitude populations. Germination rate followed a negative quadratic relationship with temperature ($\chi^2 = 358.85$, d.f. $= 2$, $P < 0.0001$), and the shape of this relationship varied between populations ($\chi^2 = 125.22$, d.f. $= 12$, $P < 0.0001$). Germination occurred over a greater range of temperatures in higher-altitude populations ($R^2 = 0.68$, $P = 0.001$; Table 2). Germination proportion at moderately low temperatures

TABLE 2. *Comparison of germination characteristics between genetic classes*

Genetic class	Germination proportion	Average germination time (d)	Germination range (°C)
CC	0·51	10·2 (0·6)	19·3
CH	0·76	8·4 (0·5)	23·8
IH	0·64	8·2 (0·6)	25·6
AH	0·73	8·1 (0·5)	25·3
AA	0·82	7·2 (0·5)	24·7
F_1	0·64	9·2 (0·5)	23·8

Average germination time is given with standard errors in brackets.

TABLE 3. *Comparison of the performance of natural and F_1 hybrid seedlings to seedlings of the parental species under cold (15/5 °C) and warm (25/15 °C) growing conditions*

(A) Trait means

Treatment	Genetic class	Trait means and standard error				
		Biomass (mg)	Height (mm)	Leaf mass (mg)	Chlorophyll content	Survival (%)
Cold	AA	23·2 (3·5)	53·4 (3·1)	3·6 (0·5)	34·4 (1·2)	100
	CC	30·6 (8·1)	52·8 (5·9)	4·6 (0·8)	30·4 (1·7)	84
	F_1	38·1 (5·4)	60·5 (3·0)	4·9 (0·6)	34·1 (1·1)	78
	IH	19·4 (2·7)	44·5 (2·7)	2·8 (0·3)	30·9 (1·4)	62
Warm	AA	381·9 (51·1)	134·5 (7·4)	20·0 (2·2)	40·3 (2·1)	52
	CC	271·1 (56·3)	104·3 (7·9)	15·5 (1·8)	36·8 (1·8)	96
	F_1	363·7 (62·7)	134·1 (7·2)	21·7 (3·0)	41·9 (2)	57
	IH	413·1 (52·3)	122·7 (7·2)	17·7 (1·7)	41·4 (1·5)	50

(B) Linear treatment contrasts

Treatment	Genetic class		Trait being compared				
			Biomass	Height	Leaf mass	Chlorophyll content	Survival
Cold	F_1	Statistic	2·64	2·75	2·33	1·02	2·8
		P	**<0·01**	**<0·01**	**0·02**	0·31	0·09
	IH	Statistic	1·3	2·45	1·68	0·63	8·8
		P	0·2	**0·02**	0·1	0·53	**<0·01**
Warm	F_1	Statistic	−0·40	−1·56	1·30	1·25	1·40
		P	0·69	0·12	0·20	0·22	0·23
	IH	Statistic	−1·43	−0·02	0·44	−0·85	3·55
		P	0·16	0·98	0·66	0·40	0·06

Part (A) gives the trait means for each genetic class (standard errors in brackets); part (B) presents the results of linear treatment contrasts between the natural and F_1 hybrid genetic class to the mid-parent average.

The test statistic is t, except survival where statistic is χ^2 (proportion test). Bold indicates P-values <0·05.
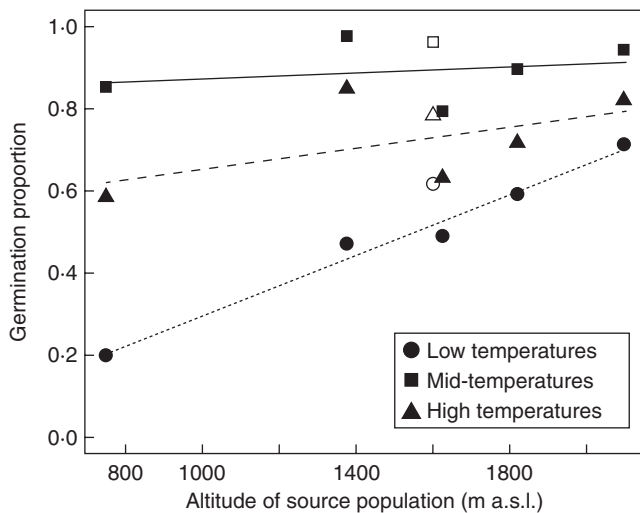

FIG. 2. Germination proportions by the end of the experiment stratified by temperature for each genetic class. F_1 hybrids (open symbols) are plotted at 1600 m a.s.l. for display purposes. Low temperatures = 7−14 °C; mid-temperatures = 14−26 °C; high temperatures = 26−35 °C.

TABLE 4. *Comparison of germination responses to different temperatures in* Senecio *populations sampled from different altitudes*

Temperature interval (°C)	F	d.f.	P	Pearson's r	P
7–9	3·11	1,3	0·18	0·60	0·28
9–11	42·62	1,3	**<0·01**	0·98	**<0·01**
11–13	16·75	1,3	**0·03**	0·98	**<0·001**
13–15	3·55	1,3	0·16	0·59	0·30
15–17	3·50	1,3	0·16	0·27	0·66
17–19	4·53	1,3	0·12	0·74	0·15

For each 2 °C interval, germination proportion per genetic class was regressed on the sampling altitude (expressed in units of 100 m increase in altitude) and Pearson's product moment correlation also calculated. Bold indicates P-values <0·05.

(9−11 °C interval and 11−13 °C interval) increased with altitude, whereas germination success was similar across all populations at very low temperatures (7−9 °C) and at higher temperatures (13−19 °C; Table 4 and Fig. 3). The natural hybrid and F_1 genetic classes were intermediate between the parental species in their ability to germinate across the 7−19 °C range (F_1: $t = −0·94$, $P = 0·34$; IH: $t = 0·37$, $P = 0·72$).

Temperature effects on seedling survival and growth

Seedlings from high-altitude populations grew better under warm conditions than those from low-altitudes but also suffered higher mortality rates; in contrast, there were no significant trends with altitude under cold conditions. A lower proportion of seedlings survived between the one leaf stage and harvest in the warm treatment compared with the cold
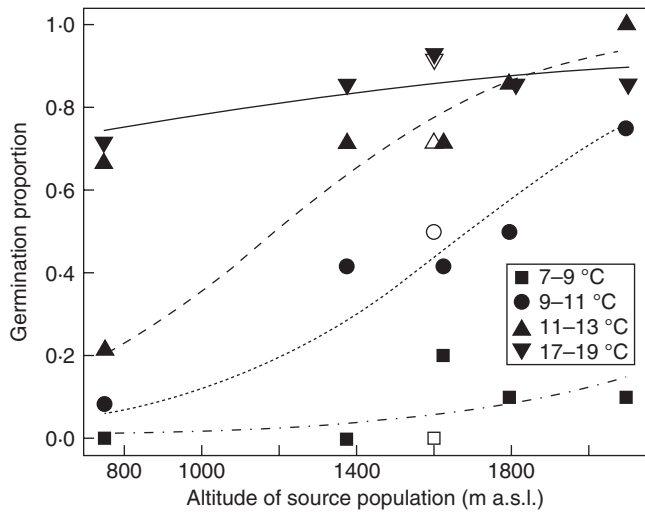
FIG. 3. Germination proportion at different temperatures. Each point represents germination proportion over a 2 °C window for one genetic class and the lines are the predictions of a GLM of germination proportion on altitude. F_1 hybrids are plotted at 1600 m a.s.l. for display purposes and represented by open symbols; temperature ranges are indicated in the key (17–19 °C values are displaced slightly on the *x*-axis as points are overlapping). The 9–11 °C and 11–13 °C intervals exhibited a significant correlation with altitude ($P < 0.01$, $P = 0.03$); other intervals were uncorrelated ($P > 0.1$). The 13–15 °C and 15–17 °C intervals have not been plotted but were also not correlated ($P > 0.1$).
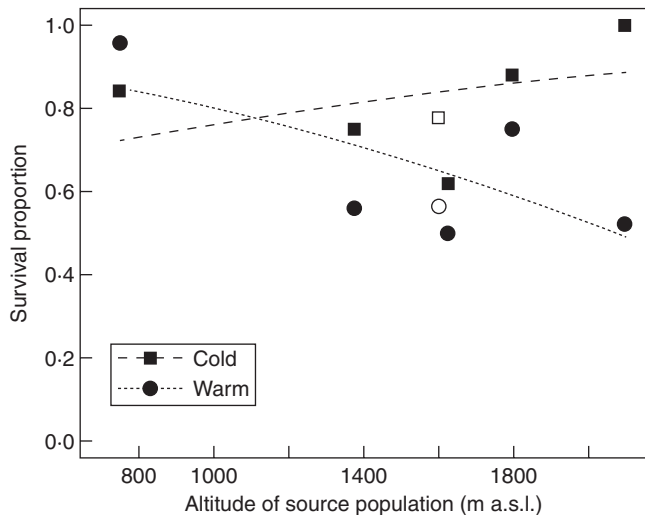

FIG 4. Proportion of seedlings surviving to harvest in cold (15/5 °C) and warm (25/15 °C) growing conditions, as indicated in the key. F_1 hybrids are plotted at 1600 m a.s.l. for display purposes and represented by open symbols. The lines are the predictions of a GLM of survival on altitude under warm ($P = 0.09$) and cold ($P = 0.39$) conditions.

treatment (*MCMC P* = 0·002; Fig. 4). In the warm treatment, the proportion of seedlings surviving decreased with increasing altitude of sampling site, but the difference fell short of significance (*MCMC P* = 0·088). In the cold treatment, there was no altitude effect (*MCMC P* = 0·394).

Seedlings from all source populations reached greater biomass in the warm treatment than the cold treatment ($P < 0.0001$; Fig. 5). Seedlings from high-altitude populations
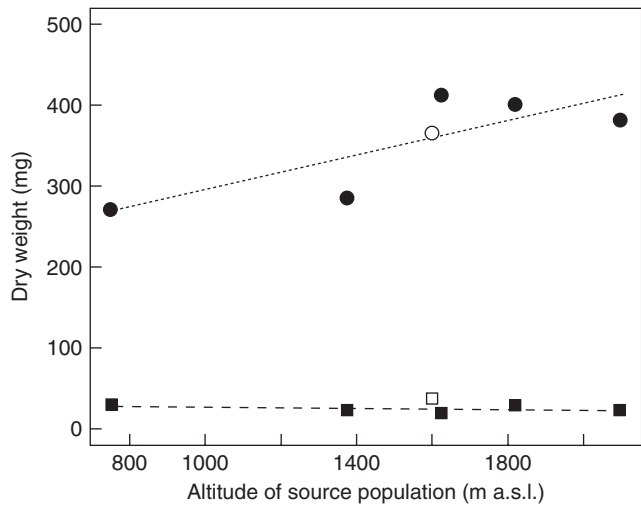

FIG. 5. Above-ground biomass of seedlings in cold (15/5 °C) and warm (25/15 °C) growing conditions. Squares and circles represent cold and warm, respectively. Open and closed symbols represent hybrid and natural populations, respectively.

had greater biomass than those from low-altitudes in the warm treatment ($P = 0.01$), while there was no altitude effect in the cold treatment. Height and leaf mass displayed similar patterns (Table 5). The SPAD-value of leaves, a measure of chlorophyll content, was significantly greater in warmer temperatures and was not affected by altitude (Table 5). There was a marginally significant trend for high-altitude populations to exhibit greater temperature-dependent plasticity of growth-related traits (traits = dry weight, height and leaf mass; $P = 0.07$, 0·09, and 0·05, respectively). Phenotypic plasticity varied significantly between traits ($F_{4,25} = 179.5$, $P < 0.001$). Biomass was the most plastic trait while chlorophyll content was quite unresponsive to growing temperature (Fig. 6).

DISCUSSION

Our study has revealed significant G × E interactions between temperature and sampling altitude for both seed germination and early seedling growth in experimental and natural hybrids from the *Senecio* hybrid zone on Mt Etna. In contrast, we found limited evidence for negative G × G interactions. Our results thus point to the likely role of exogenous selection and the evolution of local adaptation across a steep environmental gradient in structuring this plant hybrid zone.

Our study found no convincing evidence that hybrid genotypes (whether natural late-generation hybrids or experimentally generated F_1 genotypes) have uniformly lower fitness than parental genotypes. Instead, seed germination rates and, for the majority of trait and treatment combinations, the seedling traits of natural hybrids were intermediate between the parental species. Heterosis can mask hybrid unfitness, particularly in early-generation hybrids (Fenster and Galloway, 2000; Johansen-Morris and Latta, 2006; Rhode and Cruzan, 2005). However, this is unlikely to be the case here, as trait values in experimentally generated F_1 hybrids did not exceed the

TABLE 5. *Effect of temperature, seed source altitude and their interaction on seedling above-ground dry biomass; seedling height, dry mass of one mid-cauline leaf, and chlorophyll content of a mid-cauline leaf (estimated from the SPAD-value) under cold (15/5 °C) and warm (25/15 °C) growing conditions*

| | ANCOVA | | | | Regression on altitude | | | | | |
| | Temperature | | Interaction | | Warm | | | Cold | | |
Trait	LR	*P*	LR	*P*	LR	*P*	*P**	LR	*P*	*P**
Biomass	220·31	**<0·0001**	8·40	**<0·01**	6·21	**0·01**	0·07	0·22	0·64	0·60
Height	212·29	**<0·0001**	10·22	**<0·01**	8·93	**<0·01**	**<0·01**	0·02	0·88	0·94
Leaf mass	211·86	**<0·0001**	10·25	**<0·01**	6·85	**<0·01**	**0·02**	1·42	0·23	0·29
Chlorophyll	46·52	**<0·001**	<0·01	0·98	2·10	0·15	0·60	2·71	0·10	0·93

P is the significance given by a likelihood ratio (LR) test with 1 d.f. and *P** is the *P*-value obtained from an *F*-test using the mean values for each genetic class. Bold indicates *P*-values<0·05.
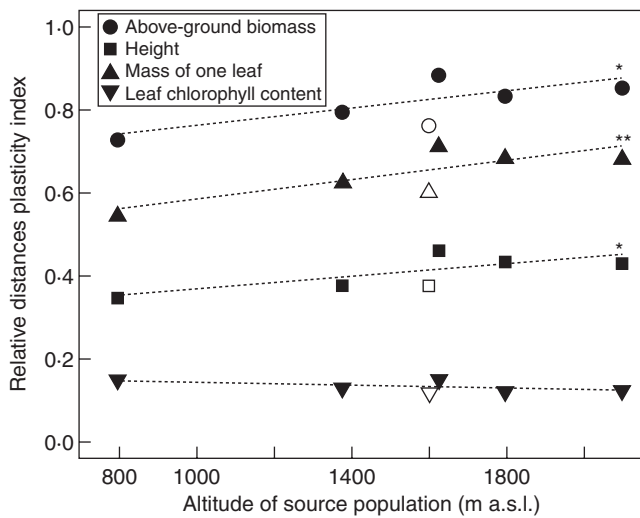

FIG. 6. Population-level plasticity of growth traits compared between parental and natural hybrid populations (filled symbols) and experimental hybrids (open symbols) using the relative distances plasticity index, where 0 = no plasticity and 1 = maximal plasticity. Growth traits are above-ground biomass, height, mass of one mid-cauline leaf, and mid-cauline leaf chlorophyll content (as indicated in the key). Asterisks indicate significant relationships with altitude: **$P = 0.05$; *$0.05 < P < 0.09$.

average of the two parental species for most treatment and trait combinations.

Instead, the impact of endogenous selection may be environmentally dependent. The seedling survival and height of intermediate altitude natural hybrids was lower than predicted from the parental performance under cold conditions but not under warm conditions. In contrast, we found evidence for F_1 heterosis for several growth-related traits, also under cold conditions only. This suggests that the effects of deleterious recessive mutations on parental fitness (which give rise to heretosis in first-generation hybrids) might only be revealed under more stressful conditions (Parsons, 1971; Charlesworth and Charlesworth, 1987; Bijlsma *et al.*, 2001). In any case these contrasting results suggest that plant performance across the *Senecio* hybrid zone on Mt Etna depends on interactions with the environment (G × E interactions) and is not solely determined by endogenous factors (G × G interactions).

We found that high- and low-altitude populations exhibited divergent seed germination responses at low temperatures. Specifically, we found that the minimum temperature at which germination occurred was lower for seeds derived from high altitude populations than seeds derived from low-altitude populations and, between 9 °C and 13 °C, seeds from higher-altitude populations germinated better than lower altitude populations. This pattern, also observed in the herb *Reynoutria japonica* on Mt Fuji, Japan (Mariko *et al.*, 1993), probably reflects adaptation to the decrease in spring temperature with altitude.

Under the warm temperature regime in our experiment, seedlings from populations more likely to experience warm temperatures in the field displayed higher survivorship than populations sampled from colder sites (although the difference was only marginally significant). In contrast, we found no evidence that high-altitude genotypes were specially adapted to cold temperatures. Our results are similar to those found for a *Mimulus* altitudinal hybrid zone, where low-altitude *M. cardinalis* showed greater survival than high-altitude *M. lewisii* when grown experimentally under a warm temperature regime representative of the native site of *M. cardinalis*, and differences in survival were observed under warm conditions but not cold (Angert, 2006).

Contrary to our expectation of local adaptation, seedlings from higher-altitude populations grew faster under warm conditions than seedlings from lower-altitude populations. This would suggest that plasticity of growth-related traits tends to increase with altitude in these species. The patterns observed in this study contrast with those typically found in temperate altitudinal gradients, where alpine plants tend to be less plastic than related low-altitude species (Körner and Larcher, 1988). We propose that this difference stems from the additional constraint of drought and light stress faced in Mediterranean ecosystems (Calloway *et al.*, 2002; Gimenez-Benavides *et al.*, 2005).

The combination of low temperature seed germination, rapid seedling growth and temperature-dependent growth plasticity observed in *S. aethnensis* may reflect an adaptation to high altitudes by enabling individuals to capitalize on short periods of favourable weather. In Mediterranean ecosystems, increases in altitude can coincide with a shift in the major environmental stress facing plants, from drought in the lowlands (Thompson, 2005; Padilla and Pugnaire, 2007;

Gimenez-Benavides *et al.*, 2008) to chilling and frost damage in montane environments (Mariko *et al.*, 1993; Galen and Stanton, 1999). In montane habitats, germination at low temperatures and the ability to grow quickly allows plants to reach a size required to flower in their first year, or to survive the next winter (Harper, 1977; Mariko *et al.*, 1993; Vera, 1997; Shimono and Kudo, 2003, Milla *et al.*, 2009). For lowland Mediterranean plants, establishment success depends on an ability to avoid water shortage during the summer months, and slow above-ground growth may thus reflect increased allocation to roots as part of a stress-tolerance strategy (Volis *et al.*, 2002; Padilla and Pugnaire, 2007; Wu *et al.*, 2008).

Previous studies on the relative importance of endogenous versus exogenous selection in maintaining the *Senecio* hybrid zone on Mt Etna have reached conflicting conclusions (James and Abbott, 2005; Brennan *et al.*, 2009). On the one hand, the steep environmental gradient and fertility of hybrids points to a key role for exogenous selection (James and Abbott, 2005); on the other hand, the genetic and phenotypic clines across the zone better match the predictions of an endogenous selection model (Brennan *et al.*, 2009). While the present study does not resolve this issue, we have uncovered significant G × E interactions that affect seed and seedling traits and that are likely to translate into differences in the relative fitness of both parents and hybrids with variation in ambient temperature across the hybrid zone. The combination of early-season seed germination and rapid seedling growth might enable high-altitude genotypes to capitalize on periods of favourable weather, which is adaptive at high altitude as the growing season is shorter, but may reduce survival at low altitudes. In our study, putatively adaptive traits, such as germinability at low temperatures or temperature-dependent growth plasticity, varied in such a way as to approximately track the temperature gradient across the Mt Etna hybrid zone. Hybrids were therefore likely to exhibit more adaptive phenotypes at intermediate altitudes, i.e. the centre of the zone, than pure parental genotypes. These patterns are consistent with the suggestions of Anderson (1948) and Moore (1977) that hybrids might flourish in disturbed habitats in environments intermediate to those occupied by their parental species, referring to the role in hybrid maintenance played by 'hybridization of the habitat'. Certainly, our results point to a potentially important role for exogenous selection acting on the early life stages in maintaining the hybrid zone.

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