

ENVIRONMENTAL FACTORS PREDICT ADAPTIVE PHENOTYPIC DIFFERENTIATION WITHIN AND BETWEEN TWO WILD ANDEAN TOMATOES

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Environmental variation is widely viewed as a major force driving morphological change and speciation. Although many environmental attributes are potentially critical for adaptive responses within and between species, the individual and relative importance of these diverse attributes remain poorly understood. Here we combine a geographical information systems (GIS)-based analysis of environmental variation with a multipopulation analysis of phenotypic, physiological, and genetic variation, to generate and test hypotheses of environmental factors likely driving adaptive divergence within and between two wild Andean plant species. First, we document large environmental differences between population locations of the two species, and among regions within species. Second, we show evidence for inter- and intraspecific differences in genetically based phenotypic and physiological variation. Third, combining these data, we report evidence for trait–environment associations both among populations within species, and between species, that are strongly indicative of recent and rapid adaptive responses. Finally, we show that these trait–environment associations cannot be simply explained by genetic relatedness within species, reinforcing our inference that local, regional, and species-wide environmental conditions are responsible for phenotypic and physiological diversification. The strongest trait–environment associations involve temperature and precipitation gradients, suggesting these climatic factors are predominant drivers of adaptive diversification in these species.

KEY WORDS: Cline, drought resistance, *Lycopersicon*, macroecology, *Solanum pimpinellifolium*, *Solanum lycopersicum* var. *cerasiforme*.

Adaptation to local environment has long been considered a major factor driving phenotypic change and speciation (Huxley 1942; Mayr 1942; Simpson 1944, 1953; Schluter 2001; Levin 2005). Ecotypic differences have been demonstrated in many different systems (e.g., Bradshaw 1984; Schlichting and Pigliucci 1998), apparently in response to environmental factors ranging from abiotic features such as climate (Joshi et al. 2001; McKay et al. 2001) and soil type (Snaydon and Davies 1982; Gauthier et al. 1998),

to biotic interactions among organisms (Parker 1995; Linhart and Grant 1996; Prati and Schmid 2000). Although gene flow between populations can retard local adaptation (reviewed in Lenormand 2002), there is also evidence that ecological adaptation can be accomplished in the presence of ongoing gene flow (Clausen et al. 1940; Antonovics and Bradshaw 1970; Via 1991; Comstock and Ehleringer 1992; Nevo et al. 1998; Schlieven et al. 2001; Linn et al. 2003)—sometimes within a few generations (Antonovics

et al. 1971; Freeman and Byers 2006; Franks et al. 2007)—suggesting that natural selection is often surprisingly strong. Nonetheless, although many diverse factors have been identified as potentially critical in influencing adaptive responses within and between species (Schluter 2000), directly linking natural environmental variation with adaptive phenotypic responses remains challenging.

One difficulty lies in identifying the critical environmental factors that likely shape species and population differentiation. Recently, “macroecological analyses” (Brown 1999; Gaston 2003) have been used to infer adaptive differentiation between populations or species. These approaches identify environmental correlates of species’ ranges using Geographical Information Systems (GIS) analyses that combine known species occurrence records with data from broadscale environmental databases (e.g., Brown et al. 1996). The expectation is that critical environmental factors should define species ranges; strong species–environment associations therefore suggest which environmental factors are likely most influential in shaping species and/or ecotypic differences. This approach can provide insight into the nature of climatic, geographic, and other factors that coincide with species or ecotypic ranges. However, these associations are purely correlational, and frequently the best-investigated study systems are not amenable to follow-up experiments designed to evaluate adaptive hypotheses emerging from these analyses (Kawecki and Ebert 2004). Therefore, it remains unclear whether species–environment associations truly capture adaptive differences and, if so, what physiological mechanisms underlie these adaptive responses.

A potentially powerful approach to resolving this disjunct is to combine species-wide GIS-based analyses of climatic variables with a direct examination of heritable morphological and physiological variation at the species and population level. This allows hypothesized adaptive differences identified in macroecological analyses to be directly evaluated with data on genetically based phenotypic differentiation within and between species. The resulting trait–environment associations are more compelling evidence of adaptively mediated differentiation, especially where there are plausible mechanistic links between the observed trait variation and environmental differences (e.g., Ackerly and Reich 1999 and references therein). To date there has been little effort to combine GIS-based climate modeling approaches with quantitative genetic analyses within and between species to understand the predominant factors, and corresponding phenotypic responses, involved in recent adaptive diversification.

In this study, our aim was to combine GIS-based macroecological analyses of abiotic environmental variation with a direct evaluation of phenotypic and physiological variation within and between two closely related wild plant species from the tomato clade, *Solanum* sect. *Lycopersicon*. The center of diversification of wild tomato species is from Ecuador to Chile, along the Andean

mountains and the adjacent Pacific coast. The recent rapid uplift of the Andes since the Eocene (~55 Mya, Campbell 1975; Stibane 1975) has created a dramatic altitudinal cline (sea level to > 6500 m) and correspondingly diverse climate zones ranging from temporal desert along the western coast (e.g., 12–23 °C average temperature range) to seasonable highlands in the Andes (e.g., temperature range: –7 to 21 °C) to tropical rainforests in the Amazon basin (e.g., temperature range: 22 to 28 °C), all within 500 km (Young et al. 2002). Similarly, despite recent divergence of the tomato group (~7 million years (MY), Nesbitt and Tanksley 2002), each species appears to display a characteristic geographical distribution pattern and habitat preference across this environmentally diverse region (Rick 1973, 1978, 1979; Taylor 1986; Smith and Peralta 2002; T. Nakazato, D. Warren, and L. C. Moyle, unpubl. data). They are also morphologically diverse, and some traits are likely adaptive responses to their habitats (e.g., Rick 1973, 1976, 1978; Patterson et al. 1978; Vallejos 1979; Bloom et al. 2004), suggesting that abiotic ecological conditions play a critical role in these species’ phenotypic evolution and speciation.

Focusing on two wild tomato species, *Solanum lycopersicum* var. *cerasiforme* (SC) and *Solanum pimpinellifolium* (SP), our goals in this analysis are threefold: (1) to evaluate whether there are systematic environmental differences between species, and between regions within species, that might be responsible for recent phenotypic divergence and speciation in the group; (2) to assess whether this environmental variation is associated with genetically based phenotypic and physiological variation within and between species (i.e., strong trait–environment associations indicative of adaptation); and (3) to independently assess genetic relatedness within and among species using putatively neutral molecular markers, to evaluate whether simple genetic relatedness is likely to explain observed trait–environment associations. The simultaneous examination of detailed geographical, climatic, phenotypic, physiological, and genetic relationships, allows us to evaluate the relative importance of each in describing and explaining patterns of adaptive differentiation within and among our focal species.

Materials and Methods

STUDY SYSTEM AND SPECIES

Wild and cultivated tomatoes form a relatively small monophyletic clade within the diverse family Solanaceae (Darcy 1978), consisting of 14 closely related species or subspecies. All members of the section are diploids ($2n = 24$; Peralta and Spooner 2001; Nesbitt and Tanksley 2002) that share a high degree of genomic synteny (Chetelat and Ji 2007), and are to some degree intercrossable (Rick 1979). Recent taxonomic revision has nested the tomato clade (formerly the genus *Lycopersicon*) within the genus *Solanum* sect. *Lycopersicon*, based on molecular genetic analyses

(Peralta and Spooner 2001; Spooner et al. 2005). We use the revised nomenclature here, however continue to refer to the clade as *Lycopersicon*, to retain continuity with the large historical literature on this group.

Our analysis focuses on two wild Andean tomato species: *S. pimpinellifolium* and *S. lycopersicum* var. *cerasiforme*. These species are very closely related to each other and to the domesticated tomato, based on classical morphological and molecular analyses (reviewed in Spooner et al. 2005). *Solanum l. cerasiforme* is most likely the sister group of the domesticated tomato (*S. lycopersicum* var. *esulentum*), and is unusual among tomato species because it occurs in wet environments of the lowland Amazon basin and other areas of the subtropics, although some accessions are found on the western coast of South America (Rick and Fobes 1975); it is unclear whether these western populations are representative of the natural range of *S. l. cerasiforme* (see further below). *Solanum pimpinellifolium* is closely related (e.g., <1% silent site divergence; Nesbitt and Tanksley 2002) to both *S. lycopersicum* varieties (Rick and Fobes 1975) but is largely restricted to the western slopes of the Andes and the adjacent coastal regions (Taylor 1986).

STUDY POPULATIONS

We obtained seed accessions from the Tomato Genetics Resource Center (TGRC) at the University of California at Davis, representing 19 populations: five populations from the Amazon basin and four from the coastal regions and the Galapagos islands for *S. l. cerasiforme*, and five populations each from mid-elevation and coastal regions for *S. pimpinellifolium* (Fig. 1, online Supplementary Table S1). Although there are many wild-collected populations available for these species (www.tgrc.ucdavis.org), we limited our study to a subset of these to enable a more detailed analysis of each population for phenotypic and molecular genetic traits. Our study populations were selected a priori on the basis of two criteria: first, to span the majority of each species' known geographic range in South America and, second, to enable a pairwise intraspecific comparison among populations found in contrasting geographical environments within each species (i.e., Amazon vs. coastal in *S. l. cerasiforme*, and coastal vs. mid-elevation in *S. pimpinellifolium*) (Fig. 1). Note that several "coastal" populations of *S. l. cerasiforme* were originally collected from sites in close contact with human settlement (online Supplementary Table S1). *Solanum l. cerasiforme* is commonly found in the Amazon basin, but its occurrence along the western coast of South America might reflect recent adventive colonization (Taylor 1986). Common garden experiments have been used to assess whether introduced plant populations differ genetically from native populations, and/or whether they show phenotype-climate matching to their new environments, to evaluate the role of recent adaptation in the persistence of exotics under

new environmental circumstances (e.g., Maron et al. 2007). For Amazon versus coastal/Galapagos accessions of *S. l. cerasiforme*, therefore, climate-specific trait differentiation between these regions might implicate the role of adaptive phenotypic change in the persistence of colonist populations; alternatively it might indicate that successful colonists are drawn from nontypical populations within the native range (Maron et al. 2007). Conversely, lack of climate-matched physiological or phenotypic differentiation between Amazon and coastal *S. l. cerasiforme* suggests that the latter might primarily be recent weedy commensal populations with little adaptation to natural local environmental conditions. These alternatives are discussed further in the Results.

Each population accession used in the study has a known geographical location (latitude and longitude; available from <http://tgrc.ucdavis.edu>), based on map interpolation of the place names of the original collection site; geographical locations are therefore generally accurate to the minute scale, that is, to within ~1.85 km.

Finally, note that our analysis uses some germplasm collections that have been maintained and cultivated as seed stocks for multiple generations. Germplasm maintenance is most likely to have the effect of alleviating or changing the selective pressures originally experienced in the natural source populations; this would tend to reduce the strength of the trait-environment associations we are examining, making our analysis relatively conservative rather than overly permissive. In addition, individual germplasm collections might become increasingly homogenized over time due to sampling and breeding effects. To indirectly examine the potential genetic consequences of extended periods of germplasm maintenance, we assessed the effect of accession collection year on the amount of genetic variation detected within each population. We found no associations between variation (proportion polymorphic loci for AFLPs, or within population variance for each quantitative trait) and time since accession collection in either species (data not shown). Regardless, the use of greenhouse-propagated seed in our experiment allows us to exclude potential maternal environmental effects on our examined quantitative traits.

GEOGRAPHICAL, CLIMATIC, AND ENVIRONMENTAL DATA

We used spatial data layers to determine environmental conditions associated with the 19 populations. Based on known population accession locations (latitude and longitude), we extracted ecogeographical data from nine GIS maps of climatic and environmental variables sourced from two databases: Worldclim interpolated climate and altitude data (Hijmans et al. 2005) and USDA NCRS soil data (US Department of Agriculture, Natural Resources Conservation Service, Soil Survey Division, World Soil Resources, Washington, D.C., <http://www.nrcs.usda.gov>). Worldclim data (altitude

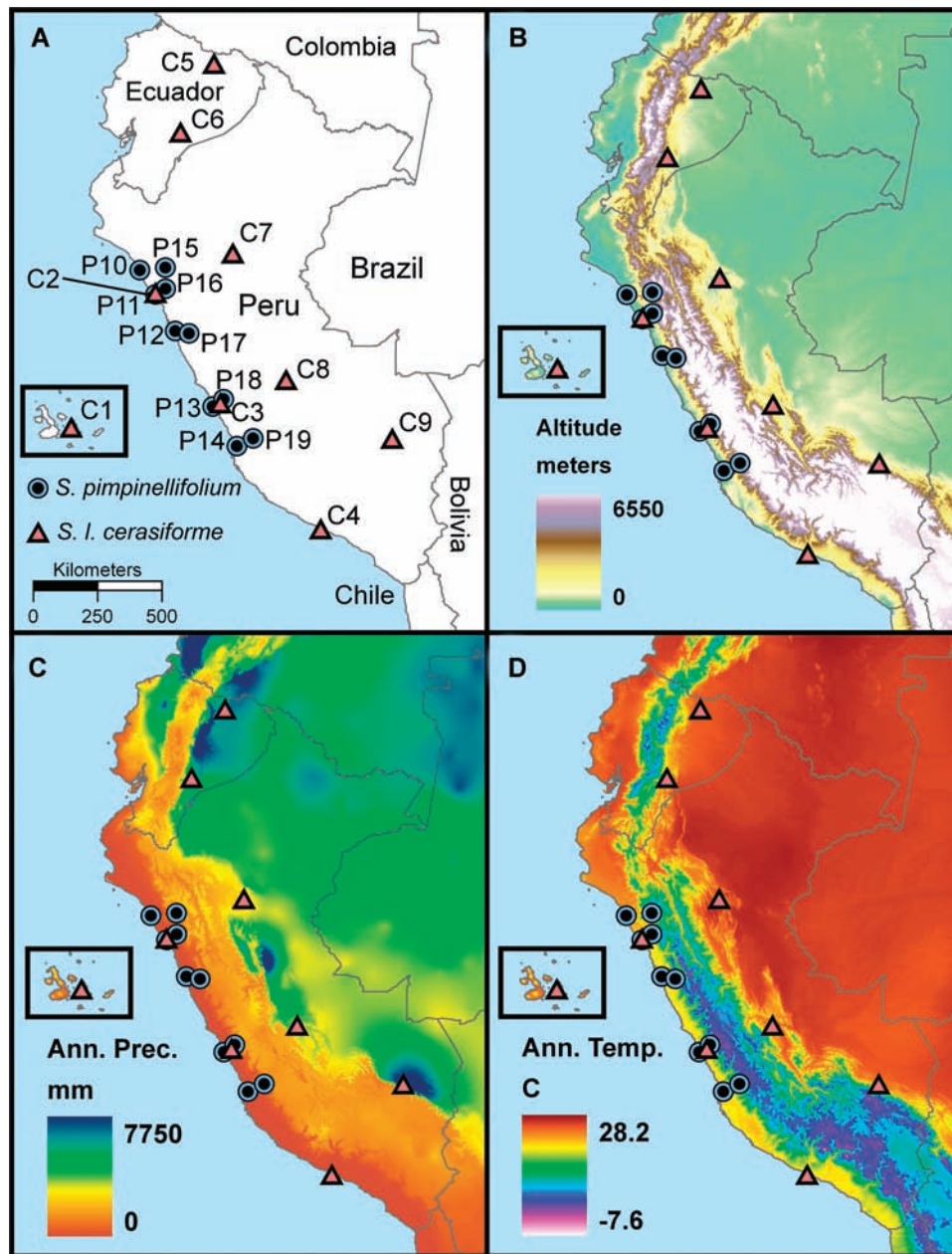


Figure 1. Map of the study region, including (A) collection locations of accessions (populations), (B) altitude gradient, (C) mean annual precipitation gradient, (D) mean annual temperature gradient. Triangles and circles represent sample locations of *S. l. cerasiforme* and *S. p. pimpinellifolium*, respectively (see legend in A); population labels correspond to locations provided in online Supplementary Table S1.

and climate maps), had a resolution of 30 arcseconds or around 1 km. Worldclim climate variables represent 50 year climate normals for the period of 1950–2000. We included the following variables: altitude (meters), mean annual temperature ($^{\circ}\text{C}$), mean annual precipitation (mm), temperature seasonality (standard deviation of monthly temperature averages ($^{\circ}\text{C}$) \times 100), precipitation seasonality (coefficient of variation (SD/mean) of monthly precipitation averages) and mean annual diurnal range ($^{\circ}\text{C}$). Soil map data were produced by the USDA NCRS and included organic carbon (kg/m^3), and soil inorganic carbon (kg/m^3). Although we

assessed differences among these edaphic variables, they showed no meaningful patterns among species or populations (data not shown). This is perhaps not surprising, given that soil characteristics often vary on scales much smaller than our GIS mapping resolution (e.g., John et al. 2007). Worldclim variables were imported as raster map layers into ArcGIS 9.1 software (ESRI 2005) for analysis. Population locations were georeferenced as points in a shapefile, and all ecogeographical data were extracted from raster layers using Hawth's analysis tools, "intersect point tool" (Beyer 2004; freeware available at <http://www.spatial ecology.com>).

Our selection of environmental variables was intended to represent several classes of factors known to be important in governing the distribution of plant species, including water, energy (Salisbury 1926; Woodward 1987; Field et al. 2005), and altitude (Humboldt 1855; Bhattarai et al. 2004). Seasonal or diurnal cycles can also influence these factors, therefore seasonality and diurnal components of temperature and precipitation were also considered in analyses. After environmental variables associated with populations were determined from GIS analysis (see Table 1, online Supplementary Table S2), these variables were used in correlations with population morphological, physiological and genetic data in analyses detailed below.

MORPHOLOGICAL AND PHYSIOLOGICAL TRAITS

Common garden experiment: We measured a suite of morphological and physiological traits on each of at least 10 plants per population accession (see below and online Supplementary Table S2). Plant material was generated using standard greenhouse cultivation (i.e., greenhouse soil mix, twice daily watering, weekly fertilization, and ambient temperature range from ~ 15 – 28°C) of seeds obtained from the TGRC. Prior to sowing, average seed weight was estimated by weighing all available seeds (23–51) for each sample. Because seed color appeared to differ between accessions, and might be an important ecological trait (Baskin and Baskin 1998, and see below), seed color was measured by capturing digital images of four randomly chosen seeds per accession; intensity of red, green, and blue components of these images was quantified based on ~ 170 pixels using ImagePro Express software (Media Cybernetics, Inc., Silver Spring, MD). Seeds were then germinated on moist sterile blotting paper and 10–19 resulting seedlings per population accession were transplanted to soil in one-gallon pots, and randomly arranged in the Indiana University greenhouse. Three seedling traits (hypocotyl length, root length, and cotyledon length; Table 1) were measured on all seedlings at 7 days after sowing (DAS) on blotting paper. At 50 DAS, plant height was measured on all plants from the base to the youngest node on the main stem. Juvenile leaf traits (leaf size, weight, and area) were measured at 60 DAS on the youngest fully expanded leaf on each of at least three plants per population accession; each leaf was collected and scanned on a flatbed scanner, and area was determined automatically using ImagePro Express. Each leaf was weighed immediately after collection (wet weight) and again after drying at 65°C for 7 days (dry weight). Two physiologically relevant measures, specific water content (SWC) and leaf mass/area (LMA, also known as specific leaf weight [SLW]), were calculated from these traits as (wet weight – dry weight)/leaf area, and dry weight/leaf area, respectively. (Note that all leaves were collected post-watering and prior to 1100 h [i.e., when fully hydrated], to minimize environmental variance in wet weight; nonetheless, specific water content is usually measured on leaves that are max-

imally hydrated after collection, so our estimates of SWC might be subject to some additional error for this reason.) As a measure of plant architecture, basal branch number was determined at 70 DAS by counting the number of basal branches emerging from the main stem. Days to flowering was measured as the number of days from 58 DAS until the first flower fully opened. Finally, for three plants from each of four accessions of *S. pimpinellifolium*, we also estimated leaf nitrogen and carbon content, using carbon and nitrogen isotope analysis of punched leaf discs sampled at 60 DAS. Nitrogen (or carbon) content was calculated by dividing absolute N (or C) by total dry sample weight. Carbon content was used to generate an estimate of carbon isotope discrimination, which is frequently used as an integrated measure of water-use efficiency (WUE) across the lifetime of the sampled tissue (Dawson et al. 2002). More negative values indicate greater carbon isotope discrimination, which reflects lower integrated WUE (Dawson et al. 2002). Isotope analysis was conducted by the UC Davis Stable Isotope Facility.

Drought response assay: To assess the relative degree of drought response in each population, at 70 DAS we conducted a manipulative study of at least five juvenile plants per population accession. These plants were from the same cohort of seedlings as plants measured for phenotypic traits (above). To assess drought resistance, irrigation was withheld from each plant and the time to wilting recorded. Days to wilting (Table 1) is the average number of days until all leaves and terminal branches lose turgidity, measured from the last irrigation at 70 DAS.

MOLECULAR GENETIC (AFLP) ANALYSIS

To generate an estimate of genetic relatedness between populations and species, three to five individuals per population accession were genotyped using standard amplified fragment length polymorphisms (AFLP) analysis. DNA for each sample was extracted from young leaves using MagAttract 96 Miniprep Core Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA (300 ng) was digested with three units each of EcoRI and MspI and 1X NEB#2 buffer in 40 μl reaction. After 1 h of incubation at 37°C , the following ligation reagents in 10 μl mixture was added to the digestion reaction and incubated for 3 h at 16°C : 1.5 μM of double-stranded EcoRI adapter (combination of oligos 5'-CTCGTAGACTGCGTACC3' and 5'-AATTGGTACGTCAGTCTAC3'), 15 μM of double-stranded MspI adapter (combination of oligos 5'-GACGATGAGTCTAGAA3' and 5'-CGTTCTAGACTCATC-3'), 1X Ligase buffer, and 0.1 μl of T4 ligase (NEB). The preselective AFLP amplification was carried out in a 20 μl reaction mixture containing 30 mM Tricine, 50 mM KCl, 2 mM MgCl_2 , 5% acetamide, 10 mM of each dNTP, 0.2 mM of each EcoRI primer (5'-GACTGCGTACCAATTCA-3') and MspI primer (5'-GATGAGTCTAGAACGGA-3'), 0.6 μl of Taq

Table 1. Species and population effects for environmental and phenotypic variables, from analysis of variance (ANOVA) analyses. For each species, the mean species value is also included.

Variable type	Variable/trait	Species effect				Population effect			
		<i>S. l. cerasiforme</i>		<i>S. pimpinellifolium</i>		<i>S. l. cerasiforme</i>		<i>S. pimpinellifolium</i>	
		Mean	F	P	F	P	F	P	
Environmental	Altitude (m)	482.44	752.90	0.95	0.344	-	-	-	-
	Mean annual temperature (°C)	21.90	18.56	9.88	0.006†	-	-	-	-
	Mean annual precipitation (mm)	1631.44	112.40	7.62	0.013	-	-	-	-
	Temperature seasonality	1214.44	1844.00	4.50	0.049	-	-	-	-
	Precipitation seasonality	43.11	88.30	6.26	0.023	-	-	-	-
Diumal range		9.52	10.96	1.62	0.220	-	-	-	-
Morphological	Average seed weight (mg)	1.50	1.10	9.23	0.007†	-	-	-	-
	Seed color (PCI)	0.66	-0.59	11.92	0.003†	3.57	0.006†	6.02	<0.001†
	Cotyledon length (mm)	7.45	6.02	9.14	0.008†	20.66	<0.001†	4.44	<0.001†
	Hypocotyl length (mm)	19.55	19.14	0.11	0.744	11.18	<0.001†	7.63	<0.001†
	Seedling root length (mm)	36.57	40.60	0.52	0.480	16.10	<0.001†	17.14	<0.001†
	Plant height (cm)	23.84	26.86	2.31	0.147	5.69	<0.001†	8.79	<0.001†
	Basal branch number	3.99	6.79	30.17	<0.001†	16.00	<0.001†	8.44	<0.001†
	Indiv. leaf area (cm ²)	13.09	5.15	32.36	<0.001†	27.71	<0.001†	8.31	<0.001†
	Leaf water content (g/cm ²)	1.52 × 10 ⁻⁰²	1.31 × 10 ⁻⁰²	12.67	0.002†	21.94	<0.001†	8.62	<0.001†
	Leaf mass/area (g/cm ²)	2.41 × 10 ⁻⁰²	3.15 × 10 ⁻⁰²	12.31	0.003†	11.81	<0.001†	5.09	<0.001†
- reproductive traits	Days to first flower	8.40	4.83	5.07	0.038	30.84	<0.001†	15.72	<0.001†
	Stigma exertion (mm)	-	-	-	-	-	-	62.80	<0.001†
Physiological	Days to wilting	5.03	10.33	26.61	<0.001†	6.34	<0.001†	21.75	<0.001†
	Leaf nitrogen content (gN/g leaf)	-	-	-	-	-	-	2.25	0.062
	Leaf carbon content (gC/g leaf)	-	-	-	-	-	-	2.13	0.076
	Leaf δ ¹³ C (‰)	-	-	-	-	-	-	2.95	0.021†

† P-values that remain significant after correction for multiple testing.

polymerase, and 2 μ l of the ligation reaction mixture using the following PCR program; 35 cycles of 30 sec at 94°C, 30 sec at 55°C, and 1 min at 72°C. The selective AFLP amplification was carried out as the preselective AFLP amplification, except that 2 μ l of 1:20 dilution of the preselective amplification was used as a template using 11 primer pairs (listed in online Supplementary Table S3). Amplified fragments were detected with the 3730 DNA Analyzer (Applied Biosystems, Foster City, CA), and length polymorphisms were automatically scored between 50 and 500 bp using Genemapper software version 4.0 (Applied Biosystems) using the default AFLP analysis settings, except with polynomial degree of 8 and window size of 1.

ANALYSES

Species and population differences in environmental, phenotypic, and physiological traits

A total of 13 climatic and environmental variables, and 18 morphological and physiological variables, were generated in the study. Within each group of variables, several measures appeared to be highly correlated and likely nonindependent or redundant. Accordingly, prior to testing for species and population level differentiation in measured traits, we performed a set of preliminary correlations to identify variables that were largely independent of each other, to reduce the number and degree of redundancy among variables to be assessed. Online Supplementary Table S2 lists all the environmental and morphological variables measured and their correlations. For environmental/climatic variables we retained only a single representative variable from sets of variables that were strongly correlated ($P < 0.01$) with each other. For example, mean annual precipitation, precipitation in the driest month, and precipitation in the wettest month, were all highly correlated; only the first was retained for formal analysis. In one case—altitude—we retained this variable despite high correlations with precipitation and temperature measures, as we considered it might capture additional environmental variation (e.g., radiation) not captured by these other factors. For morphological variables, we also removed apparently redundant measurements based on large significant correlations ($P < 0.01$, $r > 0.80$). Nonetheless, in several cases morphological traits were retained despite high correlations if they were measured at different developmental stages (i.e., seed, seedling, juvenile, reproductive), based on the reasoning that these might reflect biologically important life-history associations. In the case of seed color, all three color components (blue, green, and red) were combined in a standard principal components analysis (PCA) using individual plant measures, and values on the first principal axis (PC1) based on the regression method were used as a summary measure of overall color. Table 1 shows the six environmental variables and 16 morphological or physiological traits used in the final analyses.

Univariate ANOVAs were used to test for species-level ef-

fects on these variables using population means, and to test for population-level effects within species using trait values of individuals within populations. Student's *t*-test was used to test the differences in these variables between biogeographical regions within species (i.e., coastal vs. Amazon in SC, and coastal vs. montane in SP) using population means. Within each species, relationships among population means for specific environmental variables were assessed with standard Pearson's correlations; trait correlations within species were similarly assessed with standard correlation analyses. Because of the large number of statistical tests involved, we performed sequential Bonferroni (Dunn-Sidak) corrections (Box 9.9; Sokal and Rohlf 1995) to conservatively correct the overall experiment-wise error rate in each set of analyses. Observed *P*-values and those that remain significant after correction are reported in the Results. The relationships among climatic, morphological/physiological, and genetic traits at the population level within species, including assessments of genetic isolation by distance, were assessed with Mantel tests (Mantel and Valand 1970).

AFLP ANALYSIS

Pairwise fixation indices (F_{ST} s) between populations within the two species were estimated using AFLP-SURV version 1.0 (Vekemans 2002) based on all scored AFLP polymorphisms. Allele frequencies were estimated using the default Bayesian method (the most general setting), assuming Hardy–Weinberg equilibrium within populations. Pairwise geographic distances between-population locations were calculated based on their coordinates using GeoCalc version 0.9.50 (<http://www.fizzymagic.net/Geocaching/GeoCalc/GeoCalc.html>). All Mantel tests were conducted based on 30,000 randomizations using *zt* program (Bonnet and Van de Peer 2002). All statistical analyses were conducted using SPSS version 14.0 (SPSS Inc., Chicago, IL).

Q_{ST}/F_{ST} COMPARISON

An alternative approach to evaluate the action of divergent selection is to compare population divergence in presumptively neutral molecular makers (i.e., F_{ST}) with population divergence in quantitative traits that might be subject to selection. Quantitative genetic divergence for each trait is estimated by Q_{ST} —a measure analogous to F_{ST} , calculated from observed within and between population quantitative genetic variance (McKay and Latta 2002, and references therein). For traits under strong divergent selection, $Q_{ST} > F_{ST}$, whereas for traits under stabilizing selection $Q_{ST} < F_{ST}$ (Merila and Crnokrak 2001, McKay and Latta 2002). We calculated Q_{ST} values for each morphological and physiological trait to evaluate whether these measures were smaller or larger than F_{ST} s calculated from AFLP markers. For each species, Q_{ST} and F_{ST} were calculated across all populations (species level), as well as among populations within each region (region level).

Results

ECOLOGICAL FEATURES OF THE STUDY AREA

GIS maps identified steep gradients within and between the species ranges of *S. l. cerasiforme* and *S. pimpinellifolium*, for several of the analyzed environmental variables (Fig. 1). Most notable were large (in some cases up to ten fold) differences in mean precipitation and temperature, as well as daily and seasonal variation in these attributes. In particular, the climate of the Amazon basin populations were characterized by high temperature and precipitation, and relatively little fluctuation in these parameters as indicated by low-to-moderate seasonality in precipitation and temperature, and modest diurnal temperature ranges (Fig. 1). The Andean highlands appeared typically moderate in precipitation and low in temperature, particularly in southern Peru. Temperature seasonality was moderate, but diurnal temperature range and precipitation seasonality were generally high. In contrast, western coastal regions appear to be extremely dry but moderate in temperature and relatively low in precipitation and temperature fluctuations, both diurnally and across the year. The scant rainfall that occurs in this region is strongly episodic, positively correlated with elevation, and influenced by ENSO climate cycles (Haylock et al. 2006; Sifres et al. 2007). Variation among populations within this region also appears to be relatively small compared to other regions (Fig. 1).

Given the range differences of our two focal species—*S. l. cerasiforme* occurs predominantly in the Amazon basin, whereas *S. pimpinellifolium* is found in the coastal and mid-elevation western Andes—these large climatic and environmental differences a priori suggest ecogeographical requirements of possible adaptive significance that differ between our study species. Variable environmental conditions between geographical regions within species similarly suggest potentially large ecotypic differences among populations within species growing under these different ecological conditions.

BETWEEN-SPECIES VARIATION

As suggested by generalized geographical differences in environmental variables, our analysis revealed large environmental and climatic differences between the two species, particularly in mean and variation in temperature and precipitation. Of six environmental variables investigated, four were significantly different between species (Table 1) based on our sample of study populations, although only one difference remains significant after correction for multiple testing. In particular, habitats of *S. l. cerasiforme* and *S. pimpinellifolium* were significantly different in mean annual temperature (Table 1); they also showed over 10-fold difference in mean annual precipitation (1631.4 mm vs. 112.4 mm, respectively; Table 1). As noted above, the habitat of *S. l. cerasiforme* western coastal populations are at the extreme end of environ-

mental gradients within the species range, and might be atypical, so that these samples may not be generally representative of the species habitat preference. Climatically, these population locations are more similar to typical *S. pimpinellifolium* coastal sites (see below—*Within-species variation*). Despite this, our results show that the two species generally have substantially different habitat preferences. Note that these species differences are even more pronounced when the atypical *S. l. cerasiforme* populations are excluded from the species comparison (i.e., mean annual temperature: $f = 40.74$, $P < 0.001$; mean annual precipitation: $f = 27.44$, $P < 0.001$; temperature seasonality: $f = 27.68$, $P < 0.001$; precipitation seasonality: $f = 9.75$, $P = 0.008$; all comparisons significant after multiple-testing correction).

We detected significant between-species differences ($P < 0.05$) for all phenotypic variables, except for plant height, cotyledon length, and seedling root length; eight differences remained significant after statistical correction (Table 1), indicating that the two species not only are different in their habitat choice but also display genetically based phenotypic differences (Fig. 2). Some of these phenotypic differences are correlated with each other in apparently straightforward biological relationships. In particular, seed weight was positively correlated with both cotyledon and hypocotyl length in both species ($r > 0.67$, $P < 0.05$) indicating that seed size is a good predictor of seedling size. Accordingly, larger seedling size in *S. l. cerasiforme* is likely at least partly explained by its nearly 40% heavier seeds compared to *S. pimpinellifolium* (mean = 1.5 and 1.1 mg, respectively). In contrast, root length was largely independent ($r < 0.37$, $P > 0.1$) of seed weight and seedling size in both species.

Some of the phenotypic differences between species appear to reflect divergent adaptation to different habitat types, most notably precipitation differences but also potentially differences related to the intensity of light competition. In the former case, species differ substantially in leaf traits relevant to water relations and WUE, particularly leaf area, which is ~2.5 times smaller in *S. pimpinellifolium*, and leaf mass/area which is larger in *S. pimpinellifolium*, indicating thicker leaves. Both these traits are indicative of more drought-resistant phenotypes; smaller thicker leaves suggest *S. pimpinellifolium* likely has much lower transpiration rates at the whole plant level, and therefore loses water much more slowly under drought conditions than *S. l. cerasiforme*. This hypothesis is directly confirmed by results from our drought resistance manipulation: *S. pimpinellifolium* was substantially more drought resistant than *S. l. cerasiforme* (mean days to wilting of 10.33 vs. 5.03 days, respectively) (Fig. 1, Table 1). Given *S. pimpinellifolium*'s occurrence in the arid environments of the western Andes, these responses most likely reflect adaptive matching between phenotypic and physiological traits, and predominant environmental conditions.

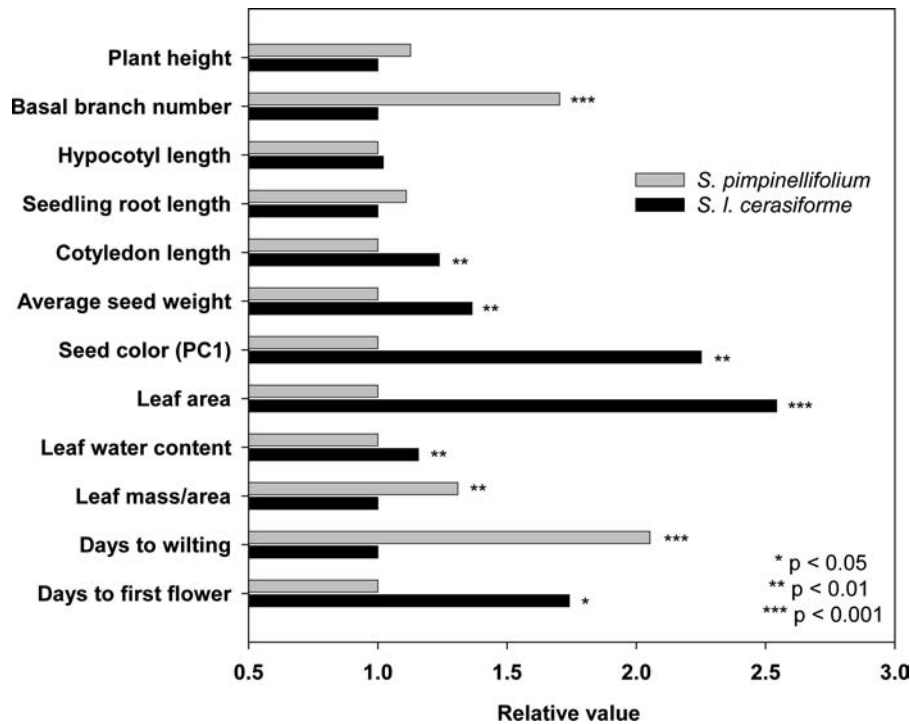


Figure 2. Differences in mean phenotypic values between *S. l. cerasifforme* and *S. pimpinellifolium*. For graphical representation, species differences have been standardized to the smaller value of the two species; the scale therefore represents the fold difference between species in the size or value of individual traits. All species differences remain significant after correction for multiple testing, except days to first flower.

Another notable, significant difference between the species was in their overall plant architecture. *Solanum l. cerasifforme* has fewer thick, vertical basal branches whereas *S. pimpinellifolium* produces more basal branches that are thinner and more lateral (Table 1). In addition, several other phenotypic differences, including much larger leaf area, suggest that *S. l. cerasifforme* invests more heavily in leaf tissue, even at the seedling stage (as shown by greater cotyledon, but not hypocotyl and root, length compared to *S. pimpinellifolium*; Table 1). These characteristic traits in *S. l. cerasifforme* may be advantageous in competition for light (especially in the absence of water limitations), although this hypothesized adaptation needs to be more directly investigated in future studies. Nonetheless, this would be consistent with our finding that *S. l. cerasifforme* populations occur in locations with significantly higher vegetation cover than *S. pimpinellifolium*. This difference is also apparent from accession records and images of the native habitat made at the time of collection, and available on the TGRC website (<http://tgrc.ucdavis.edu>).

Finally, in addition to the large number of detected species effects, we also detected significant population effects for all phenotypic variables within *S. l. cerasifforme*, and for all but leaf nitrogen and carbon content within *S. pimpinellifolium* (Table 1). Therefore substantial heritable phenotypic variation exists not only between species, but also among populations within species.

WITHIN-SPECIES VARIATION

Comparisons between populations from different ecotypic regions within each species range (i.e., coastal vs. Amazon in *S. l. cerasifforme*; coastal vs. montane in *pimpinellifolium*) indicated substantial differences that were similar in kind to those detected between species. First, several climatic and environmental variables differed significantly between populations located in contrasting geographical regions within each species range (Table 2). In particular, the coastal populations of *S. l. cerasifforme* had significantly lower annual temperature and higher temperature seasonality, than those of the inland Amazon basin populations. The coastal populations of *S. pimpinellifolium* experience higher temperature means and seasonality, but lower diurnal temperature range and lower precipitation seasonality compared to the highland populations (Table 2); five of the six detected differences were robust to our statistical correction for multiple tests. Second, these climatic differences were accompanied by varying degrees of phenotypic differentiation between regions within each species. In *S. l. cerasifforme*, coastal versus inland differences were significant only for seed color (prior to correction for multiple testing), despite markedly different climates between the two geographical regions. In contrast, coastal and highland populations of *S. pimpinellifolium* differed in multiple phenotypic and physiological traits ($P < 0.05$), including seedling root length, cotyledon

Table 2. Comparison of environmental and phenotypic factors between populations in contrasting regions of *S. l. cerasiforme* and *S. pimpinellifolium*.

Variable	<i>S. l. cerasiforme</i>				<i>S. pimpinellifolium</i>			
	Mean		<i>t</i>	<i>P</i>	Mean		<i>t</i>	<i>P</i>
	Coastal	Inland			Coastal	Highland		
Altitude (m)	394.75	552.60	−0.651	0.553	33.40	1472.40	−13.490	<0.001†
Mean annual temperature (°C)	19.15	24.10	−6.475	<0.001†	19.78	17.34	3.089	0.015†
Mean annual precipitation (mm)	295.75	2700.00	−2.810	0.026	12.20	212.60	−3.789	0.019
Temperature seasonality	1996.25	589.00	9.281	<0.001†	2213.20	1474.80	3.362	0.010†
Precipitation seasonality	58.75	30.60	1.086	0.314	53.20	123.40	−7.535	<0.001†
Diurnal range	7.88	10.84	−2.359	0.050	8.78	13.14	−5.858	<0.001†
Average seed weight (mg)	1.41	1.56	−0.603	0.565	1.23	0.96	2.48	0.06
Seed color (PC1)	−0.74	0.59	−2.599	0.035	0.19	−0.19	0.563	0.589
Cotyledon length (mm)	7.02	7.79	−0.802	0.449	6.39	5.65	3.340	0.010
Hypocotyl length (mm)	19.17	19.85	−0.334	0.748	20.42	17.86	1.850	0.101
Seedling root length (mm)	43.18	31.29	1.446	0.191	47.51	33.68	2.408	0.043
Plant height (cm)	23.33	24.24	−0.341	0.743	26.55	27.16	−0.191	0.853
Basal branch number	4.12	3.88	0.281	0.787	7.41	6.17	2.377	0.045
Leaf area (cm ²)	15.15	11.44	1.196	0.310	4.69	5.61	−1.890	0.095
Leaf water content (g/cm ²)	0.02	0.01	1.653	0.142	0.01	0.01	−0.148	0.886
Leaf mass/area (g/cm ²)	0.02	0.03	−0.874	0.411	0.03	0.03	1.883	0.096
Days to first flower	8.86	8.04	0.314	0.762	4.44	5.21	−0.351	0.734
Stigma exertion (mm)	–	–	–	–	1.05	0.75	0.463	0.656
Days to wilting	4.80	5.22	−0.540	0.606	12.12	8.53	2.452	0.040
Leaf nitrogen content (gN/g leaf)	–	–	–	–	0.035	0.034	0.264	0.799
Leaf carbon content (gC/g leaf)	–	–	–	–	0.421	0.431	−0.103	0.334
Leaf δ ¹³ C (‰)	–	–	–	–	−27.77	−26.95	−1.719	0.124

†*P*-values that remain significant after correction for multiple testing.

length, and plant stem number, as well as days to wilting, although none of these differences remained after correction for multiple testing.

Some of these suggestive intraspecific differences might reflect adaptive responses to regional environmental conditions. To more directly associate phenotypic and climatic variation, we assessed the correlation between each climatic or environmental factor and those phenotypic and physiological traits that exhibited significant population differentiation within each species. We detected several strong trait–environment associations, consistent with adaptive matching between phenotypes and environmental factors acting on local populations, particularly in *S. pimpinellifolium* (Table 3). Some of the strongest associations detected were between phenotypic and physiological variation and climatic variables affecting water availability. Interestingly, days to wilting was negatively correlated with annual precipitation in *S. pimpinellifolium* ($r = -0.718$, $P = 0.019$, Fig. 3A), indicating that individual populations that occur in arid environments are likely more drought resistant, although this relationship is not significant after our conservative correction for multiple testing. No such correlation exists in *S. l. cerasiforme*, despite much greater variation in

natural precipitation among populations, suggesting that populations of *S. l. cerasiforme* found in coastal western South America are not locally adapted to water conditions (see further, below). In addition, in *S. pimpinellifolium*, mean annual precipitation was also negatively correlated with hypocotyl length and seedling root length (Table 3, Fig. 3B), indicating a connection between seedling size traits and arid environments. In *S. l. cerasiforme*, seedling root length was marginally correlated with mean annual precipitation ($P = 0.072$, Table 3, Fig. 3C), perhaps suggesting this is a general adaptive response to drier conditions or associated environmental variation. Interestingly, although we found marginally significant population effects for isotope-based estimates of WUE (Leaf $\Delta^{13}\text{C}$; Table 1), we found no difference between biogeographic regions for these physiological traits (Table 2) and no associations between these and variation in precipitation in *S. pimpinellifolium* (Table 3); partly this might be due to low power to detect these differences (i.e., only two populations were analyzed per biogeographic region), although other studies have also failed to detect strong environmental associations with these estimates of integrated WUE (Dawson et al. 2002, and references therein).

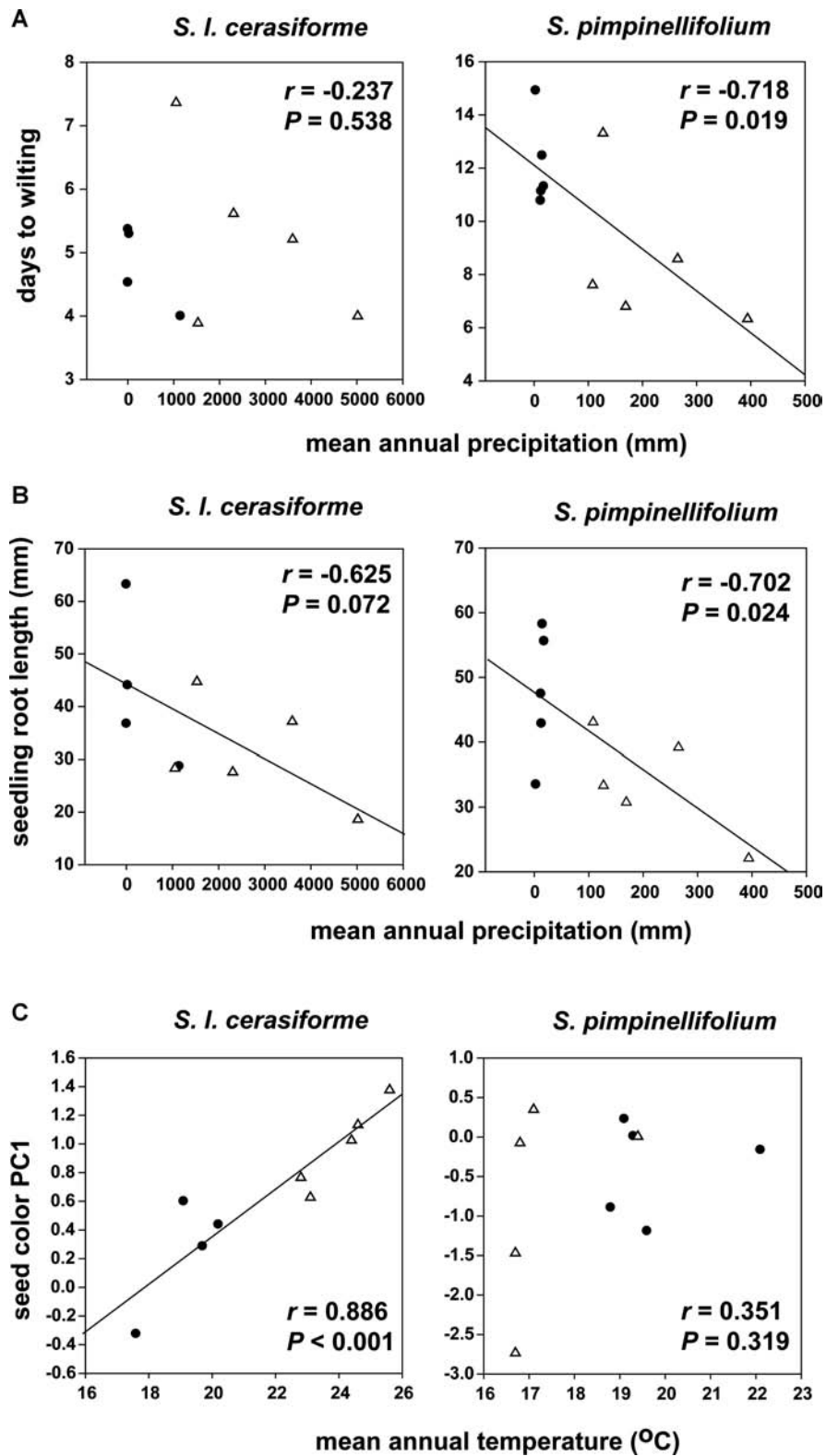


Figure 3. Correlations between phenotypic and environmental variables for *S. l. cerasiforme* and *S. pimpinellifolium*, representing (A) associations between mean annual precipitation in the native source population and estimated drought resistance (days to wilting); (B) associations between mean annual precipitation in the native source population and seedling root length; and (C) associations between mean annual temperature and a composite measure of seed color intensity that combines seed color in red, blue, and green regions of the spectrum. Within each species, populations from different regions are denoted with different symbols (SC: circles, coastal; triangles, Amazon; SP: circles, coastal; triangles, mid-elevation).

Table 3. Trait–environment correlations between environmental and phenotypic variables. For each comparison, upper and lower numbers correspond to Pearson's coefficient for *S. l. cerasiforme* (SC) and *S. pimpinellifolium* (SP), respectively.

Trait	Species	Altitude (m)	Mean annual temperature (°C)	Mean annual precipitation (mm)	Temperature seasonality	Precipitation seasonality	Diurnal range
Average seed weight (mg)	SC	−0.523	0.403	−0.192	−0.371	−0.413	0.028
	SP	−0.652*	0.539	−0.549	0.279	−0.688*	−0.599
Seed color (PC1)	SC	−0.321	0.886**†	0.363	−0.807**†	−0.795*†	0.256
	SP	−0.31	0.351	−0.248	0.202	−0.342	−0.297
Cotyledon length (mm)	SC	−0.42	0.503	−0.187	−0.373	−0.504	0.065
	SP	−0.756*	0.573	−0.586	0.405	−0.774**	−0.721*
Hypocotyl length (mm)	SC	−0.277	0.21	−0.257	−0.319	−0.238	0.052
	SP	−0.468	0.198	−0.847**†	0.457	−0.374	−0.442
Seedling root length (mm)	SC	−0.487	−0.389	−0.625	0.526	0.233	−0.307
	SP	−0.572	0.468	−0.702*	0.614	−0.441	−0.457
Plant height (cm)	SC	0.097	0.042	0.649	−0.185	−0.372	−0.408
	SP	0.096	−0.398	−0.422	0.146	0.229	−0.006
Basal branch number	SC	−0.175	−0.102	0.292	0.256	0.195	−0.151
	SP	−0.539	0.189	−0.794**†	0.663*	−0.468	−0.532
Indiv. leaf area (cm ²)	SC	−0.806**	−0.354	−0.504	0.352	−0.096	−0.443
	SP	0.514	−0.335	0.416	−0.396	0.343	0.532
Leaf water content (g/cm ²)	SC	−0.566	−0.511	−0.691*	0.549	0.089	−0.461
	SP	0.019	0.28	0.411	−0.002	−0.106	0.266
Leaf mass/area (g/cm ²)	SC	0.756*	0.064	0.396	−0.105	0.299	0.308
	SP	−0.59	0.462	−0.194	0.5	−0.569	−0.556
Days to first flower	SC	−0.27	0.007	−0.583	0.064	−0.311	−0.14
	SP	0.047	0.032	0.366	−0.371	−0.163	−0.031
Stigma exertion (mm)	SC	–	–	–	–	–	–
	SP	−0.101	0.262	−0.406	−0.011	−0.088	0.061
Days to wilting	SC	0.016	0.245	−0.237	−0.264	−0.03	0.452
	SP	−0.696*	0.402	−0.718*	0.567	−0.574	−0.735*
Leaf nitrogen content (g/g)	SC	–	–	–	–	–	–
	SP	−0.035	−0.290	−0.388	0.561	0.035	0.029
Leaf carbon content (g/g)	SC	–	–	–	–	–	–
	SP	0.375	−0.337	0.086	0.008	0.304	0.507
Leaf δ ¹³ C (‰)	SC	–	–	–	–	–	–
	SP	0.497	−0.3	0.251	−0.403	0.378	0.577

* $P < 0.05$, ** $P < 0.01$, † indicates P -values that remain significant after correction for multiple testing.

Further analysis indicates that early seedling traits related to drought are unlikely to be mechanistically associated with the later juvenile drought responses observed in our manipulation experiment. In particular, in *S. pimpinellifolium*, the partial correlation between days to wilting and seedling root or hypocotyl length was not significant ($P > 0.40$) and was only marginal with cotyledon length ($P = 0.099$), once the effect of mean annual precipitation was statistically removed. In *S. l. cerasiforme*, seedling root length also was not significantly associated with days to wilting, if mean annual precipitation was factored out statistically. Therefore, the observed variation in juvenile drought resistance in *S. pimpinellifolium* (i.e., days to wilting) is likely attributable to factors other than characteristics of early seedling development. In addition, in contrast to the pattern observed for between-species differences,

individual leaf size (estimated as area from an average of three leaves/plant) does not seem to be directly involved in observed drought resistance variation, as indicated by a lack of association between individual leaf area and mean annual precipitation or days to wilting. Instead, total plant leaf area might be responsible for drought resistance differences observed within *S. pimpinellifolium*: the *S. pimpinellifolium* highland populations appear to have greater total plant leaf area in comparison to coastal populations (T. Nakazato, pers. obs.), although this difference was not quantitatively evaluated in our study. Coastal *S. pimpinellifolium* populations also appeared to quickly lose older leaves without wilting upon drought treatment, whereas highland ones had the tendency to wilt as a whole plant (T. Nakazato, pers. obs.). Therefore, reducing leaf area by leaf abscission (drought deciduous

response) may be an important mechanism of juvenile and adult drought tolerance/avoidance in *S. pimpinellifolium*, as in other xerophytes (Monneveux and Belhassen 1996); in the case of the coastal *S. pimpinellifolium* populations, drought-induced leaf abscission might be an adaptation to a more episodic rainfall pattern along the coast, where an ability to reduce leaf area to cope with prolonged aridity after a rainfall event would be beneficial. This hypothesis can be directly evaluated in future studies.

In comparison to *S. pimpinellifolium*, there were few associations between climatic variables and phenotypic population differences within *S. l. cerasiforme*. The notable exception was seed color, which was highly significantly positively correlated with mean annual temperature and temperature seasonality (Table 3; Fig. 3C), such that plants from cooler climates have darker seeds. (No such association was detected among *S. pimpinellifolium* populations; Table 3.) It is possible that this trait–climate association is due to the involvement of color in moderating seed temperature and/or water relations during germination. Variation in seed color has been shown to affect seed dormancy and germination (Baskin and Baskin 1998), although its physiological and genetic mechanisms are poorly understood. In tomato, variation in pigmentation of the seed testa has been associated with differences in microbial protection, seed longevity, and germination control (Downie et al. 2003, and references therein). For example, tomato anthocyanin mutants have lighter colored seeds and increased seed coat permeability that allows more rapid germination, perhaps due to the absence of condensed tannins in the inner epidermal testa layer (Atanassova et al. 2004). Conversely, tomato “dark seed” mutants have reduced germination percentages and/or rates under controlled conditions (Downie et al. 2004) and some dark seed mutants also show enhanced testa toughness (Downie et al. 2003). These observations suggest that, in tomato, dark seed coloration might promote seed dormancy via slowed response to germination cues, perhaps due to chemical or structural changes in the seed testa. Nonetheless, it has been difficult to infer one selective force that might be primarily responsible for natural variation in this trait because studies have also shown general associations between cold, salt, and drought tolerance during germination, suggesting that the same genes might control tomato seed germination under each of these stress conditions (Foolad et al. 2003); interestingly, our analysis suggests that temperature is the strongest correlate of population differentiation in seed color. Our observed association between temperature and seed color in *S. l. cerasiforme* is consistent with between-species differences in seed color, where seeds of *S. pimpinellifolium*, which generally occurs in cooler climates, are darker than those of *S. l. cerasiforme* (Table 1).

Apart from this association, however, our general failure to detect strong trait–environment associations within *S. l. cerasiforme* (especially associations with local and regional water conditions) suggests that climatic factors are poorer predictors of the

geographical occurrence of this species, at least for the populations we have analyzed here. *Solanum l. cerasiforme* accessions often occur adjacent to agricultural fields or along irrigation ditches as weeds (Taylor 1986), a microclimate in which they might not experience severe water stress. By preferentially growing in these water-favorable microenvironments, *S. l. cerasiforme* could avoid the necessity of local adaptation to natural water conditions. Given this, we might not expect broadscale environmental variation in natural precipitation to be associated with appropriate matching phenotypes in this species. This is especially true for the coastal accessions we have analyzed here, as several are known to have been collected from sites adjacent to human settlement (see Methods; online Supplementary Table S1). To indirectly assess the specific influence of these atypical, possibly adventive coastal populations of *S. l. cerasiforme* on our results for this species, we also evaluated environment–phenotype correlations using only Amazon populations of *S. l. cerasiforme*. We found a similar number of significant correlations as the species-level analysis, however the Amazon only correlation coefficients were on average greater than those observed when using all populations from *S. l. cerasiforme* (when the correlations were in the same direction; data not shown). In combination with our finding of very few significant physiological and phenotypic differences between geographical regions in this species, our data indicate little evidence that coastal populations of *S. l. cerasiforme* are persisting in their atypical environments due to recent adaptation to local environmental conditions (unlike, for example, introduced North American populations of St. John’s Wort; Maron et al. 2007). Rather, it is most likely that human commensalism allows them to persist in microhabitats that are not representative of natural climatic conditions. In this way, *S. l. cerasiforme* illustrates the importance of interpreting results from ex situ analyses of trait–environment associations in light of additional information from direct field observations.

GENETIC RELATIONSHIPS BETWEEN AND WITHIN SPECIES

The strong climate–trait associations we detected clearly suggest the influence of natural selection in shaping phenotypic variation within and between species, especially for *S. pimpinellifolium*. However, without an independent estimate of relatedness we cannot exclude the possibility that these associations are due primarily to historical relatedness among populations found in similar climates rather than recent adaptation. Using 11 AFLP primer pairs (online Supplementary Table S3), we found 2367, 2377, and 2704 polymorphic loci among *S. l. cerasiforme* and among *S. pimpinellifolium*, and among all samples, respectively. Wright’s fixation indices (F_{ST}) among all populations within each species were significant ($P < 0.01$) but small (0.052 and 0.023 within *S. l. cerasiforme* and *S. pimpinellifolium*, respectively), indicating

relatively little molecular genetic differentiation among populations of these species, despite substantial phenotypic differentiation (Table 1). For *S. pimpinellifolium*, this estimate is substantially lower than previously estimated from a single-copy nuclear intron region (global $F_{ST} = 0.467$; Caicedo and Schaal 2004), and also lower than an estimate from six SSR (simple sequence repeats) markers (global $F_{ST} = 0.17$; Nuez et al. 2004). Differences in the markers used and the genomic regions surveyed may be partly responsible for this difference (see below).

No significant correlation was detected between pairwise F_{ST} and geographic distances among populations of *S. l. cerasiforme* ($r = 0.090$, $P = 0.388$), however a significant association was detected in *S. pimpinellifolium* ($r = 0.482$, $P = 0.002$), indicating genetic isolation by distance in this species. Isolation by distance has been previously detected across the species range of *S. pimpinellifolium* using allozymes (Rick et al. 1977) and a single-copy nuclear intron region (Caicedo and Schaal 2004), but not using AFLPs or SSRs across a smaller geographical region (Nuez et al. 2004). In comparison to *S. pimpinellifolium*, our findings indicate that a stepping-stone equilibrium model of gene flow is probably inappropriate for *S. l. cerasiforme*, perhaps because there is distant-independent gene flow between populations (e.g., due to anthropogenic dispersal) and/or there has been insufficient time to establish equilibrium conditions in this species. These explanations are likely especially true of the atypical populations of coastal *S. l. cerasiforme*, which might be recent commensal colonists to this region. When we examined the correlation between geographic distance and F_{ST} separately in coastal and Amazon population groups, neither association was significant, however the Amazon correlation is in the expected direction for IBD whereas the coastal correlation is not ($r = 0.279$ and $r = -0.233$ for Amazon and coastal groups, respectively; online Supplementary Table S4).

No significant correlations were found between F_{ST} and population differences in any of the environmental factors or phenotypic traits within either species (online Supplementary Table S5), except for mean annual temperature for *S. l. cerasiforme* and leaf nitrogen content for *S. pimpinellifolium*. (Note that, given 46 tests

of association between genetic differentiation and environmental or phenotypic traits within species, two to three are expected to be significant by chance alone; as expected these results do not remain significant after statistical correction.) Based on the environmental variables studied here, therefore, environmental affinity generally appears to be independent of simple genetic distance, suggesting little niche conservatism at the population level. Similarly to habitat preference, evolution of phenotypic traits within these species appears to be independent of or at least unconstrained by simple genetic relatedness.

Finally, our comparisons of Q_{ST} (for each quantitative trait) with F_{ST} (from AFLP markers) were consistent with our inference of divergent selection acting on many of the examined quantitative genetic traits: in every case (with one exception), our Q_{ST} estimate was larger than the corresponding F_{ST} estimate (Table 4). The one exception was for juvenile plant height among coastal *S. pimpinellifolium* populations, where Q_{ST} was smaller than F_{ST} (0.016 vs. 0.023, respectively). Although the observed difference is small, this result might suggest that juvenile plant height is under stabilizing selection among SP coastal populations. Nonetheless, because inferences from these Q_{ST}/F_{ST} comparisons rely on several simplifying assumptions (e.g., regarding the underlying genetic architecture and gene action of the quantitative traits being compared—see Merila and Crnokrak 2001; Latta and McKay 2002) more specific inferences should be interpreted with caution. In addition, the use of highly mutable genetic markers, such as microsatellites or AFLPs, can lead to underestimation of molecular F_{ST} (Ritland 2000). Here our estimates of F_{ST} are very low, likely reflecting the high mutability of AFLP-based genetic differences. Nonetheless, given these caveats, overall these comparisons are consistent with our inference of divergent selection acting on quantitative trait differences between populations, both at the whole-species and regional levels.

Discussion

The pronounced topographical variation and latitudinal extension of the Andes has created a variety of unique climatic zones,

Table 4. F_{ST} and Q_{ST} values for species-wide and within-region analyses. F_{ST} is estimated from AFLP data; Average Q_{ST} is the mean Q_{ST} value over all quantitative traits (*S. l. cerasiforme* $N = 10$ traits; *S. pimpinellifolium* $N = 14$); Range Q_{ST} gives the minimum and maximum Q_{ST} values for all quantitative traits. With one exception, Q_{ST} was larger than F_{ST} in all cases (see text).

Species	Analysis level	F_{ST}	Average Q_{ST}	Range Q_{ST}
<i>S. l. cerasiforme</i>	Whole species	0.052	0.353	0.150–0.494
	Within Coastal	0.0565	0.299	0.059–0.562
	Within Amazon	0.0402	0.296	0.093–0.629
<i>S. pimpinellifolium</i>	Whole species	0.023	0.311	0.122–0.881
	Within Coastal	0.023	0.238	0.016–0.867
	Within Mid-elevation	0.021	0.302	0.028–0.891

providing opportunities for organisms to rapidly diversify and adapt to their local environments (e.g., Moore and Donoghue 2007). Its high-altitude region is by far the most plant species-rich tropical alpine area in the world (Smith and Cleef 1988; Burnham and Graham 1999), despite the young age of this habitat (2–4 Mya; Gregory-Wodzicki 2000). The coastal deserts of the Andean region are also relatively species rich, due partly to the influence of the persistent coastal fog associated with the Humboldt Current, which moderates water demand and contributes significant moisture via condensation in coastal bluffs or “loma” habitats (Marquet et al. 1998). Similarly, despite recent divergence of the wild tomatoes as a whole (~7 MY, Nesbitt and Tanksley 2002), there appears to be tremendous variation in their habitat choice. In particular, *S. l. cerasiforme* and *S. pimpinellifolium* studied here provide a convincing case of recent differential ecological adaptation within this group, both between species and intraspecifically—among populations within species.

RAPID SPECIES DIVERSIFICATION IN RESPONSE TO CLIMATE

The first level at which we observe apparent adaptive divergence is between the two study species. These two species clearly display heritable differences in a number of morphological and physiological traits. The coincidence between this substantial interspecific phenotypic divergence and differences in habitat preference suggests that environmental conditions are driving divergent evolution of phenotypic traits. Most striking is the difference in precipitation conditions between the two species’ habitats, and the phenotypic and physiological changes that have accompanied this differentiation. In particular, the twofold difference in days to wilting suggests that *S. pimpinellifolium* is much more adapted to xeric environments than *S. l. cerasiforme*. Reduced leaf area and higher leaf mass/area in *S. pimpinellifolium* implicate leaf structure as an important determinant of drought resistance. This hypothesis is consistent with the common observation that plants in arid environments have smaller and thicker leaves compared to ones in wet environments (Givnish 1984; Fonseca et al. 2000), possibly an adaptation to maintain turgidity during drought by reinforcing leaf structure (Maximov 1929; Oertli 1989). In addition, an ongoing study of water usage among multiple wild tomato species (T. Nakazato and P. Davis, unpubl. data) indicates that there is large variation in leaf size among species, and reduction in leaf size is indeed the primary strategy to minimize water loss for xeric-adapted tomato species.

The nature of species differences also provides insight into the predominant drivers of ecological diversification that influence each of these taxa. In *S. pimpinellifolium*, several traits are predominantly associated with mean annual precipitation rather than temperature, seasonality variables, and soil variation, strongly suggesting that local water availability is one of the major en-

vironmental agents driving adaptation in this species. Although selection for smaller and thicker leaves may be operating in *S. pimpinellifolium* in response to water conditions, the larger and thinner leaves observed in *S. l. cerasiforme* are potentially selected in response to competition for light. Several pieces of evidence are consistent with this provisional hypothesis. First, the native range of *S. l. cerasiforme* is characterized by more dense vegetation cover in comparison to other tomato species, particularly in the Amazon Basin. Second, species in shady environments have previously been found to have lower leaf mass/area and to have higher relative growth rate (Garnier 1992; Lambers and Poorter 1992; Reich et al. 1992; Saverimuttu and Westoby 1996). Third, the larger seeds of *S. l. cerasiforme* may also be advantageous in low light conditions. Plant species experiencing dense shade and light competition tend to have larger seeds than plants that occur in open habitats (Saverimuttu and Westoby 1996). Although the mechanisms underlying increased shade tolerance for large-seeded plants are not clear, it has been suggested that seedlings from larger seeds can grow more quickly to reach higher photosynthetically active radiation levels (Westoby et al. 1996). Alternatively, the differences in seed size, and in fruit size between the two species—those of *S. pimpinellifolium* are smaller—might indicate differences in seed dispersal agents, that is, coevolution with different types of animals. Unfortunately, available information on animal/plant relationships in the tomatoes is mostly anecdotal (Rick 1966); the larger fruit of *S. l. cerasiforme* might be an adaptation to dispersal by mammals, including humans, whereas the smaller seeds of *S. pimpinellifolium* could be an adaptation to bird dispersal. Finally, the significant negative correlation we detected between leaf area and leaf mass/area in both species is also common in other species (Cunningham et al. 1999; Fonseca et al. 2000), and potentially reflects different resource allocation strategies for either maximizing protective tissue in dry environments or maximizing leaf area in shady environments. In *S. l. cerasiforme*, therefore, light intensity may be a more important selective agent than the water availability that clearly influences *S. pimpinellifolium*.

Adaptation of *S. l. cerasiforme* to high humidity has previously been noted in terms of its high tolerance to water logging and resistance to various fungal infections (Rick 1973). Given that most *Lycopersicon* species inhabit the more or less xeric western Andes, adaptation of *S. l. cerasiforme* to the wet environments of the Amazon basin is likely a derived state. Previously it has been hypothesized that the ancestors of *S. lycopersicum* originated from Peru, and migrated north and eastward to colonize the Amazon basin and subsequently the tropics of the other parts of the world (Jenkins 1948; Rick 1976). Our data are consistent with several key phenotypic innovations, specifically involving changed water and competition responses, being involved in this habitat shift from dry to wet environments in *S. l. cerasiforme*. Moreover, it is

possible that relaxed requirements on *S. l. cerasiforme* for drought resistance in relatively water-abundant habitats might itself have allowed novel adaptive responses to new environmental conditions, especially light competition.

Overall, given the close evolutionary relationship between our two study species (e.g., clock-based estimates have placed the divergence between *S. l. cerasiforme* and *S. pimpinellifolium* at ~1 MY; Nesbitt and Tanksley 2002), our data indicate that there has been rapid phenotypic, and apparently adaptive, differentiation between these species, most likely in response to variation in several key environmental factors.

SUBSTANTIAL ADAPTIVE DIVERSIFICATION WITHIN SPECIES

The strong trait–environment associations observed at the species level are also mirrored at the population level within species, especially within *S. pimpinellifolium*. First, our GIS data showed that there is substantial variation in environmental conditions within each species' range, providing ample opportunities for local intraspecific adaptation. Second, substantial trait variation among populations within both species indicates that detectable phenotypic differentiation is occurring rapidly within species, even where our genetic data confirm that the studied populations are closely related. Third, strong trait–environment associations are detectable at the regional and population level within both species, although more notably within *S. pimpinellifolium*. Finally, the lack of correlation between genetic similarity and environmental and phenotypic variation in both species suggests that morphological and physiological evolutionary responses are most likely due to adaptive differences based on climate rather than historical genetic relatedness between populations found in similar climates. Because of the difficulty of definitively establishing the relative contributions of natural selection and genetic drift to observed differences between populations, additional molecular genetic data would be valuable in confirming this likely scenario. Our overall conclusion based on our findings here is that ecological conditions are likely a major force driving recent and rapid phenotypic and physiological divergence within both species examined.

Rapid phenotypic evolution in response to local environments may not be uncommon in nature. For example, New England mussels (*Mytilus edulis*) show heritable shell thickening upon exposure to cues from their predators, Asian shore crabs (*Hemigrapsus sanguineus*), which were introduced within the last 15 years (Freeman and Byers 2006). The Andean lupines (*Lupinus* spp.) show remarkable morphological diversity and the highest known speciation rate in plants (2.49–3.72 species per MY), also likely driven by the dynamic ecological landscape of the Andes (Hughes and Eastwood 2006). Our data similarly indicate that adaptive responses to key environmental factors in this geographical region can be rapid.

PHENOTYPIC, PHYSIOLOGICAL, AND EVOLUTIONARY DIVERGENCE THROUGHOUT THE TOMATO CLADE

Our study documents substantial phenotypic variation between and within *S. l. cerasiforme* and *S. pimpinellifolium* that appears to be adaptive to local environmental conditions. Our observed association between a direct physiological response to drought (days to wilting) and natural patterns of precipitation experienced in the field is particularly persuasive. Nonetheless, other detected associations between phenotypes and environmental variables might not necessarily imply causal relationships. Many environmental variables are correlated in nature (e.g., precipitation and vegetation); to decouple correlated environmental variables and elucidate the causal nature of ecological adaptation, careful phenotypic analyses under controlled conditions, as well as transplant experiments, would be a valuable complement to the analyses we present here. Nonetheless, several provisional conclusions emerge from these results. First, although the resolution of GIS data is too coarse to describe microclimatic variation at very local scales (i.e., < 1 km), in these species this approach appears powerful enough to identify key environmental gradients that are shaping population and species' adaptive responses, at least within the typical species' ranges. Second, by combining GIS-based analyses—that identify climatic correlates of species and ecotypic differences—with phenotypic analyses, we are able to directly assess hypotheses that emerge from a macroecological approach. Our phenotypic and physiological data confirm that species, and populations within species, exhibit strong patterns of differentiation, and several notable trait–climate associations consistent with adaptive responses to observed regional and local environmental variation. Conversely, in populations that are thought to be very recent colonists (*S. l. cerasiforme* western coastal populations), we found little evidence for local adaptation, indicating these populations likely escape local environmental conditions by acting as human commensals. Third, integrating these approaches with an independent assessment of genetic relatedness allows us to confirm that ecological differentiation has occurred on short evolutionary timescales; these data also support our hypothesis that trait–environment associations are due to adaptive differentiation, rather than merely reflecting historical patterns of relatedness. Finally, the predominant influence of precipitation and temperature, and perhaps light conditions/vegetation cover, suggests that relatively few (in this case, largely abiotic) environmental conditions can be involved in determining different species and population habitat preferences. Overall, combining a macroecological understanding of this environmental variation with population-level analyses of phenotypic and genetic variation provides insight into predicting and evaluating patterns and rates of diversification in this group, in addition to identifying the specific environmental factors and possible underlying phenotypic mechanisms likely involved in natural adaptive diversification.

The *Lycopersicon* group is ideally suited to further expanding this kind of integrated approach. Strong environmental gradients that appear to span inter- and intraspecific species' ranges (Fig. 1), make them ideal for studying responses to local and regional climatic differences. In addition, the apparent ecological diversity in this system is matched by many logistical advantages, including extensive historical occurrence records for all species in the group, as well as matching publicly available germplasm for hundreds of known wild accessions. As a first step in expanding our analyses, we are currently completing a biogeographic study of > 1000 accessions of 10 wild tomato species based on GIS data. Our preliminary findings suggest that each species has a unique geographic distribution pattern corresponding to a unique combination of environmental variables (T. Nakazato, D. Warren, and L. C. Moyle, unpubl. data). Past empirical and observational studies also indicate that other wild tomato species display morphological traits that appear to be adaptive (e.g., Rick 1973, 1976, 1978; Patterson et al. 1978; Vallejos 1979; Bloom et al. 2001), suggesting rich material for similar future phenotypic and physiological studies in additional taxa. Extremely deep roots of *S. chilense* are potentially adaptive for acquiring water stored in deep soil in the extremely xeric environments of Northern Chile, for example, and *S. cheesmaniae* appears to have developed high salt tolerance to inhabit the coastlines of the Galapagos islands (Rick 1973). Our results, in addition to these suggestive associations between environment and phenotypes in other species, indicate that ecological factors are a major determinant of species distribution and adaptive patterns across all of the wild Andean tomato species.

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Supplementary Material

The following supplementary material is available for this article:

Table S1. Accessions used in the study.

Table S2. All measured climatic and soil variables, and morphological and physiological traits, and Pearson correlation coefficients for associations among them.

Table S3. Sequences of the 11 AFLP primers used for selective amplifications.

Table S4. Correlations between pair-wise genetic similarity and physical distance within species, and within ecotypic regions, of each species.

Table S5. Correlations between fixation index (F_{st}) and differentiation in morphological and environmental traits.

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