

# Morphological differentiation in a common garden experiment among native and non-native specimens of the invasive weed yellow starthistle (*Centaurea solstitialis*)

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**Abstract** Understanding the differences between weedy and non-weedy plant populations is important because they may provide clues to genetic factors that create invasive species, as well as important insights into local adaptation. We studied weedy, non-native (California and Argentina) and non-weedy, native populations (Republic of Georgia and Turkey) of *Centaurea solstitialis* in a common garden setting. Specimens grown from non-native seed stock were generally taller, had longer leaves with more surface area, and flowered earlier than plants grown from native seed stock. Plants from California tended to be much taller, on average, than plants from any other country, and plants from the Republic of Georgia tended to bolt much later than plants from other countries. When we compared neutral genetic variation at microsatellite or simple sequence repeat markers using AMOVA to quantitative morphological variation, we found that quantitative variation was much more likely to be partitioned among regions than genetic variation. We also evaluated  $F_{ST}$  values

against  $Q_{ST}$  ( $F_{ST}/Q_{ST}$  analysis) and found evidence for possible selection on plant height and leaf length in the non-native regions. Our results suggest that local adaptation may play a role in the success of *C. solstitialis* as an invasive weed.

**Keywords** Invasive species · Local adaptation · Post-invasion selection · Neutral EST-SSR markers ·  $F_{ST}/Q_{ST}$  · AMOVA

## Introduction

Local adaptation is one of the driving forces of evolution. The study of native and non-native plant populations that show evidence of morphological differentiation may be integral to our understanding of the genetic origins of local adaptation in general, and invasive plant biology in particular. Comparative studies of native and non-native populations of a single species are also critical to our understanding of the factors that contribute to the creation of an invasive species (Hierro et al. 2006; Kane and Rieseberg 2008; Lai et al. 2008).

*Centaurea solstitialis* (Compositae) is a prime example of a species that has both weedy and non-weedy populations in distinct geographic regions. For example, it is considered a noxious invasive plant in the western United States, where it covers an estimated 5.7 million hectares in California alone since it was established in the mid-1800s (Pitcairn and

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Schoenig 2006). However, Uygur et al. (2004) have shown that in the geographic center of its native range in Turkey, the species occurs at densities only 4% of those measured in California. *Centaurea solstitialis* was introduced originally from Europe to Chile and Argentina in the mid-1800s as a contaminant of alfalfa seed (Gerlach 1997; Hierro et al. 2009) and thence probably introduced multiple times to California (Gerlach 1997; Sun 1997). The first herbarium record for *C. solstitialis* in California is from 1869, and it is now considered the most abundant weed in the state (Pitcairn and Schoenig 2006).

Several studies have looked at the differences between native and non-native populations of this noxious weed (Hierro et al. 2006; Widmer et al. 2007; Hierro et al. 2009), but there are only a few genetic studies of the species (Sun 1997; Sun and Ritland 1998). We conducted a common garden experiment on specimens from Turkey and the Republic of Georgia (Georgia), the central region of the native range of the species, and from central Argentina and the United States (California), non-native regions where the species is considered a weed. We compared levels of differentiation among populations for quantitative morphological traits to those obtained from neutral genetic markers. Differentiation among populations for neutral molecular markers would generally be caused by evolutionary forces such as founder effect and subsequent genetic drift. Substantially more differentiation among populations for a phenotypic trait would suggest the added force of natural selection causing differentiation in that trait. Thus, the analyses in this study are used to shed light on the extent to which local adaptation has played a role in the success of *C. solstitialis* in its non-native range, and to identify candidate morphological and life history traits that may have been influenced by natural selection and contributed to fitness advantages and local adaptation in invaded regions.

## Methods

### Morphological analysis

Seed was collected from open-pollinated individual plants from 16 sites; four from each of two native regions (Turkey and Georgia) and four from each of two invaded regions (California and central

Argentina). Sites within political regions were separated by at least 50 km. Seed was handled according to Hierro et al. (2009). Populations within Turkey and California experience “Mediterranean climate” of winter rains and summer drought, while those in Georgia and Argentina have more summer rains, allowing us to make preliminary inferences on the effects of climatic factors on morphological traits. Populations from both the native and the non-native regions spanned roughly the same range of latitude (37°–41°), though the non-native regions were split between the northern and southern hemisphere. We planted five seeds each from five individual plants, which were from each of the four collection sites within each political region (400 plants total) in approximately 50 cm<sup>3</sup> peat pots containing standard potting soil, with one seed per pot. The seeds were sprouted in a growth room in a randomized design of rotating trays to new positions in the room at 2 or 3 days intervals. After 2 weeks, once the plants were well established, they were transplanted into larger 5,800 cm<sup>3</sup> plastic pots with standard potting soil and transferred to the greenhouse for the remainder of the experiment.

During the experiment, numerous traits were scored including days to germination, days until the plant achieved the third true leaf, plant height at various intervals, length and width of the fifth true leaf, leaf shape, days until bolting, days until flowering, flower number, and plant size at senescence. Data for flowering traits are not shown here because they were incomplete and not independent of bolting time. Only data for plant height at senescence is shown here. Traits such as the bolting time, the point when the flowering shoot first emerged from the vegetative rosette, were measured from the date the first individual of the collection bolted, and thus this trait was adjusted by the days to germination for each plant. Each plant was kept until it flowered and senesced, which was more than 1 year in some cases. We conducted two types of comparisons on these traits. First, we compared native versus non-native populations, and then we compared each political region to all others. Traits were analyzed with one-way ANOVA, where region was treated as a fixed factor. The mean of the populations within each region was used for these analyses. Analyses were performed with SPSS version 15 and higher. When variances within each group were equal as determined by a Levene test for homogeneity of variances, the assumptions of the

standard ANOVA test were met and the standard F statistic was used to determine the significance of differences between regions. The significance of each test was evaluated by a sequential Bonferroni correction based on the number of comparisons in each table (Rice 1989). If a significant difference between regions was found using the F statistic, post hoc multiple comparison tests were made using the Bonferroni multiple comparisons test. When the data variances were not equal and the assumptions of the ANOVA test could not be met, then the Welch statistic was employed. The Welch statistic is a nonparametric test for the equality of means designed for use when the variances are not equal (SPSS v. 16). In these cases, Tamhane's T2 statistic was used to find differences between regions in post hoc multiple comparison tests.

### Genetic analysis

Five weeks into the experiment, and after the plants were all well established, the fifth true leaf of each plant was removed, photographed, immediately frozen and stored at  $-80^{\circ}\text{C}$ , and later used for DNA extractions. The same plants were used for both morphological and genetic analysis, unless the plant senesced before a tissue sample could be collected. We developed seven trinucleotide EST-SSR loci from the Compositae Genome Project website (<http://compgenomics.ucdavis.edu/>) and assayed these using hotstart and touchdown polymerase chain reactions (PCRs) with annealing temperatures ranging from 50 to  $60^{\circ}\text{C}$ . All reactions were performed in 25  $\mu\text{l}$  volumes with 5  $\mu\text{l}$  of diluted DNA ( $\sim 20\text{--}100\text{ ng}$ ), 0.1  $\mu\text{M}$  of the forward primer, 0.4  $\mu\text{M}$  of the reverse primer, and 0.3  $\mu\text{M}$  of a fluorescently labeled M13 primer, 2.5  $\mu\text{l}$  10 $\times$  reaction buffer, 2.5 mM combined dNTPs, 3 mM  $\text{MgCl}_2$ , and 1.25 units of *Taq* polymerase (Promega GOTaq Flexi DNA Polymerase). The PCR products were assayed on the 3100 Avant automatic gene sequencer (ABI).

We compared morphological variation between regions to neutral genetic variation in the EST-SSR loci between regions using an AMOVA analysis as well as a standard  $F_{\text{ST}}/Q_{\text{ST}}$  comparison. AMOVA analysis and  $F_{\text{ST}}$  values were determined using Arlequin v. 3.1 and higher (Excoffier et al. 2005; Excoffier and Lischer 2010). The use of analysis of molecular variance (AMOVA) to evaluate genetic differences is analogous to the use of ANOVA to evaluate quantitative differences (Excoffier et al.

1992) and can be compared to our ANOVA results. To evaluate if there is evidence of selection on any of the quantitative traits we analyzed, we compared a measure of genetic differentiation ( $F_{\text{ST}}$ ) to quantitative differentiation ( $Q_{\text{ST}}$ ). Differentiation based on genotypic data ( $F_{\text{ST}}$ ) derived from neutral molecular markers would presumably be most influenced by forces of founder effect and genetic drift.  $Q_{\text{ST}}$  would be influenced by these same forces as well as natural selection (Whitlock 2008). The following expression was used to calculate  $Q_{\text{ST}}$  values for each individual trait for a pairwise comparison among the four regions (groups), Turkey, Georgia, Argentina and California:

$$Q_{\text{ST}} = \frac{\text{variance among groups}}{\text{variance among groups} + 2(\text{variance within groups})}$$

In theory, the magnitude of the difference between  $Q_{\text{ST}}$  and  $F_{\text{ST}}$  indicates the level of selection acting on the population for that morphological trait (Merilä and Crnokrak 2001; O'Hara and Merilä 2005; Whitlock 2008).

For this analysis, we pooled individuals from each of our collection sites into a single regional population in order to compare these genetic results to our morphological study. In this sense,  $F_{\text{ST}}$  is perhaps more accurately referred to as  $F_{\text{RT}}$  (Hartl and Clark 2007), and is a measure of the regional differences in fixation compared to the total fixation. Likewise,  $Q_{\text{ST}}$  values were calculated among regions, not individual collection sites. We did this in part to cancel out differences in  $Q_{\text{ST}}$  among populations due to latitudinal effects. Colautti et al. (2008) show that latitudinal effects can confound results of common garden studies investigating genetic differences between native and non-native populations of plants, and by pooling our populations, we ensure that all of our populations fall within a certain latitudinal range ( $37^{\circ}\text{--}41^{\circ}\text{N}$  and S).

## Results

### Morphological analysis

Plants from non-native regions (California and Argentina) tended to be taller, have longer leaves, and they tended to have fewer leaf indentations and thus more leaf surface area than plants from native regions (Georgia and Turkey; plant height: 102.48 cm vs.

73.54 cm,  $P < 0.001$ ; leaf length: 18.13 cm vs. 13.09 cm,  $P < 0.001$ ; indentations: 2.09 vs. 2.88,  $P < 0.001$  for non-native and native regions, respectively; Table 1). Plants from California and Argentina also tended to grow slower in early stages, as they took longer to achieve the third true leaf (6.46 days vs. 4.92 days,  $P < 0.001$ ). In addition, plants from non-native regions flowered earlier, achieving their first bolt nearly twice as early as individuals from native regions (average 73.63 days vs. 150.41 days,  $P < 0.001$ ). In contrast, there were no significant differences in the number of days to germination (4.22 days vs. 3.76 days for non-native and native regions, respectively,  $P = 0.044$ ) between plants from non-native or native regions (Table 1).

Individuals from California proved to be taller than plants from any other country (109.29 cm,  $P < 0.001$  for each comparison) (Fig. 1, Table 1). California specimens also tended to have the longest leaves (19.66 cm,  $P < 0.001$  for all comparisons). In turn, individuals from Georgia and Turkey had, on average, more indentations on each leaf (2.76 and 2.99 respectively), and that differed significantly from each invasive country (Georgia and each invasive region  $P < 0.001$ ; Turkey and each invasive region,  $P < 0.001$ ). Individuals from Turkey also tended to achieve the third true leaf more rapidly (4.13 days,  $P < 0.001$  for all comparisons) and to germinate faster (3.13 days), and this differed significantly with the germination of individuals from Argentina (4.72 days,  $P < 0.001$ ), California (3.8 days,  $P < 0.001$ ), and Georgia (4.52 days,  $P < 0.001$ ; Fig. 1, Table 1). These data indicate that individuals from Turkey were generally the fastest growing individuals, but the smallest individuals with an average height of 68.65 cm (though this was not significantly different from Georgian individuals at  $P = 0.496$ ; all other comparisons,  $P < 0.001$ ), and the ones with the second smallest leaf size at 13.99 cm ( $P < 0.001$  for all comparisons). Finally, individuals from California bolted much faster than individuals from other regions (49.52 days,  $P < 0.001$  for each comparison); but more striking, individuals from Georgia bolted much later than individuals from any other region (Fig. 1). The average bolt time in Georgia was 204 days, compared to 105.95 days in Argentina ( $P < 0.001$ ), 95.95 days in Turkey ( $P < 0.001$ ), and 49.52 days in California ( $P < 0.001$ ), though the variance in bolt time was very large in all regions (Table 1).

## Genetic analysis

The amplifications of the fragments for the simple sequence repeat (SSR) loci were consistent and repeatable. Heterozygosity values were generally high, ranging from 0.486 to 0.786, and the single locus  $F_{ST}$  values calculated for a given pair of populations did not differ significantly from each other, suggesting that none were affected by selection or genetic draft. Thus, the markers appear to be neutral.

AMOVA analysis revealed that there was no significant difference between native and non-native seed stock (Table 1) based on these neutral markers, though ANOVA analysis revealed significant differences for quantitative traits such as days to third true leaf, days to bolt, height, length of the fifth true leaf, and number of indents. Most of the variation exists within populations ( $\Phi_{ST} = 0.07$ ), rather than among native and non-native regions ( $\Phi_{CT} = 6.92 \times 10^{-3}$ ), though this figure was not significant even with 10,100 permutations (Table 1). When we compared political regions by AMOVA analysis, we still found that most of the variation exists within populations ( $\Phi_{ST} = 0.12$ ) and relatively little is partitioned among regions ( $\Phi_{CT} = 0.05$ ) (Table 1).

$Q_{ST}$  values for most traits were relatively low indicating low amounts of morphological differentiation, except for size-related traits, such as the length of the fifth true leaf, where all  $Q_{ST}$  values exceeded 0.1, and were as high as 0.661 for the comparison between California and Georgia (Table 2). Indeed,  $Q_{ST}$  values for leaf length were higher than  $F_{ST}$  values between California and all other regions, as well as for Argentina and all other regions.  $Q_{ST}$  values for plant height were also higher than  $F_{ST}$  values between California and Turkey, as well as Argentina and Turkey.

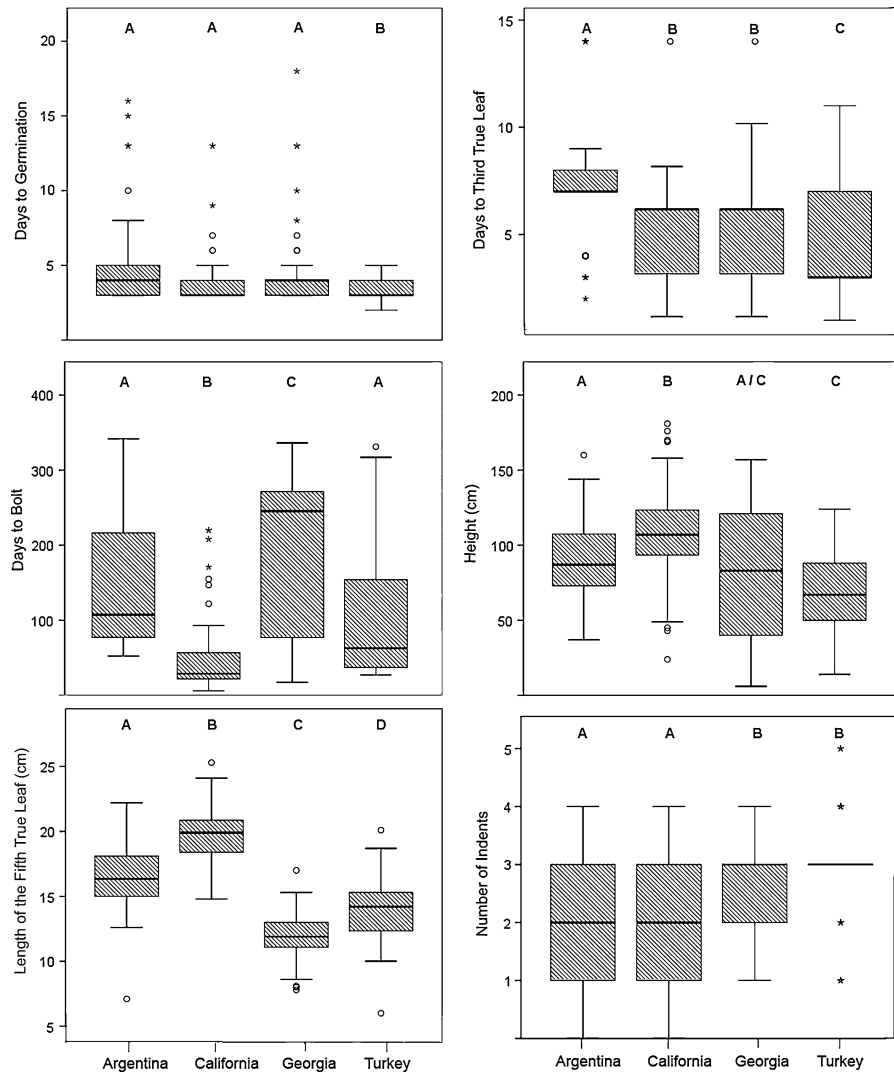
Differences in development times were also apparent.  $Q_{ST}$  values for germination time and the number of days until the third true leaf appeared were both higher than the  $F_{ST}$  value between Argentina and Turkey. Most striking, however, was the difference between Georgia and all other regions in the number of days to bolt.  $Q_{ST}$  values for the number of days to bolt exceeded  $F_{ST}$  values between Georgia and all other regions. The  $Q_{ST}$  value for the number of days to bolt also exceeded the  $F_{ST}$  value between California and Argentina.

**Table 1** Morphological and genetic variation between non-native and native ranges, and among regions

	Non-native		Native		F	Welch	df	P value				
	Mean	Variance	Mean	Variance								
<b>ANOVA</b>												
Days to germination	4.22	4.44	3.76	4.13	4.08	31.22	1,320	0.044				
Days to third true leaf	6.46	5.42	4.92	5.86		38.24	1,294.43	<0.001*				
Days to bolt	73.63	5602.82	150.41	12920.41		43.14	1,194.812	<0.001*				
Height at senescence (cm)	102.48	1232.96	73.54	843.29		280.40	1,314.74	<0.001*				
Length of 5th true leaf (cm)	18.13	7.50	13.09	5.20		37.21	1,184.08	<0.001*				
Number of indents	2.09	1.45	2.88	0.91			1,267.27	<0.001*				
<b>AMOVA</b>												
Within populations	n = 130		n = 106		$\varphi_{ST} = 0.07$		470	<0.001*				
Within regions					$\varphi_{SC} = 0.06$		2	<0.001*				
Among regions					$\varphi_{CT} = 6.92 \times 10^{-3}$		1	0.34				
<b>ANOVA</b>												
	Argentina		California		Georgia		Turkey		F	Welch	df	P value
	Mean	Variance	Mean	Variance	Mean	Variance	Mean	Variance				
Days to germination	4.72	7.02	3.80	1.98	4.52	7.42	3.13	0.54	16.37	3,149.8	<0.001*	
Days to third true leaf	7.14	5.36	5.84	4.74	5.85	6.01	4.13	4.42	23.55	3,159.61	<0.001*	
Days to bolt	105.95	8256.27	49.52	2310.44	204.60	9745.64	95.95	10453.02	41.63	3,117.96	<0.001*	
Height at senescence (cm)	90.63	652.55	109.29	837.99	79.71	1872.64	68.65	636.15	23.813	3,109.22	<0.001*	
Length of 5th true leaf (cm)	16.39	652.54	19.66	837.99	12.01	1872.66	13.99	636.15	167.33	3,276	<0.001*	
Number of indents	2.12	5.53	2.07	4.27	2.76	3.19	2.99	5.13	13.01	3,150.25	<0.001*	
<b>AMOVA</b>												
Within populations	n = 65		n = 65		n = 42		n = 64		$\varphi_{ST} = 0.12$	456	<0.001*	
Within regions									$\varphi_{SC} = 0.07$	12	<0.001*	
Among regions									$\varphi_{CT} = 0.05$	3	<0.001*	

The mean value is given for each morphological trait, as well as the ANOVA F statistic or Welch statistic (depending on whether the data fit the homogeneity of variance assumption of the F statistic), the degrees of freedom, and the P value. \* A significant P value, that is, one below the alpha level determined by a sequential Bonferroni correction. The AMOVA  $\Phi$  statistics and the number of individuals included in the genetic analysis are given

**Fig. 1** Boxplots for each trait scored. Circles above the boxes denote single individuals that were outliers, and stars denote extreme outliers. Letters above the boxes represent groups that were significantly different in an ANOVA test using the F statistic or the non parametric Welch statistic when homogeneity of variances was not met



**Table 2**  $F_{ST}$  and  $Q_{ST}$  values for each pairwise comparison

	$F_{ST}$	Germination	Days to third true leaf	Days to bolt	Height	Length 5th true Leaf	Number of indents
Argentina versus California	0.06*	0.024	0.041	<b>0.075</b>	0.052	<b>0.217</b>	0.000
Argentina versus Georgia	0.08*	0.001	0.036	<b>0.121</b>	0.012	<b>0.358</b>	0.039
Argentina versus Turkey	0.06*	<b>0.082</b>	<b>0.191</b>	0.001	<b>0.087</b>	<b>0.121</b>	<b>0.070</b>
California versus Georgia	0.13*	0.015	0.000	<b>0.351</b>	0.079	<b>0.661</b>	0.051
California versus Turkey	0.1*	0.043	0.075	0.044	<b>0.211</b>	<b>0.464</b>	0.087
Georgia versus Turkey	0.1*	0.062	0.068	<b>0.129</b>	0.013	0.104	0.007

\*  $F_{ST}$  values denote significance at the 0.05 level.  $Q_{ST}$  values in bold are those that exceed neutral  $F_{ST}$  values

This constituted one of the few instances in which  $Q_{ST}$  exceeded  $F_{ST}$  in these two non-native regions (the other instance being length of the fifth true leaf).

**Discussion**

Our common garden experiment revealed significant morphological differences between populations of

*C. solstitialis* in native and non-native regions. Individuals from California tended to be taller, have longer leaves with fewer indentations and more surface area, and they tended to bolt earlier than specimens from other countries (Fig. 1). We found evidence that quantitative morphological variation is more likely to be partitioned among regions than neutral genetic variation (Table 1). There is also evidence that differentiation in certain quantitative morphological traits is greater than differentiation in neutral genetic markers, suggesting possible selection driving differentiation for those traits; this is particularly true for size-related traits such as the length of the fifth true leaf and height.  $Q_{ST}$  values for leaf length exceed  $F_{ST}$  values in every comparison involving a non-native region, suggesting greater differentiation for this trait than we would expect under genetic drift alone. Our data also suggest weak evidence that plant height is selected for when comparing  $F_{ST}/Q_{ST}$  between California and Turkey, and Argentina and Turkey (Table 2).

Four previous studies have assessed morphological differences between native and non-native seed stock of *C. solstitialis* in common garden settings. Widmer et al. (2007) found that plants from the non-native region (California and Idaho, USA) tended to be larger after 2 weeks of growth and larger at maturity than plants grown from native seed (France, Greece, Turkey, Italy, Sicily, Sardinia, and Russia). Our study also found this pattern; plants grown from non-native seed stock were taller, had larger leaves, and had more surface area per leaf at the conclusion of our study. These are all traits that suggest increased vigor in non-native seed stock (Bossdorf et al. 2005). Both of these studies seem to contrast the studies of Hierro et al. (2006) and Andonian and Hierro (2011), which found no difference in plant biomass among plants from native or nonnative seed stock grown in a common garden. While we looked at several different morphological traits, Hierro et al. (2006) and Andonian and Hierro (2011) only recorded plant biomass and fecundity, which may account for the differences in our results. In addition, the plants in the studies by Hierro et al. (2006) and Andonian and Hierro (2011) were grown in small pots and these plants may not have reached their full size potential as they did in the experiment reported here (Hierro pers. comm.). For example, the number of flower heads per plant averaged 7.67 in the California populations of Hierro

et al.'s (2006) study, but 119.6 in the current study (data not shown). We also looked at different native populations than Andonian and Hierro (2011), and we tested for differences among political regions (Turkey, Georgia, California, Argentina) rather than simply native versus introduced regions, which would have pooled geographically distant populations in our study.

Our study looked at one native and non-native population each from a summer drought and summer rain climate, and noted an interesting characteristic about *C. solstitialis* from the summer rain region of the Republic of Georgia. Individuals from Georgian seed stock took twice as long to bolt on average than the next slowest group (Argentina and Turkey) and four times the period to bolt as did specimens from Californian seed stock, though variance in this trait was very large for all regions. Such a large time difference suggests that some lines or subspecies of *C. solstitialis* may be biennial, and that these biennial lines predominate in Georgia while lines with an annual life history pattern predominate in our other collections. Because *C. solstitialis* from summer drought areas such as California and Turkey have a higher proportion of individuals that exhibit a clear annual life history strategy, our data may indicate that a biennial life history strategy is part of the plant's response to the local climate. Argentina also has summer rains (Hierro et al. 2009), but *C. solstitialis* in this region is not necessarily adapted yet to this climatic pattern, though some weak evidence suggests that there is selection for longer days to bolt in relation to California (Table 2). Further study must be done to confirm that Georgian populations have a biennial life history and to investigate a possible mechanism for this difference among regions. Climate may also explain differences in the number of indents in leaves from native and non-native regions. Vogel (1970) suggested that heavily dissected leaves increase convective exchange which reduces leaf tissue damage due to high light and heat stress. The number of indents in the leaves (these data were collected from the same leaf on every plant grown in an indoor greenhouse setting) was consistently different among regions, and may indicate some relaxation of selection in non-native regions for tolerance to heat stress, or it may indicate differences due to latitudinal effects that our experimental design was not able to correct.



None of these data or hypotheses leads to overwhelming evidence for local adaptation or selection on non-native populations of *C. solstitialis*. Indeed,  $F_{ST}/Q_{ST}$  analyses are wrought with problems (Merilä and Crnokrak 2001; O'Hara and Merilä 2005; Whitlock 2008; Edelaar and Björklund 2011). Most recently, Edelaar and Björklund (2011) suggested that because highly variable markers such as microsatellites can underestimate  $F_{ST}$  values, they are an inappropriate marker to use in  $F_{ST}/Q_{ST}$  comparisons, unless migration rates are sufficiently high. We used the program Migrate-n (Beerli 2009; Beerli and Palczewski 2010) to estimate migration rates, and found that migration rates for some loci were one order of magnitude higher than an estimated mutation rate of  $10^{-3}$  for microsatellites (data not shown); however, this value is not high enough to meet Edelaar and Björklund's (2011) criteria for the appropriate use of highly variable markers (when migration rate is at least 2 orders of magnitude greater than mutation). Our results regarding evidence of selection, therefore, should only be considered in relation to the ANOVA results comparing quantitative traits across regions. Because we observed these morphological differences in size between native and non-native seed stock in a common garden experiment, it suggests that the difference in growth patterns between native and non-native regions may be more genetically induced than environmental. Our evidence of selection on these traits from the  $F_{ST}/Q_{ST}$  analysis, however, may be biased by artificially low  $F_{ST}$  values due to the use of EST-SSR markers.

Evidence is mounting that rapid evolution contributes, at least in part, to the success of a non-native species in a new region, though in many cases it is not clear whether these changes result from local adaptation, genetic drift, or founder effects (Bossdorf et al. 2005). The extent to which local adaptation affects the success of an invader is still debated, and is likely to be different for every situation. For example, Dlugosch and Parker (2007) found evidence in ancient colonization patterns in *Hypericum canariense* that non-native populations were favored in environments to which they were pre-adapted, yet in regions where the species is considered invasive, they have found evidence for increased growth rates and local adaptation compared to native populations (Dlugosch and Parker 2008a). Dlugosch and Parker (2008b) suggest that a loss of variance upon introduction may be more

important to a species' ability to adapt than a loss of neutral genetic variation, but were able to identify only one study that reported unequal variances among their populations which was driven by greater variation in native regions. We found unequal variances among regions for many of the quantitative traits we considered, however there was no single region that drove that difference (Table 1).

Both ecological and evolutionary differences between native and non-native populations have been supported in *C. solstitialis* (Hierro et al. 2006; Widmer et al. 2007; Hierro et al. 2009; Andonian and Hierro 2011, this study), and both may have significant effects on the invasion capabilities of the species. Genotype-by-environment interactions may also favor different theories in different common gardens. Further study on the extent to which each component contributes to the success of *C. solstitialis* are necessary, and may shed light on possible control methods and their impacts (Müller-Schärer et al. 2004). Our data indicate that the success of *C. solstitialis* as an invasive species may be driven by local adaptation; the species may thus be a good candidate species to look for further evidence of morphological differentiation in order to gain understanding of the extent to which local adaptation occurs in invasive species.

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