

CONTRASTING PLANT PHYSIOLOGICAL ADAPTATION TO CLIMATE IN THE NATIVE AND INTRODUCED RANGE OF *HYPERICUM PERFORATUM*

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How introduced plants, which may be locally adapted to specific climatic conditions in their native range, cope with the new abiotic conditions that they encounter as exotics is not well understood. In particular, it is unclear what role plasticity versus adaptive evolution plays in enabling exotics to persist under new environmental circumstances in the introduced range. We determined the extent to which native and introduced populations of St. John's Wort (*Hypericum perforatum*) are genetically differentiated with respect to leaf-level morphological and physiological traits that allow plants to tolerate different climatic conditions. In common gardens in Washington and Spain, and in a greenhouse, we examined clinal variation in percent leaf nitrogen and carbon, leaf $\delta^{13}\text{C}$ values (as an integrative measure of water use efficiency), specific leaf area (SLA), root and shoot biomass, root/shoot ratio, total leaf area, and leaf area ratio (LAR). As well, we determined whether native European *H. perforatum* experienced directional selection on leaf-level traits in the introduced range and we compared, across gardens, levels of plasticity in these traits. In field gardens in both Washington and Spain, native populations formed latitudinal clines in percent leaf N. In the greenhouse, native populations formed latitudinal clines in root and shoot biomass and total leaf area, and in the Washington garden only, native populations also exhibited latitudinal clines in percent leaf C and leaf $\delta^{13}\text{C}$. Traits that failed to show consistent latitudinal clines instead exhibited significant phenotypic plasticity. Introduced St. John's Wort populations also formed significant or marginally significant latitudinal clines in percent leaf N in Washington and Spain, percent leaf C in Washington, and in root biomass and total leaf area in the greenhouse. In the Washington common garden, there was strong directional selection among European populations for higher percent leaf N and leaf $\delta^{13}\text{C}$, but no selection on any other measured trait. The presence of convergent, genetically based latitudinal clines between native and introduced *H. perforatum*, together with previously published molecular data, suggest that native and exotic genotypes have independently adapted to a broad-scale variation in climate that varies with latitude.

KEY WORDS: Common gardens, exotic plants, *Hypericum perforatum*, latitudinal clines, phenotypic plasticity, rapid evolution.

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Climate can be a potent selective agent on life history and physiology of plants (Linhart and Grant 1996). Pioneering work by Turesson (1930) and Clausen et al. (1940), together with more recent studies, has demonstrated that populations from diverse altitudes or latitudes are often genetically differentiated in adaptive traits (Mooney and Billings 1961; Gurevitch et al. 1986; Neuffer and Hurka 1986; Galen et al. 1991; Körner et al. 1991; Winn and Gross 1993; Jonas and Geber 1999; Olsson and Ågren 2002; Stinchcombe et al. 2004). This differentiation in traits among populations is powerful evidence of the selective effects of the environment that predictably varies across latitudinal or altitudinal gradients.

Although clinal differentiation in morphological and physiological traits is well documented for native plants (Linhart and Grant 1996), less is known about exotics. In fact, the role of adaptive evolution in successful plant invasions is unclear (Blossey and Nötzold 1995; Parker et al. 2003). A fundamental unanswered question is how populations locally adapted to conditions in the native range persist in the introduced range, where there may be a mismatch between adaptive traits and the environment.

Several nonmutually exclusive scenarios are possible. First, novel conditions may lead to in situ selection on genetic variation generated by mutation and recombination, causing rapid adaptive evolution in the introduced range (Reznick et al. 1997; Thompson 1998; Huey et al. 2000; Bone and Farres 2001; Sakai et al. 2001; Lee 2002). Second, the new environment may simply filter out maladapted genotypes early in the invasion process (Neuffer and Hurka 1999).

Finally, phenotypic plasticity may enable exotics to cope with new conditions in the introduced range (Novak et al. 1991). Plasticity may predispose plants that inhabit diverse environmental conditions in the native range to exploit a wide range of conditions in which they are introduced (Crawley 1987; Williamson and Fitter 1996). In other words, high plasticity may be a key adaptation in itself that permits weedy native and exotics alike to exhibit wide environmental tolerances (Baker 1974; Wu and Jain 1978; Schlichting 1986; Rice and Mack 1991; Williams et al. 1995; Sultan 2000, 2001; Richards et al. 2006).

One way to gain insight into the role of these processes is to compare patterns of local adaptation and phenotypic plasticity among native and exotic conspecifics. If native populations exhibit genetically based latitudinal clines in adaptive traits and these clines are replicated in the introduced range, then either individuals have by chance been introduced to similar latitudes as their native home site (climate matching), or initial filtering and/or adaptive evolution are important mechanisms shaping the response of exotics to novel environmental conditions in the introduced range (Huey et al. 2000). Alternatively, if native populations exhibit genetically based geographic clines but these are absent among introduced populations, then phenotypic plasticity may be

of paramount importance in invasion. We know of few studies that have made these comparisons.

In previous work on the perennial forb, St. John's Wort (*Hypericum perforatum*), we found strong latitudinal clines in size and fecundity among native European and introduced North American populations in northern (Sweden and Washington) and southern (Spain and California) common gardens in the native and introduced range (Maron et al. 2004a). Neutral genetic markers revealed that plants growing at particular latitudes in North America did not necessarily originate from similar latitudes in Europe (Maron et al. 2004a). Thus, climate matching and/or strong initial filtering out of genotypes (i.e., initial selection in North America favoring genotypes originating from similar latitudes in Europe as where they landed in North America) are not likely to be responsible for the clines in fitness among North American populations. Instead, these results suggest that introduced St. John's Wort has rapidly evolved adaptations to novel climatic conditions in North America.

In this paper, we compare morphological and physiological traits among native and introduced populations of St. John's Wort that were grown in common gardens in Washington and Spain. Specifically, we compared percent leaf nitrogen and carbon, $\delta^{13}\text{C}$ values, and specific leaf area (SLA) among native and introduced genotypes originating from divergent latitudes in western Europe (plus Kyrgyzstan) and western North America, respectively. In a greenhouse, we compared root and shoot biomass, root/shoot ratio, total leaf area, and leaf area ratio (LAR) among these populations. One goal was to determine what morphological or physiological traits might underpin clines in size or fecundity. Strong links between physiological traits and fitness are rarely demonstrated, especially in introduced organisms (but see Dudley 1996a,b; Arntz and Delph 2001).

We hypothesized that native and exotic genotypes from cooler, wetter northern populations would have greater percent leaf N and SLA but lower $\delta^{13}\text{C}$ values than plants from hotter and drier southern populations. $\delta^{13}\text{C}$ provides an integrated measure of water use efficiency (WUE), the gain in carbon per unit of water loss, with more negative $\delta^{13}\text{C}$ values corresponding to lower WUE (Farquhar et al. 1989; Ehleringer and Monson 1993; Dawson et al. 2002). In northern environments, where water is less limiting than in the south, we predicted that plants would have higher photosynthetic rates (and therefore higher percent leaf N), and lower WUE, which would allow for higher photosynthetic assimilation and biomass production (Lambers et al. 1998). In contrast, in warmer and drier southern sites, where water rather than photosynthetic capacity may limit growth, we expected populations to have higher WUE and lower SLA. Small, thick leaves rich in structural tissues such as cellulose and poor in proteins (thus with higher leaf percent C concentrations) are all adaptations to reduce water loss and costs of leaf replacement. We further

postulated that if these leaf-level traits are important in allowing exotic plants to cope with novel climatic conditions where they are introduced, we would see evidence of directional selection on these traits within the introduced range or these traits would exhibit phenotypic plasticity in response to climate differences.

To test these predictions, we determined if there were genetically based latitudinal clines in leaf-level morphological and physiological traits among native and introduced populations and explored whether these clines were convergent. As well, we assessed whether there was directional phenotypic selection on leaf traits of native genotypes that were experimentally introduced into a common garden in Washington. Finally, we quantified levels of plasticity in leaf-level physiological traits across common gardens that varied in climate (Washington vs. Spain), compared plasticity between native and introduced populations and determined whether this plasticity was adaptive.

Methods

St. John's Wort is native to Europe, North Africa, and Asia and was introduced to North and South America, South Africa, New Zealand, and Australia. It is commonly found in disturbed sites, along roadsides, in overgrazed rangeland, and in abandoned fields. Plants germinate in the spring; young plants initially send out prostrate shoots that eventually bolt, flower, and set seed in late summer. After setting seed, plants senesce until winter rainfall stimulates new growth. St. John's Wort has an extremely diverse mode of reproduction. Individuals vary from obligate outcrossers to obligate apomicts. The majority of plants from most populations are, however, facultative pseudogamous apomicts; individuals produce upwards of 90% of their seed apomictically but some fraction of seeds are produced via outcrossing (Arnholdt-Schmitt 2000; Matzk et al. 2001, 2003).

We obtained leaf trait data from plants grown in field common gardens, and biomass allocation data from greenhouse-grown plants, to determine whether clinal patterns in traits among native European and introduced North American plants were similar. We used leaf trait and performance measures (size and fecundity) of European populations in a common garden in Washington to determine whether there was directional phenotypic selection on these traits. Finally, to quantify phenotypic plasticity, explore how this varied between native and introduced populations, and determine its adaptive value, we compared differences in leaf traits and performance among populations grown in common gardens in Washington and Spain.

FIELD COMMON GARDENS

We collected mature seed capsules of *H. perforatum* from at least 10 individuals from 18 source populations across Europe and 17 source populations from western North America (Table 1). All source populations occurred below 1000 m and were separated

from each other by at least 20 km. European collection sites average 30.9–160.8 mm of rainfall during May–August, the peak growing season for St. John's Wort. For the same period, average rainfall ranges from 9.8 to 44.6 mm for North American collection sites. Seed collection methods are outlined in Maron et al. (2004a).

We germinated seeds and grew plants for several months in greenhouses at the University of Washington and the University of Barcelona prior to transplanting into plots in common gardens located in Snohomish, Washington (latitude 47°52') and the Universitat Autònoma de Barcelona campus field station in Bellaterra (Barcelona), Spain (latitude 41°34'). Seedlings were outplanted into the Washington garden in May 2000 whereas the Spain common garden was initiated in May 2002. At the Washington site, the winters are cool and wet, and summers can be either hot and dry or cooler with rain. Mean annual precipitation is 1230 mm. The Spanish site experiences a typical Mediterranean climate with cool winters and hot and dry conditions in summer. Mean annual precipitation is 590 mm.

In Washington, we outplanted 10 individuals, each from a different maternal family (except for four population where we drew from a pooled sample of seeds), from each of 35 source populations (18 native European populations and 17 introduced populations from western North America; Table 1). In Spain we used 10 individuals from each of 29 source populations (15 European and 14 western North American; Table 1). Where possible, we used the same maternal families in both common gardens. Because St. John's Wort produces the majority of its seed apomictically, maternal sibs planted across gardens were likely clones (Arnholdt-Schmitt 2000; Mayo and Langridge 2003). Information pertaining to the spatial distribution of plants in gardens and other details can be found in Maron et al. (2004a,b).

We assessed plant fecundity by estimating the cumulative number of seed capsules produced by plants across multiple years (2000–2002 in Washington, 2002–2004 in Spain). We estimated plant size (in summer 2002 in Washington and summer 2004 in Spain) as $V = ((W_1 + W_2)/4)^2 \times \pi \times H$, where V is the plant volume, W_1 and W_2 are the width of each plant in perpendicular directions, and H is the height of the tallest stem. Estimates of plant volume were highly correlated with plant biomass based on separate experiments (J. Maron, unpubl. data).

In July 2002 and 2004, after plants had grown in Washington and Spain, respectively, for two seasons, we harvested unshaded healthy leaves from the tops of the tallest shoots from an average of 5.4 individuals per population (range: 3–7) in Washington and an average of 4.7 individuals per population (range: 2–7) in Spain. We could not sample leaves for all individuals due to end of summer plant senescence (in Washington) and plant mortality (in Spain). Leaves were air dried, then ground, and analyzed at the Colorado Plateau Stable Isotope Laboratory, Northern

Table 1. Location of seed source populations. Location refers to country (for native European populations) or state (for introduced North American populations) of origin. Experimental sites: WA, Washington; SP, Spain; GR, Greenhouse.

Location	Collection site	Latitude/Longitude	Experimental site
Native range			
Germany	Hamburg	53°55'N/10°58'E	WA
The Netherlands	DeSteege	52°00'N/6°05'E	WA, SP
England	Silwood Park	51°40'N/0°66'W	WA, SP
Germany	Bonn	50°73'N/7°1'E	WA, SP, GR
Czech Republic	Prague	50°08'N/14°46'E	WA, SP, GR
France	Neuves Maisons	48°60'N/4°03'E	WA
Germany	St. Blasien	47°73'N/8°0'E	SP
Switzerland	Arlesheim	47°48'N/7°61'E	WA
Switzerland	Adliswil	47°30'N/8°56'E	WA, SP, GR
France	Pontailleur-sur-Saône	47°30'N/5°41'E	WA
Italy	Bolzano	46°51'N/12°15'E	WA, SP
Italy	Badia Polesine	45°08'N/11°48'E	WA, SP, GR
France	Clapier	43°70'N/7°41'E	WA, SP, GR
France	Assas	43°70'N/3°88'E	WA, SP
Kyrgyzstan	Suu Ravine	42°65'N/74°51'E	WA, SP
France	Cerbère	42°45'N/3°16'E	WA, SP, GR
Spain	Centellas	41°80'N/2°22'E	WA, SP, GR
Spain	Granera	41°73'N/2°06'E	WA, SP, GR
Spain	La Selva del Mar	41°21'N/1°13'E	WA, SP, GR
Introduced range			
Washington	Mima Mounds	46°63'N/122°95'W	WA, SP, GR
Oregon	Corvallis	44°61'N/123°20'W	WA, SP, GR
Oregon	Winchester	43°26'N/123°36'W	WA, SP
Oregon	Sunny Valley	42°70'N/122°88'W	WA, SP, GR
Oregon	Gold Hill	42°45'N/123°66'W	WA, SP
Oregon	Ashland	42°20'N/122°71'W	WA, SP, GR
California	Gasquet	41°85'N/122°63'W	WA
California	Weed 1	41°43'N/122°25'W	WA
California	Weed 2	41°33'N/122°38'W	WA, SP
California	Mt. Shasta	41°30'N/122°25'W	WA, SP, GR
California	Eel River	40°83'N/124°10'W	WA
California	Holiday	40°62'N/122°30'W	WA, SP, GR
California	Covelo	39°88'N/123°25'W	WA, SP
California	Sierra Field Station	39°25'N/121°60'W	WA, SP, GR
California	Meadow Vista	39°00'N/121°08'W	WA, SP, GR
California	Bass Lake	38°66'N/119°50'W	WA, SP
California	Cazadero	38°55'N/123°13'W	WA, SP, GR

Arizona University (Flagstaff, AZ) for determination of $\delta^{13}\text{C}$ as well as percent leaf N and percent leaf C. Analyses were conducted using a Carlo Erba model NA2500 elemental analyzer coupled to a Finnigan Delta Plus isotopic ratio mass spectrometer. To estimate SLA (leaf area/leaf mass), in Washington in July 2006, we harvested one healthy leaf from every plant at the sixth node from the top of the tallest shoot. We flattened each leaf and measured its length and width (at the widest point) and then dried and weighed leaves individually. We converted leaf length and width to leaf area based on the following regression developed for a subsample of greenhouse-grown plants (see below for methods): individual

leaf area = width \times length \times 0.785 + 0.001; $n = 182$, $R^2 = 0.99$, $P < 0.0001$. In Spain we used identical methods to estimate SLA.

GREENHOUSE COMMON GARDEN

In February 2002, we initiated a greenhouse experiment to determine if there were clinal patterns in root and root/shoot biomass among native and introduced populations. We created conditions in the University of Washington greenhouse to simulate a dry site. Pots were kept uniformly moist for 30 days after seeding to promote germination and seedling establishment. Thereafter, plants were watered when soil moisture dropped below a

predetermined threshold, which was just before water stress caused permanent wilting. Soil moisture levels were monitored daily in a subset of pots using a HydroSense soil moisture probe (Spectrum Technologies, Plainfield, IL). This probe measures dielectric permittivity to estimate volumetric soil water content. We used seed from 10 maternal families from each of nine source populations per continent (Table 1). Seed from each maternal family (five seeds per pot) were planted in separate one-gallon pots filled with Turface (Profile Products LLC, Buffalo Grove, IL), a calcid clay substrate. We mixed 4.6 g/pot of slow release 17:6:12 (N-P-K) + minors osmocote fertilizer (Scots-Sierra Horticultural Products Co., Marysville, OH) into the growth medium of each pot. Seeds were germinated in a small plug of moist potting soil placed in the center of each pot. One week after germination ceased, seedlings were selectively thinned to one individual per pot. Pots were arranged in 10 blocks with each population represented by one plant in each block (18 plants per block). In total there were 2 continents \times 9 populations per continent \times 10 blocks = 180 plants. Positions of blocks and pots within blocks were regularly rerandomized on greenhouse benches to reduce position effects. Pyrethrol and BT (gnatrol) were applied once at the beginning of the experiment to control greenhouse pests. Plants experienced ambient light during the day, plus supplemental greenhouse lighting during the evenings (1700–2100 h). Greenhouse lights were also used on rainy days during the first six weeks of the experiment, when natural light levels were low. Immediately before harvest we removed, air dried, and pressed one leaf from the sixth node from the apex of the tallest stem of each plant. Leaf area was determined by digitizing pressed leaves using a flatbed scanner and analyzing total area using NIH Image version 1.62 (available on the Internet at <http://rsb.info.nih.gov/nih-image/>). In May 2002, during the fifteenth week of the experiment, we destructively harvested all plants. We divided each plant into stem, leaf and root tissues, and determined the oven-dried weight of each component separately. Based on these data, for each individual plant we calculated total leaf area, which was SLA multiplied by the total leaf weight. We also calculated LAR, which is the ratio of total leaf area to total shoot dry weight.

STATISTICAL ANALYSES

We took a two-step approach to analyze data collected within common garden and greenhouse experiments. First, we used nested ANOVA to explore how trait variation differed among populations and between continents of population origin. We performed these tests using the PROC GLM module within SAS using Type III sum of squares (vers. 9.1), where continent of population origin (Europe vs. North America) was treated as a fixed factor and population nested within continent was treated as a random factor. For greenhouse data we also added block as a random factor. Second, we performed ANCOVA (in Systat, ver. 11) on population means,

using continent of population origin as a fixed factor and latitude as a covariate. Because preliminary analyses revealed no continent \times latitude interaction for any trait, this term was dropped from analyses. Response variables in these analyses were percent leaf N and C (arcsine square-root transformed), $\delta^{13}\text{C}$ and SLA for data collected from field common gardens and root and shoot biomass, root/shoot ratio, total leaf area, and LAR for data collected from the greenhouse experiment.

One potential difficulty in interpreting results from these analyses is that we sampled European populations across a larger latitudinal range than we did for North American populations. Thus, it is possible that significant clinal patterns observed when all data are analyzed together might result from relatively strong effects imposed by native European populations and relatively weak effects from introduced North American populations (yet effects not divergent enough to produce significant latitude by continent interactions). To explore this possibility, we regressed latitude on population means of trait values for North American populations in isolation.

To determine if there was directional selection on traits in the introduced range, we performed phenotypic selection analysis based on data collected on European plants in the Washington garden (Lande and Arnold 1983). Our goal was to mimic the original invasion of multiple European genotypes into one North American site to examine the selection these genotypes might have experienced. We excluded introduced genotypes from this analysis because they may have already passed through a “selection filter.” Ideally, this test would be conducted on plants grown in the field from seed, but this was not possible as we did not want to create a new invasion with novel genotypes. However, in a separate experiment we found no strong effect of latitude of origin on seed germination or initial seedling establishment (Maron 2007). Estimates of selection can be biased if there is substantial mortality and traits are measured at the end of the experiment, after this mortality has occurred (Lynch and Arnold 1988). In Washington, this was unlikely to be a problem, as mortality during the course of the experiment was quite low; after 29 months in the field only 4.6% of plants had died (Maron et al. 2004a).

In JMP (ver 4.0) we performed linear multiple regressions of relative fitness on the standardized values for the following traits: percent leaf N and C, $\delta^{13}\text{C}$, and SLA. To avoid pseudoreplication, analyses were conducted using population means rather than individuals as replicates. We standardized traits and fitness metrics to have a mean of zero and a standard deviation of one. Partial regression coefficients from these multiple regressions indicate the directional selection gradient (β) on each trait. We also used simple linear regressions to calculate standardized selection differentials (S) for each trait in isolation. We lacked sufficient power to estimate stabilizing or disruptive selection, because these analyses require testing quadratic (γ_{ii}) and cross-product functions

(γ_{ij}) of traits and our sample size was limited to 18 populations. Correlations among traits were also calculated using population means.

Finally, to examine how phenotypic plasticity might enable plants to invade climatically distinct continents, we determined if traits varied plastically across our two garden environments, whether there was genetic variation for phenotypic plasticity, and the adaptive significance of this plasticity where possible. We considered conditions in the Spanish and Washington gardens examples of some of the contrasting environmental conditions the species might encounter when transported to new areas. Strictly, such an analysis should be conducted on replicate clones or siblings planted in both gardens. In our case, however, we analyzed genetic variation for plasticity at the population level even though plants in the two gardens were not always siblings or clones. Analyzing plasticity at the population or species level is less precise than when experiments are conducted on clones (Schlichting 1986). However, population level or even species level comparisons of plasticity are commonly used to make more general inferences about the role of plasticity in natural communities (Richards et al. 2006). Accordingly, considering genetic variation at the population rather than family level allowed us to include many populations, which made for a more representative sample from each region. Furthermore, because St. John's Wort produces a high fraction of seeds apomictically (Arnholdt-Schmitt 2000; Matzk et al. 2001, 2003), analysis of plasticity at the population level is appropriate because most genetic differentiation is partitioned among rather than within populations (Maron et al. 2004a).

To examine the magnitude of plasticity for leaf-level traits across gardens, we performed ANOVAs on each trait (SAS ver. 9.1, Proc GLM). Garden location, continent of population origin, and their interaction were treated as fixed effects, with population nested within continent and its interaction with garden location treated as random effects. The relative magnitude of plasticity for plants from each continent is reported as the proportional difference in least-squares means between gardens (i.e., $X_{Wa} - X_{Sp} / (1/2 (X_{Wa} + X_{Sp}))$). Percent leaf N and C were arcsine square-root transformed.

To assess the potential adaptive significance of plasticity, we took a twofold approach. We first determined whether populations differed significantly in plasticity for each trait, because genetically based variation is a prerequisite for testing the adaptive significance of plasticity (Steinger et al. 2003). To do this, we conducted ANOVAs on European and North American populations separately, testing the effects of population, garden location, and their interaction on each trait. Significant population \times garden interactions indicate genetic variation for plasticity at the among-population level. If there was significant variation among populations in plasticity, we then performed an across-environment genotypic selection analysis to assess the adaptive significance of

such a variation (Caruso et al. 2006). Using multiple regression, we regressed standardized mean population fitness across both gardens on a standardized mean population value for a particular trait across gardens and a standardized plasticity index for that trait. We calculated a standardized plasticity index for traits as the population mean value in Spain–Washington, because population means were generally higher in Spain for traits of interest; (Caruso et al. 2006). Standardized mean population fitness was calculated for European and North America populations separately. A significant positive regression coefficient for plasticity indicates that more plastic populations maintained the highest fitness across gardens. By necessity, population means for both traits and fitness measurements included only plants that survived to reproduction. To ensure strong selection at earlier life stages did not alter our results, we repeated these analyses with fitness values adjusted to account for potential differences in mortality among populations by multiplying population level mortality rates by reproductive output. This revealed qualitatively similar results as reported.

Results

CLINES IN LEAF PHYSIOLOGICAL TRAITS IN FIELD COMMON GARDENS

Populations of St. John's Wort originating across a wide latitudinal distribution throughout Europe (native range) and North America (introduced range) exhibited strong population differentiation in percent leaf N and C and $\delta^{13}\text{C}$ when grown in common gardens in Washington and Spain (Table 2). As well, there was significant population differentiation for SLA in the Spain garden, although this was not the case in the Washington garden (Table 2). The genetically based differentiation in leaf percent N among populations occurred along a latitudinal gradient; there was a significant positive relationship between latitude of population origin and percent leaf N (Figs. 1, 2; Table 3). In both common gardens, individuals from populations originating from more northerly latitudes in Europe and North America had higher levels of percent leaf N than did plants from more southerly populations (Figs. 1, 2). The slopes of these latitudinal clines were similar in both common garden locations, despite the fact that common gardens differed in latitude and climate. Not only were there significant clines in this trait when all populations were analyzed together but even introduced populations in isolation formed significant or marginally significant clines in percent leaf N (Washington garden: effect of latitude, $F_{1,15} = 4.53$, $P < 0.05$; Spain garden: effect of latitude, $F_{1,12} = 4.44$, $P = 0.06$).

In contrast to the results for percent leaf N, there were no latitudinal clines in percent leaf C, $\delta^{13}\text{C}$, or SLA that were expressed consistently across both gardens (when native and introduced populations were analyzed together; Table 3). In Washington, there was a cline in percent leaf C, but this was not the case in Spain (Table 3). When analyzed in isolation, native

Table 2. Results from ANOVA testing for the effect of continent of population origin (i.e., native or introduced) and population nested within continent on St. John's Wort percent leaf N and C, $\delta^{13}\text{C}$, and SLA in a common garden in (A) Washington (introduced range) and (B) Spain (native range). In Washington, error degrees of freedom for Continent for SLA are 261. In Spain, error degrees of freedom for SLA for Continent and population (Continent) are 17 and 72, respectively.

(A)													
Source	df	% Leaf N			% Leaf C			Leaf $\delta^{13}\text{C}$			SLA		
		SS	F	P	SS	F	P	SS	F	P	SS	F	P
Continent	1	0.00037	1.1	0.299	0.00019	2.66	0.11	10.83	7.68	0.009	0.012	1.91	0.18
Pop. (Continent)	33	0.010	1.87	0.007	0.0026	2.54	0.0001	50.09	6.68	0.001	0.21	1.11	0.32
Error	136	0.022			0.0042			30.9			1.54		

(B)													
Source	df	% Leaf N			% Leaf C			Leaf $\delta^{13}\text{C}$			SLA		
		SS	F	P	SS	F	P	SS	F	P	SS	F	P
Continent	1	0.00068	0.96	0.33	0.0027	19.01	0.0001	3.6	1.30	0.26	0.0018	1.1	0.74
Pop. (Continent)	27	0.02	3.13	0.0001	0.004	1.81	0.017	79.78	4.64	0.0001	0.029	2.13	0.014
Error	107	0.025			0.0087			68.13			0.059		

European populations exhibited a negative relationship between latitude of population origin and $\delta^{13}\text{C}$ ($F_{1,16} = 8.3, P < 0.02$) and a marginally significant positive relationship between latitude of population origin and percent leaf C (Fig. 1) in Wash-

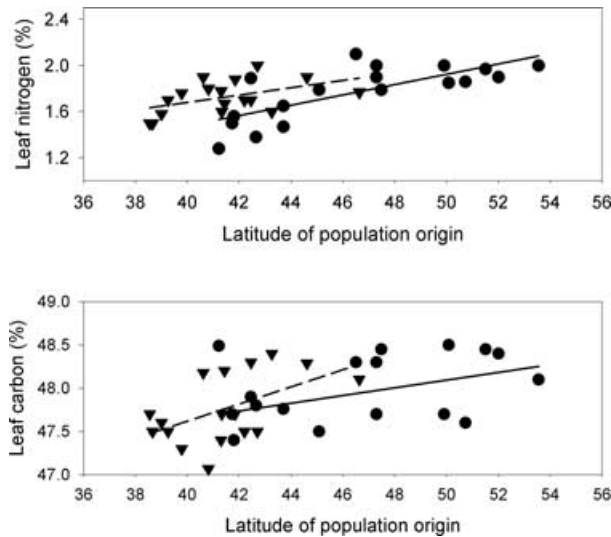


Figure 1. Mean percent leaf nitrogen (top panel) and mean percent leaf carbon (bottom panel) for native European (circles) and exotic North American (triangles) populations grown in a common garden in Washington. Lines through points indicate a significant (or marginally significant) effect of latitude of population origin on trait value. Effect of latitude for native populations in isolation for percent leaf N ($F_{1,16} = 17.4, P < 0.0008$) and percent leaf C ($F_{1,16} = 3.4, P = 0.085$).

ington. However, European populations showed no clines in $\delta^{13}\text{C}$ ($F_{1,13} = 1.0, P = 0.33$) or percent leaf C (Fig. 2) in the common garden in Spain. Introduced North American populations, in isolation, exhibited a significant positive relationship between latitude of origin and percent leaf C in Washington

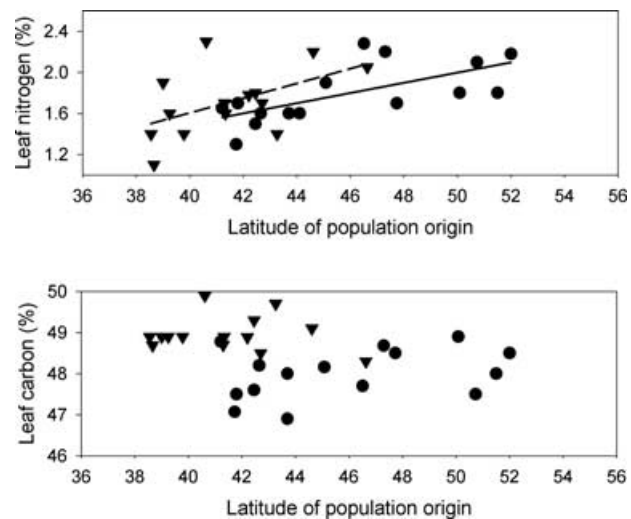


Figure 2. Mean percent leaf nitrogen (top panel) and mean percent leaf carbon (bottom panel) for native European (circles) and exotic North American (triangles) populations grown in a common garden in Spain. Lines through points indicate a significant effect of latitude of population origin on trait value. Effect of latitude for native populations in isolation for percent leaf N ($F_{1,13} = 10.6, P < 0.007$) and percent leaf C ($F_{1,13} = 1.9, P = 0.19$).

Table 3. Results from ANCOVA testing for the effect of continent of population origin (i.e., native or introduced) and latitude of population origin on percent leaf N and C, $\delta^{13}\text{C}$, and SLA in a common garden in (A) Washington (introduced range) and (B) Spain (native range). ANCOVA performed on population means. Error degrees of freedom for SLA in Spain is 16.

(A)													
Source	df	% Leaf N			% Leaf C			Leaf $\delta^{13}\text{C}$			SLA		
		SS	F	P	SS	F	P	SS	F	P	SS	F	P
Continent	1	0.0002	7.05	0.012	0.085	0.55	0.46	5.63	2.47	0.13	0.0006	0.72	0.40
Latitude	1	0.0009	25.44	0.0001	1.29	8.3	0.007	0.004	0.002	0.97	0.0003	0.30	0.59
Error	32	0.0011			4.95			73.1			0.028		

(B)													
Source	df	% Leaf N			% Leaf C			Leaf $\delta^{13}\text{C}$			SLA		
		SS	F	P	SS	F	P	SS	F	P	SS	F	P
Continent	1	0.0002	1.78	0.19	0.0018	9.68	0.0045	0.68	1.16	0.29	0.0003	1.63	0.22
Latitude	1	0.0013	13.01	0.0013	0.0004	2.38	0.13	0.0016	0.0028	0.96	0.0003	2.19	0.16
Error	26	0.0025			0.0048			15.29			0.0025		

($F_{1,15} = 7.24, P < 0.017$) but not in Spain ($F_{1,12} = 0.13, P = 0.72$). However, North American populations produced no significant latitudinal clines in $\delta^{13}\text{C}$ (Washington: $F_{1,15} = 1.6, P = 0.22$, Spain: $F_{1,12} = 0.88, P = 0.37$) or SLA (Washington: $F_{1,15} = 1.3, P = 0.27$, Spain: $F_{1,6} = 0.25, P = 0.63$).

After statistically controlling for latitude of population origin, there were no significant differences in leaf traits based on continent of origin that were consistent across both gardens (Table 3). Percent leaf N was higher for native versus introduced plants grown in Washington (Table 3), but this was not the case for plants grown in Spain (Table 3). Percent leaf C was lower for native than introduced plants grown in Spain but this was not the case in Washington (Table 3).

CLINES IN PLANT TRAITS IN A GREENHOUSE COMMON GARDEN

In the greenhouse experiment, both native and introduced populations exhibited significant latitudinally based clines in root ($F_{1,15} = 70.9, P < 0.0001$) and above ground ($F_{1,15} = 24.8, P < 0.0003$) biomass (Fig. 3), and in total leaf area ($F_{1,15} = 37.8, P < 0.0001$) but we detected no cline in root/shoot ratio ($F_{1,15} = 2.97, P = 0.10$) or LAR ($F_{1,15} = 0.13, P = 0.72$). Where there were significant latitudinal clines, there was no difference in the slope of clines between native and introduced populations (as indicated by a lack of latitude \times continent interaction; $P > 0.05$). Plants from more northerly populations had greater root and above ground biomass and greater total leaf area than did plants from more southerly populations

(Fig. 3). Introduced populations in isolation also exhibited significant clines in root biomass ($F_{1,7} = 38.5, P < 0.0004$), and marginally significant clines in total leaf area ($F_{1,7} = 5.2, P = 0.057$) and aboveground biomass ($F_{1,7} = 4.4, P = 0.07$). After controlling for latitude, introduced populations had greater root ($F_{1,15} = 71.83, P < 0.0001$) and shoot ($F_{1,15} = 32.5, P < 0.0001$) biomass, and also greater total leaf area ($F_{1,15} = 34.9, P < 0.0001$) than did those from native populations. For plants from both Europe and North America there were also significant differences among populations in shoot ($F_{16,148} = 8.08, P < 0.0001$) and root biomass ($F_{16,149} = 6.17, P < 0.001$).

PHENOTYPIC SELECTION AND PLASTICITY

Although some leaf traits included in the selection analysis were significantly correlated, the correlation coefficients among these traits were mostly statistically nonsignificant (Table 4). Interestingly, percent leaf N and $\delta^{13}\text{C}$ were not positively related, as one might expect if higher N translates into greater assimilation rate per unit of water loss (Table 4). Selection differentials indicated that when European *H. perforatum* populations were grown in Washington, there was strong directional selection for lower $\delta^{13}\text{C}$ values (and thus WUE) and percent leaf N (and percent leaf C when size was used to estimate fitness), as indicated by significant selection differentials for these traits (Table 5). Native plants with more negative $\delta^{13}\text{C}$ values and higher levels of percent leaf N were larger and more fecund than plants with less negative $\delta^{13}\text{C}$ values and lower levels of percent leaf N. The directional selection gradient on $\delta^{13}\text{C}$ was statistically significant (when size was used

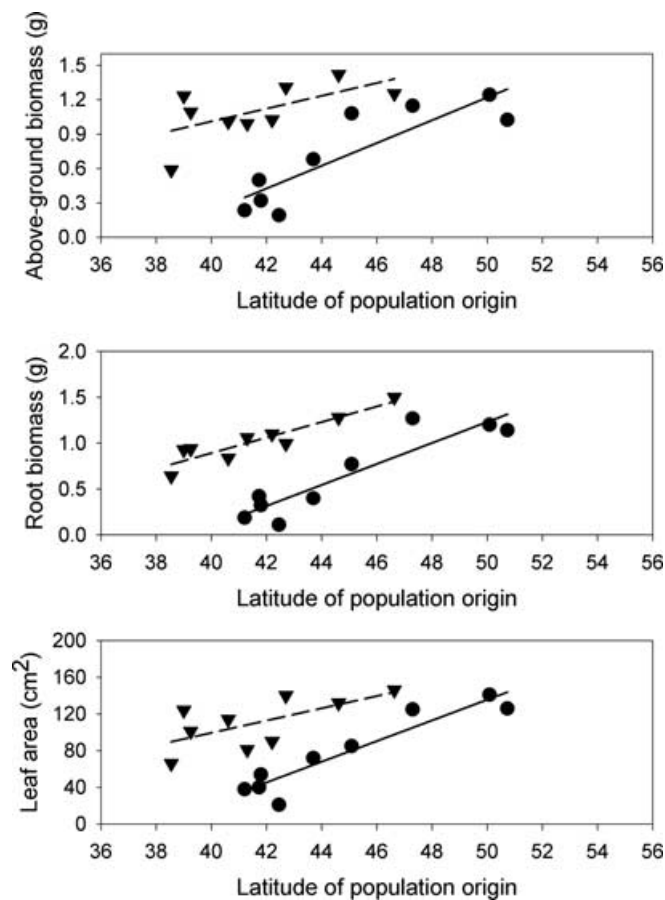


Figure 3. Aboveground biomass (top panel), root biomass (middle panel), and total leaf area (SLA \times total dry weight leaf biomass) of plants from native European (circles) and exotic North American (triangles) populations grown in a greenhouse. Lines through points indicate a significant effect of latitude of population origin on trait value.

to estimate fitness) or marginally significant (when fecundity was used to estimate fitness; Table 5), whereas selection gradients for percent leaf N and C and SLA were not statistically significant.

Across gardens, there was significant plasticity for $\delta^{13}\text{C}$ (effect of garden; ANOVA, $F_{1,27.22} = 177.62$, $P < 0.0001$) and SLA (effect of garden; ANOVA, $F_{1,16.875} = 148.58$, $P < 0.0001$), and a significant continent of population origin \times garden interaction (indicating that the magnitude of plasticity varied by continent of

Table 4. Pearson correlation matrix showing correlations among population means for physiological traits measured in the Washington common garden ($N = 18$). Correlation coefficients in bold are significant at $P < 0.05$ (after Bonferroni correction).

	% Leaf N	% Leaf C	Leaf $\delta^{13}\text{C}$
% Leaf C	0.353		
Leaf $\delta^{13}\text{C}$	-0.408	-0.645	
SLA	0.220	-0.542	0.301

Table 5. Standardized selection differentials (S_i) and standardized directional selection gradients (β) on percent leaf N, percent leaf C, stable isotopes of C ($\delta^{13}\text{C}$) and specific leaf area (SLA) for native populations grown in a common garden in Washington ($N = 18$). Standardized selection differentials estimated as the covariance between relative fitness and leaf traits. Standardized directional selection gradients obtained from linear multiple regressions of relative fitness (fecundity or size) on leaf traits. S_i and β values in bold are significant at $P < 0.05$ in whole models that were also significant. * $P = 0.08$.

Relative fitness				
Traits	S Fecundity	β Fecundity	S Size	β Size
Leaf % N	0.5329	0.3291	0.4735	0.1780
Leaf % C	0.1208	-0.0836	0.5753	-0.1570
Leaf $\delta^{13}\text{C}$	-0.6148	-0.5479*	-0.7220	-0.5627
SLA	-0.0354	0.0466	-0.1065	0.0452

population origin) for percent leaf C ($F_{1,29.16} = 33.36$, $P < 0.001$). In contrast, the continent \times garden interaction was not significant for $\delta^{13}\text{C}$ ($F_{1,27.22} = 0.79$, $P = 0.38$), percent leaf N ($F_{1,29.98} = 2.12$, $P = 0.15$), and SLA ($F_{1,16.87} = 1.42$, $P = 0.25$). In Spain, both European (EU) and North American (NA) populations had higher $\delta^{13}\text{C}$ (higher WUE; 3% EU, 1% NA) and lower SLA (26% EU, 24% NA) than did their counterparts in Washington. Percent leaf C was 2.6% higher for North American plants grown in Spain versus Washington. Percent leaf N did not vary consistently across gardens ($F_{1,29.98} = 1.23$, $P = 0.28$).

Plasticity for $\delta^{13}\text{C}$ varied significantly among both EU and NA populations (EU populations, $F_{13,104} = 2.6$, $P < 0.004$; NA populations, $F_{13,107} = 3.0$, $P < 0.0008$), whereas plasticity of other physiological traits did not. For European populations, plasticity in $\delta^{13}\text{C}$ appeared adaptive as populations with the highest levels of plasticity maintained the highest fitness across both gardens (significant effect of plasticity on mean fitness across gardens, for capsule number, $\beta = 0.68 \pm \text{SE } 0.29$, $P < 0.04$; volume, $\beta = 0.69 \pm 0.18$, $P < 0.003$). In contrast, plasticity in North American populations did not appear adaptive, as the most plastic populations did not maintain the highest average fitness across gardens (for capsule number, $\beta = -0.03 \pm 0.42$, $P = 0.95$; volume $\beta = 0.08 \pm 0.44$, $P = 0.42$). The direction of plasticity in SLA suggests plasticity in this trait is also adaptive, because thicker leaves in the Spanish garden would likely reduce water loss in this dry climate. However, because all populations showed similar levels of plasticity for this trait, we could not test its adaptive significance statistically.

Discussion

Although there is a long and rich history of examining levels of local adaptation in native plants (reviewed by Linhart and Grant

1996), we know surprisingly little about the prevalence or mechanisms of local adaptation in exotic populations (McKay et al. 2003). We found that St. John's Wort from diverse native European populations formed significant latitudinal clines in percent leaf N in common gardens in both Washington and Spain. Furthermore, we found latitudinal clines in $\delta^{13}\text{C}$ and a marginally significant latitudinal cline in leaf percent C in Washington, and in root and shoot biomass and total leaf area in a greenhouse study. In general, as predicted, plants from northern European populations had higher values of percent leaf N, root and shoot biomass, and total leaf area; and more negative values of $\delta^{13}\text{C}$ (lower WUE) compared to plants from southern European populations. Contrary to our original prediction, plants from northern latitudes had lower rather than higher leaf percent C compared to those from southern locales. This clinal differentiation among populations in physiological traits suggests that plants have adapted to differences in climate that vary with latitude. In particular, the generally higher $\delta^{13}\text{C}$ and lower percent leaf N in southern versus northern European populations may indicate that water limitation in drier and hotter southern regions is more limiting to growth (cell expansion and enlargement) than gas exchange. Increased WUE, in particular, can be an important adaptive response to dry conditions (Dudley 1996a). In contrast, the generally cooler and wetter climate of Northern Europe may have selected for a growth strategy that maximizes photosynthetic assimilation and biomass accumulation (Lambers et al. 1998). The fact that there was no cline in LAR despite the fact that plants from southern locales had less total leaf area than plants from northern locales, suggests that plants from the south are reducing their total biomass in direct proportion to their smaller leaf area.

Among introduced populations, we also found clines in percent leaf N within both field gardens, and clines in percent leaf C, root biomass, and total leaf area in single gardens (Washington field garden or greenhouse). These clines had a similar slope as those found among native populations. Clines in morphological traits have been documented for other exotic plants (Reinartz 1984; Lacey 1988; Weber and Schmid 1998; Kollmann and Bañuelos 2004), but it is not known whether these clines mimic those that exist in the native range. Our previous work on St. John's Wort revealed that introduced genotypes are not always most closely related to individuals originating from a similar latitude in Europe (Maron et al. 2004a). Thus, climate matching (i.e., native European genotypes being introduced only to similar latitudes in North America as where they originated in Europe) and/or strong initial filtering out of genotypes do not by themselves account for the observed clinal variation in physiological or morphological traits. It therefore seems likely that introduced genotypes have undergone adaptive evolution in response to broad-scale climatic conditions across portions of western North America. Because St. John's Wort has only been present in western North Amer-

ica for 100–150 years (Campbell and Delfosse 1984), leaf-level traits have evolved relatively rapidly. Rapid evolution of physiological traits has been previously demonstrated when plants are exposed to extreme conditions, such as toxic soils (Antonovics et al. 1971). Our results suggest that plants can also rapidly evolve physiological adaptations in response to new climatic conditions when introduced.

One limitation of our study was that there was a significant amount of pathogen-induced mortality among plants in our common garden in Spain (Maron et al. 2004b). On average 55% of the individuals from each population were killed prior to when we measured leaf traits. This reduction in sample size may be why we found only marginally significant clines in percent leaf N, and no clines in percent leaf C in Spain despite the fact that these traits exhibited clines in the Washington garden. It also might explain why we failed to detect a significant cline in $\delta^{13}\text{C}$ among European populations in Spain even though these plants exhibited a strong cline in $\delta^{13}\text{C}$ in Washington.

Evidence for selection on physiological traits is still limited (but see Dudley 1996a); most phenotypic selection studies of plants have focused on how selection operates on life history or morphological traits rather than on physiological traits (Arntz and Delph 2001). We examined selection on physiological traits among native European genotypes that were experimentally introduced into North America. Our approach was to treat our common garden in Washington as, in essence, an experimental invasion composed of a diverse set of genotypes from Europe that had a greater or lesser degree of "pre-adaptation" to the abiotic conditions in Washington, based on their latitude of population origin. We recognize that our common garden did not fully simulate a true invasion, as it was initiated with young plants (rather than their propagules), and the founding "population" consisted of plants from multiple native populations originating from a wide latitudinal distribution across Europe. Although conservative, our approach represents a first step in determining how selection acts on traits that may provide adaptation to climatic conditions among newly introduced plant genotypes.

We found significant genetically based differences in percent leaf N and $\delta^{13}\text{C}$ and strong selection differentials for percent leaf N and $\delta^{13}\text{C}$. Plants with higher percent leaf N and lower $\delta^{13}\text{C}$ (i.e., lower WUE) had significantly higher fecundity and biomass compared to plants with lower percent leaf N and higher $\delta^{13}\text{C}$ (i.e., higher WUE). A similar negative relationship between WUE and biomass has been found for several species (Condon et al. 1987; White et al. 1990) although the opposite result has also been found (Virgona et al. 1990; Donovan and Ehleringer 1994). In cases where there is a negative relationship between WUE and biomass, stomatal limitation is likely driving the relationship rather than photosynthetic capacity (Hubrick et al. 1986). Given that we found a significant

directional selection gradient on WUE but not on percent leaf N, it is likely that selection is acting directly on WUE and perhaps indirectly on percent leaf N, which is weakly correlated with WUE ($P = 0.09$).

How invaders that may be locally adapted to a specific set of environmental conditions in their native range thrive in a diversity of habitats in the introduced range is one of the major unanswered questions in invasion ecology. One avenue is for an invader to become locally adapted to specific sites in the introduced range, suggesting an important role for evolutionary change (Lee 2002). A widely proposed alternative mechanism is phenotypic plasticity (Williams et al. 1995; Sexton et al. 2002), whereby a general purpose genotype is able to succeed across variable conditions in both the introduced and native ranges (sensu Baker 1974). In the case of St. John's Wort, we found that most morphological and physiological traits either had undergone significant local adaptation in the new range (percent leaf N) or exhibited significant phenotypic plasticity ($\delta^{13}\text{C}$ and SLA). Populations expressed higher $\delta^{13}\text{C}$ (higher WUE) and lower SLA in the drier Spanish garden, as might be expected if plasticity in these traits contributed to the success of this species across variable environments. For European populations, plasticity in $\delta^{13}\text{C}$ was positively correlated with mean fitness across the two experimental gardens. Populations that modulated WUE according to environmental cues maintained the highest average fitness across the contrasting rainfall and temperature regimes represented in the two common gardens. We found no significant plasticity for percent leaf N and plasticity for percent leaf C only among North American populations. In contrast to plastic traits, differences in percent leaf N among populations appear to be relatively canalized, with the same latitudinal clines regardless of whether plants were grown in Spain or Washington.

It has been proposed that plasticity may function as a buffer against natural selection, slowing local adaptation (Bradshaw 1965). This hypothesis may help explain the evolution, or lack thereof, of morphological and physiological clines in various leaf traits in St. John's Wort. For traits that exhibited significant phenotypic plasticity ($\delta^{13}\text{C}$ and SLA) introduced populations have not evolved strong latitudinal clines. In contrast, a trait that was relatively canalized (percent leaf N) has responded rapidly to selection regimes in the new range, evolving latitudinal clines that mimic those found among native populations. Previous studies indicate that there is often little population differentiation in traits of introduced plants that exhibit substantial phenotypic plasticity (Parker et al. 2003).

We did not find overall differences in levels of plasticity between European and North American populations (with the exception of percent leaf C where plasticity was higher among North American than European populations) as one might expect if there was strong directional selection on plasticity in the new range. Despite adaptive plasticity for some traits, as a whole local adaptation

appears to be the primary mechanism behind the successful invasion of St. John's Wort. The fact that slopes of latitudinal clines in fecundity and size switched from positive to negative between northern and southern common gardens (Maron et al. 2004a) suggests that there is no "general purpose genotype" for this species. Rather, these patterns are what one would predict if there is little plasticity in traits most important to fitness. It is likely that strong selection on physiological and morphological traits resulted in clines in fitness previously observed, with plants from northern populations locally adapted to northern climates and plants from southern populations locally adapted to southern climates.

Introduced genotypes of plants undoubtedly experience novel selection pressures in the introduced range. Results from this study highlight the fact that such selection can lead to rapid evolution, which appears to have contributed to the invasive success of St. John's Wort. Still not well resolved is the overall importance of rapid evolution to plant invasions in general, and how evolutionary and ecological processes interact to shape the outcome of exotic plant introductions.

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