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# An Evolutionary Approach to Understanding the Biology of Invasions: Local Adaptation and General-Purpose Genotypes in the Weed *Verbascum thapsus*

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**Abstract:** *The role of evolution in the invasion of non-native species has important implications for conservation, weed science, risk assessment, and policy. In this paper we first discuss why an evolutionary perspective can be helpful and outline a range of potentially useful approaches from population biology and ecological genetics. As a case study, we then ask how adaptation and genetic structure may promote or constrain the expansion of an invasive weed, Verbascum thapsus, into high elevations in the Sierra Nevada of California. We used growth-chamber and common-garden experiments to assay a range of morphological and physiological traits that could influence plant fitness at high elevation. There was a significant relationship between elevation and leaf size and reflectance above 800 m, although we found no relationship between elevation and most other traits (growth rate in warm or cool temperatures, freezing tolerance when grown in warm or cool temperatures, leaf number, rosette morphology, plant height). We did see marked genetic differentiation among populations, possibly reflecting founder effects and a history of genetic drift. When we partitioned the phenotypic variance, there was almost no variation among maternal families within populations, limiting the potential for selection to act. The majority of the variance for all traits was among individuals within families, suggesting that environmental conditions strongly influenced the phenotype. Overall, the increasing success of V. thapsus at high elevations appears to conform more to Baker's concept of a general-purpose genotype than to invasion by rapid adaptation.*

Una Aproximación Evolutiva para Entender la Biología de las Invasiones: Adaptación Local y Genotipos de Propósito General en la Hierba *Verbascum thapsus*

**Resumen:** *El papel de la evolución en la invasión de especies no-nativas tiene implicaciones importantes para la conservación, la ciencia de las malezas, la evaluación de riesgos y la política. En este manuscrito discutimos en primer lugar el porqué una perspectiva evolutiva puede ser útil y delineamos un espectro de estrategias de biología poblacional y genética ecológica que pueden ser empleados. Como caso de estudio, posteriormente nos preguntamos como la adaptación y la estructura genética puede promover o restringir la expansión de una hierba invasora, Verbascum thapsus, en sitios de altas elevaciones de la Sierra Nevada de California. Usamos experimentos en cámara de crecimiento y jardín común para evaluar un rango de características morfológicas y fisiológicas que pueden influenciar sobre la adaptabilidad de las plantas a elevaciones altas. Hubo una relación significativa entre la elevación y el tamaño de las hojas y la reflectancia arriba de 800 m, sin embargo, no encontramos relación alguna entre la elevación y la mayoría de las otras características (la tasa de crecimiento a temperaturas calientes y frías, la tolerancia al congelamiento en plantas cultivadas a temperaturas calientes y frías, el número de hojas, la morfología de la roseta, la altura de la planta). Observamos una diferenciación genética marcada entre poblaciones, lo que refleja posible-*

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mente los efectos de fundadores y una historia de deriva genética. Cuando dividimos la varianza fenotípica, casi no hubo variación ninguna entre las familias maternas dentro de las poblaciones, limitando el potencial para la selección natural. La mayor parte de la varianza de todas las características ocurrió entre individuos dentro de las familias, sugiriendo que las condiciones ambientales influyen fuertemente sobre el fenotipo. En general, el éxito creciente de *V. thapsus* a elevadas alturas parece amoldarse más al concepto de Baker de un genotipo de propósito general que al concepto de invasión por adaptación rápida.

## Introduction

Biological invaders are now widely recognized as one of our most pressing conservation threats (Vitousek et al. 1996; Parker et al. 1999; Mack et al. 2000). Yet only a small percentage of introduced species succeed in establishing themselves, and only a few of those become the widespread, high-density pest species recognized for their conservation impact (Williamson & Fitter 1996; Smith et al. 1999). Is there some distinguishing feature of these widespread, high-impact invasions? This is a key conservation question, not only because we need to predict which species will be successful invaders, but also because we need to quantify and understand how sources of uncertainty limit our ability to make predictions (Ruesink et al. 1995).

Evolution may play an important role in reducing our ability to predict whether, where, and when an introduced species will invade. Traditional ecological approaches estimate potential distributions based on the characteristics of a species' native range using climate-matching models such as CLIMEX (McFadyen & Skarratt 1996; Curnutt 2000; Holt & Boose 2000). Such approaches project the range over which a species should be invasive if its physiological tolerances do not change over time. But there is no guarantee that these tolerances, range limits, and habitat specificities will not change. We currently have no general consensus on how often adaptation of such characteristics plays a role in invasions or even what proportion of invaders show evolutionary changes in phenotype from their ancestors in the native range.

Many before us have developed evolutionary hypotheses about the success of colonizing species (Darwin 1859; Carson 1965; Lewontin 1965; Mayr 1965; Baker 1974; Brown & Marshall 1981; Parsons 1983; Barrett & Richardson 1985; Mack 1985; Barrett et al. 1989; Barrett 1992; Baker 1995), yet despite its importance there is a notable absence of an evolutionary perspective in much of the modern literature on invasion biology. To illustrate this point, we performed a five-year BIOSIS database search (1997–2001), yielding 99 papers on the biology of invasive species (key phrases: *invasion biology*, *invasion ecology*, or *exotic species*). Only 5% of the studies used evolutionary ecology approaches, or even referred to evolutionary keywords such as *genetic*, *fitness*, or *adaptation*.

Given that evolutionary ecology studies are rare in the invasion literature, here we explore what could be gained from taking an evolutionary perspective. To begin, we present two contrasting (but not mutually exclusive) views of the mechanism by which an introduced plant spreads throughout a landscape, and we then outline what can be learned from an ecological genetics approach to invasion biology. We then present a case study in which we used this evolutionary framework to develop hypotheses about how adaptation and genetic structure may promote or constrain the successful expansion of *Verbascum thapsus* L. (Scrophulariaceae), common mullein, into high-elevation sites in the Sierra Nevada of California.

## Invasion by Adaptation versus the General-Purpose Genotype

A common feature in the spread of non-native species is an initial time lag when the species are present but not invasive (Cousens & Mortimer 1995; Kowarik 1995; Mack et al. 2000). Evolution could play an important role in such time lags, because natural selection should act powerfully on organisms to overcome limits to self-sustained population growth. Recent reviews emphasize the potential importance of rapid evolution in ecological processes, and non-native species provide many of the examples studied to date (Thompson 1998; Sakai et al. 2001). Widely successful invaders may expand their range into a broad array of sites by undergoing local adaptation. For those species, high levels of genetic variance within populations allow a more rapid response to selection; therefore, the conditions that favor invasion by adaptation include high outcrossing rates, high numbers of founders in new populations, and the creation of novel genotypes through gene flow among independent introduction foci (Fig. 1).

Evolution may increase not only an invader's distribution, but also its impact. Local adaptation could lead to increased absolute fitness in the new habitat, resulting in higher population densities and increased dominance of the landscape. Analogous to time lags in invasion success, time lags in impact could be widespread and common, but we would be able to detect them only with long-term experimental studies that explicitly incorporate genetics.

Many researchers have found evidence for local adap-

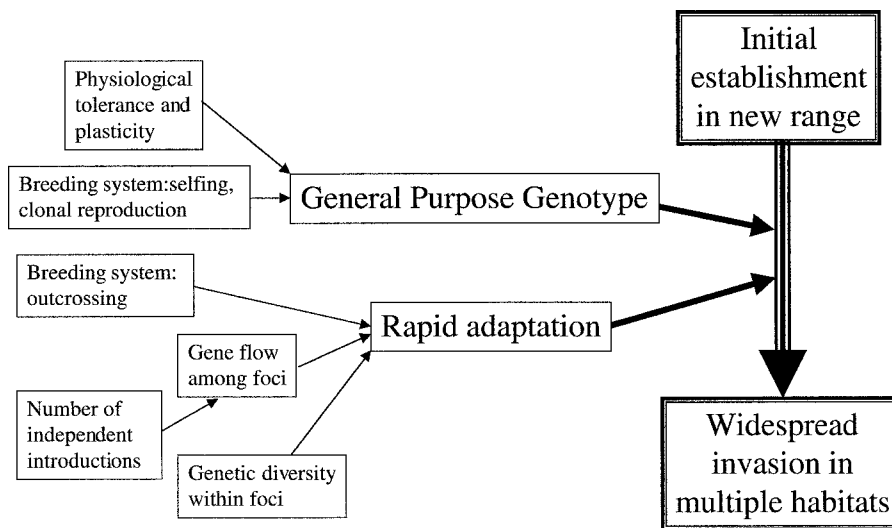


Figure 1. Factors influencing the process by which an introduced species moves from initial establishment in a new range to widespread invasion of multiple habitats, particularly natural (i.e., not anthropogenic) ecosystems. Two alternative but not mutually exclusive mechanisms are presented: rapid adaptation and the general-purpose genotype. Characteristics of the invading species (e.g., breeding system) or of the invasion process (e.g., number of introductions) that influence these two mechanisms are outlined.

tation among populations for a range of phenotypic traits, including morphology, physiology, and life history (Turesson 1922; Clausen et al. 1940; Hiesey & Milner 1965; Rice & Mack 1991b; Gurevitch 1992; Monson et al. 1992; Dudley & Schmitt 1995; Cordell et al. 1998; Huey et al. 2000). Laboratory or common-garden studies determine whether observed differences among field populations have a genetic basis, whereas reciprocal transplant experiments can show whether organisms are most fit in their natal sites (Clausen et al. 1948). Reciprocal transplants themselves do not reveal which traits are responsible for differences in fitness, but they can be coupled with selection analysis to assess these underlying mechanisms (e.g., Jordan 1992; Bennington & McGraw 1995). To show that local adaptation has played a role in driving invasion, then, one would like to know whether invading populations are differentiated for ecologically important traits and, subsequently, whether adaptation of these traits has increased the demographic success of the invader, which can be determined through reciprocal transplant experiments coupled with selection analysis. Reciprocal transplant experiments may be logistically impossible (in the case of many animals) or may be seen as ethically untenable for noxious invaders. Perhaps for these reasons, few such experiments have been performed for the purpose of understanding the genetic basis of invasion (but see Rice & Mack 1991b).

Not all widespread invaders may owe their success in multiple habitats to local adaptation. In 1965 Herbert Baker coined the term “general purpose genotype” to describe colonizing species that thrive in a wide range of environmental conditions through phenotypic or developmental plasticity (Baker 1965). Such species are able to flourish in many regions, thereby explaining their success as invaders. In contrast to the invader that spreads by rapid adaptation,

the species possessing a general-purpose genotype does not rely on the generation of new forms through recombination; in fact, such recombination could be detrimental. A general-purpose genotype allows for the success of populations founded by small numbers of individuals through reproductive systems such as autogamy that do not promote genetic exchange but do provide reproductive assurance (Fig. 1). Baker asserted that certain characteristics of some plant species, such as hybridization and polyploidy, may predispose them to be general-purpose genotype colonizers (Baker 1965, 1995).

Baker’s original ideas focused primarily on plants considered either agricultural weeds (“agrestals”) or colonizers of roadsides and waste places (“ruderals”), but today invasion ecologists are most interested in species capable of invading not only disturbed habitats but also native communities. These aggressive invaders are only a small subset of the introduced species capable of maintaining self-sustaining populations in disturbed sites, and it is an open question whether high levels of ecological flexibility (i.e., phenotypic or developmental plasticity) also aid species that are successful invaders of undisturbed natural communities. To measure the extent of plasticity of the phenotype (reviewed by Bradshaw 1965), we need to rear organisms in a range of relevant environments. Beyond such descriptions, we need to address the difficult key question of whether plasticity itself confers higher fitness (Scheiner 1993; Via et al. 1995) and invasiveness. One approach would be to compare levels of plasticity among populations that vary in invasiveness, or between invasive and noninvasive taxa. By focusing on particular traits that allow invasion into different environments, one could also directly test the link between invasiveness and plasticity at the genotype level by using manipulative approaches (Schmitt et al. 1999).

### The Structure of Phenotypic Variance

Beyond measuring plasticity or looking for adaptive evolutionary change over time, there are many insights one can gain from applying classic evolutionary ecology approaches to the study of invasive species (Fig. 1). Using even the simplest quantitative genetics designs, such as common gardens with family-structured material, one can extract information about levels of genetic variation within populations and the degree of differentiation among populations (Lawrence 1984; Mazer & Lebuhn 1999). Because the rate of evolution depends both on the strength of selection and on the amount of heritable variation available to be selected (Fisher 1930), levels of genetic variation within populations can indicate the potential for evolution in the traits of invaders (Rice & Mack 1991a). Many invasive species are likely to undergo extreme and repeated bottlenecks in the process of spreading across a landscape; therefore, founder effects and genetic drift may lead to significant differences among populations and little genetic variance within populations (Brown & Marshall 1981; Barrett & Husband 1990; Wang et al. 1995; Pascual et al. 2001). But these patterns depend on the process of spread—how propagules are vectored—and therefore on how many individuals found new populations and how rapidly populations increase in size (Nei et al. 1975).

The specifics of the original introduction are also critically important. The number of initial colonizers and the amount of genetic variance in this “inoculum” set the stage for later evolution. If multiple introduction events have occurred from separate regions of the native range, these multiple foci will have a profound influence on the structure of genetic variation in the new range. The amount and pattern of genetic variance, as well as the specific origin of invader populations, play a role not only in predicting evolutionary change in the invader but also in determining the vulnerability of the invader to control methods such as classical biological control (e.g., Nissen et al. 1995). Unfortunately, we still have a poor understanding of the dynamics or dispersal history of most invasions, even for economically important weeds. Studies such as those by Novak and Mack on *Bromus tectorum* (Novak & Mack 1993; Novak et al. 1993; Novak & Mack 2001) or Neuffer and colleagues on *Capsella bursa-pastoris* (Neuffer 1996; Neuffer & Hurka 1999; Neuffer et al. 1999) are notable exceptions. We need long-term studies that track the geographic distribution, ecological dynamics, adaptive evolution, and genetic structure of invaders as they spread.

### The Invasion of *Verbascum thapsus* in California

Although its exact invasion history is still unknown, *Verbascum thapsus* (common mullein, Scrophulariaceae) was probably introduced into North America from Eu-

rope multiple times, both accidentally and intentionally as a medicinal herb (Gross & Werner 1978). In California *V. thapsus* was naturalized in Siskiyou County by 1880 (Watson 1880), and herbarium collections were made starting in the early 1900s. Little is known about the timing and pattern of its spread within the region, but it was collected at 1615 m in the Tahoe National Forest in 1934 and probably has inhabited some high-elevation sites for at least seven decades (CalFlora Database, <http://www.calflora.org>). *Verbascum thapsus* is a serious weed pest of roadsides and industrial areas (Semenza et al. 1978), but because it is often restricted to disturbed sites, it has not been considered a major noxious invader in most areas of California (Hoshovsky 1986). However, in areas with thin soils and open vegetation, or in forested sites after fire, *V. thapsus* can form thick stands (Pitcairn 2000). It is reported to displace native herbs and grasses in undisturbed meadows in the Owens Valley (Pitcairn 2000).

*Verbascum thapsus* is also one of the few non-native plant species to invade at high elevations, and because of this it has become a priority for control efforts at Yosemite National Park and surrounding areas in the Sierra Nevada (P. Moore, personal communication). The perception that the *V. thapsus* invasion and the concomitant need for control efforts have been increasing at high-elevation sites led to alternative hypotheses for why this invader might be expanding: (1) the distribution may simply be dispersal-limited and may not yet have reached its potential extent or (2) populations may be adapting to the conditions at high elevation (e.g., episodic freezing, short growing seasons, high UV light levels), resulting in plants with increased fitness and higher population densities or growth rates. As a case study of an evolutionary approach to invasion biology, we explored the hypothesis that populations of *V. thapsus* in the Sierra Nevada are showing signs of local adaptation to high-elevation conditions. In growth-chamber and common-garden experiments, we compared relevant morphological and physiological traits of plants collected across a wide range of elevations. We then used a quantitative genetics approach to determine whether populations were genetically differentiated for these phenotypic traits and partitioned the variance to assess the role of founder effects, the potential for selection to act within populations, and levels of plasticity or environmental influence over the phenotype.

### Methods

*Verbascum thapsus* is a monocarpic perennial, but the details of its life history vary with location (Reinartz 1984). In alpine sites of California, plants may germinate in fall or in spring (Semenza et al. 1978). In coastal sites, which have a Mediterranean climate of dry summers and

wet winters, most germination likely occurs with the first rains in October or November, and plants most commonly experience two winters as rosettes.

The very small (0.06–0.1 mg) seeds are released from dry capsules and have no specialized adaptations for dispersal (Gross & Werner 1978). Long-distance seed movement along with soil and road materials may found new populations. Seeds may survive for decades, and recruitment from the seedbank is important in areas where *V. thapsus* is an early successional pioneer species (Gross 1980; Baskin & Baskin 1981).

### Field Collections and Planting

In July–September of 1999, we identified 10 representative populations of *V. thapsus* in California ranging in elevation from 75 to 2260 m. We searched extensively in the vicinity of Yosemite National Park and Inyo National Forest and included every population we found above 700 m. We used Trimble Pro-XRS global positioning system (Trimble-Pro-XRS, Sunnyvale, CA) to record each population's location and elevation (Table 1). Most populations were near roads, but some were found on the gravel beds of riverbanks, in meadows, or in forest gaps. Most populations were made up of small numbers of adult plants (mean 59, range 8–230). The populations may have been small because the species is just now getting a foothold in the region or because control and eradication efforts on these public lands eliminate populations as they get large and conspicuous.

We randomly selected 8–11 maternal plants in each population (Table 1), and after plants had produced mature fruit in the field, we measured total plant height. We collected the entire infructescence from each plant, which contains several hundred separate fruits and several hundred thousand seeds (Gross & Werner 1978).

For our experiments, we used seeds selected haphazardly from these bulk collections; the chances of two seeds coming from the same fruit (with the same outcrossed pollen parent) were very small. *Verbascum thapsus* has a mixed mating system. It is actively pollinated by insects, but also shows delayed selfing of unvisited flowers (Donnelly et al. 1998). Therefore, the offspring of a single, maternal parent would be half-sibs through outcrossing or full-sibs through selfing.

We used a randomized block design with six blocks, with one offspring from each maternal parent in each block. On 5–9 November 1999, we planted seeds in containers (37 mm in diameter), with a soil mixture of 15% turf, 10% sand, and 75% Pro-Mix potting soil. Seeds were incubated at 25 °C for 10 days. We overplanted and then thinned to one randomly chosen seedling at the cotyledon stage. We watered plants to maintain soil water potential in the root zone at  $> -0.5$  MPa.

After 2 weeks, the length of one cotyledon on each plant was measured with digital calipers. Because cotyledons are produced primarily from maternal provisions and are correlated with seed size, we used cotyledon length to test for maternal effects, which can influence the interpretation of the results of common-garden experiments (Roach & Wulff 1987; Donohue & Schmitt 1998). Analysis of variance (ANOVA) revealed a significant effect of population on cotyledon length ( $df = 9$ ,  $F = 3.2$ ,  $p < 0.001$ ); out of 45 two-way comparisons between populations, however, Scheffe's post-hoc test revealed only one significant difference (Lee Vining vs. Highway 120,  $p = 0.02$ ). In addition, mean cotyledon length did not covary with elevation ( $R^2 = 0.02$ ,  $p = 0.70$ ). This absence of pattern in early germinants suggests that size-related traits (early growth rate, final size) were unlikely to be influenced by maternal effects, although one cannot rule out the possible role of mater-

**Table 1.** Characteristics of sampled populations of *Verbascum thapsus*, including elevation, location (lat. and long.), population size (number of flowering adults), habitat, exposure (amount of direct sun), mean annual maximum and minimum temperatures as estimated from the nearest climatological data from the Western Regional Climate Center, and number of maternal families sampled for experiments.

Population	Elevation (m)	Latitude	Longitude	Population size	Habitat type	Exposure	Mean annual maximum and minimum temperature (°C)	No. maternal families
Santa Cruz Island	75	39°0'14.04''N	119°26'39.84''W	175	roadside	open	21/11	10
Highway 140	390	37°39'20.80''N	119°54'58.59''W	37	river bank	open	23/8	10
Boulder Creek	722	37°14'45.95''N	122°8'47.72''W	54	roadside	shade	23/6	10
Oakhurst	819	37°21'46.79''N	119°38'22.11''W	20	roadside	open	23/6	9
Bass Lake	1046	37°21'8.43''N	119°36'11.65''W	8	roadside	open	23/6	8
Wawona	1238	37°32'42.71''N	119°38'42.53''W	16	river bank	partial shade	21/4	10
South Entrance	1562	37°30'23.17''N	119°37'56.93''W	13	meadow	partial shade	17/2	10
Mariposa Grove	1808	37°30'9.28''N	119°35'43.43''W	44	forest gap	shade	14/1	11
Lee Vining	2235	37°56'0.47''N	119°9'7.20''W	230	roadside	open	16/2	11
Highway 120	2262	37°55'50.52''N	119°9'59.99''W	12	roadside	open	16/2	9

nal effects for other traits such as freezing tolerance. Maternal effects would inflate the chances of finding significant variation among populations and with elevation.

We used offspring to investigate the evidence for genetically based differences in a series of phenotypic traits. We focused on physiological and morphological traits that had clear links to plant performance at high elevation: growth rates under both warm and cold conditions, freezing tolerance when grown under warm and cold day/night air temperatures, leaf reflectance, rosette size, and rosette shape.

### Growth under Warm versus Cold Conditions

On 15 November, we separated the blocks into growth chambers under 2 day/night air-temperature treatments, 25/15° C (warm), and 15/5° C (cold), on a schedule of 14 hours of light and 10 hours of darkness. We chose temperatures that spanned the range of spring conditions experienced by plants across our sites from Santa Cruz Island (west, elevation 75 m) to Lee Vining (east, elevation 2240 m), based on climatological data from the Western Regional Climate Center (<http://www.wrcc.dri.edu>). Plants were re-randomized within chambers after 4 weeks. At 8 weeks, all plants were photographed with a digital camera. We determined total leaf area with image software from the National Institutes of Health, which provided an estimate of growth rates in warm and cold temperatures.

### Freezing Tolerance under Warm versus Cold Conditions

We measured the relative freezing tolerance of plants from the different populations, and by comparing the freezing tolerance of offspring from the same maternal family grown under cold versus warm conditions, we measured the ability of genotypes to enhance freezing tolerance in response to a shift to cooler day and night temperatures. Chlorophyll A fluorescence from Photosystem II (PSII) is a valuable tool for indicating stress tolerance in plants (Bolhar-Nordenkamp & Oquist 1993; Loik & Harte 1996). We first generated a dose-response curve to determine the appropriate treatment temperature to which leaves should be exposed for population comparisons. Using a hole-punch, we removed leaf discs 1 cm in diameter from a sample of plants grown at 25/15° C and 15/5° C. We cooled the leaf discs from 20° C at a constant rate of 3° C per hour. Leaf and air temperatures were monitored with copper constantan thermocouples (1.0 mm in diameter) appressed to the leaf and in the air above leaves. Subsets of leaf discs were removed at 5-degree intervals from -5 to -25° C. Discs underwent a 24-hour thawing period on ice, and then a Hansatech FMS 1 fluorometer (Kings Lynne, England) was used to measure the ratio of variable fluores-

cence to maximum fluorescence in the fast kinetics of PSII ( $F_V/F_M$ ). This ratio is proportional to the quantum yield (Bolhar-Nordenkamp & Oquist 1993); changes in  $F_V/F_M$  are correlated with tolerance of both high- and low-temperature extremes (Loik & Harte 1996; Loik et al. 2000).

The 588 plants were tested at -13° C based on the above protocol, one block at a time over 1 week (11-18 February). Leaf discs were cooled from 20° C to -13° C, then held at -13° C for 1 hour. After the plants thawed on ice in the dark for 24 hours,  $F_V/F_M$  values were determined for each individual.

### Leaf Reflectance

Leaf pubescence is an important trait influencing the physiology of plants in stressful environments and tends to be more common at high elevations (Halloy & Mark 1996). Increased leaf pubescence, which moderates plant reflectance, affects energy budgets, photosynthetic productivity, and UV absorption (Ehleringer & Mooney 1978; Sandquist & Ehleringer 1998). We used spectroscopy to assay leaf pubescence, measuring reflectance spectra for individual plants of *V. thapsus*. At 12 weeks (8-9 February), we obtained leaf-reflectance spectra for one block of 98 individuals (8-11 from each population). From each spectrum we then extracted the percent reflectance at 310, 360, 440, 480, 500, and 625 nm relative to a white standard. Reflectances at all wavelengths were highly correlated and produced consistent results with respect to elevation.

### Rosette Shape and Plant Size

After plants were tested for freezing tolerance, four blocks (two from the 25/15° C chamber, two from the 15/5° C chamber) were moved from the growth chambers out onto benches in an open-air growing facility in Santa Cruz, California. On 21 March, we transplanted them into 3.8-L (15.5-cm diameter) pots of Premier Pro-Mix HP potting soil and placed them on benches in a randomized array. On 29 June, we transplanted them into 11.4-L (26.0-cm diameter) pots and randomized again. Plants were watered occasionally during the dry season to maintain soil water potential at >-0.5 MPa and were given a controlled-release general fertilizer.

At 16 months (March and April 2001), we measured rosettes for a series of morphological traits that might adapt in response to elevation. We measured two aspects of rosette size: (1) number of leaves produced and (2) leaf length. A preliminary study showed that leaf length increased with the rank (i.e., age) of the leaf, but comparisons among plants were consistent across leaf ranks. Therefore, we measured the length and width (highly correlated) of the thirtieth leaf on each plant.

We also quantified rosette shape. *Verbascum thapsus* plants varied in their tendency to form a “head” of tightly packed leaves in the center of the rosette. Some plants have tight heads, whereas others are open. This compactness may influence photosynthetic rates at low ambient temperatures (Körner 1999). We quantified rosette shape by counting the number of leaves on which the tip pointed in from the vertical as a proportion of the total number of leaves. Data were collected one block at a time. Three out of four blocks were completed in 10 days or less; the last one was completed in 17 days. After bolting and seed maturation, we measured the height of each plant.

### Data Analysis

We looked for evidence of adaptation to elevation by performing parametric regressions ( $n = 10$ ) of the mean population values on elevation for each of the above traits. For these regressions, individual plant values were incorporated only indirectly through the population mean because individuals within populations were not independent samples. For morphological characters, the separate blocks were measured over a period of time over which some characters changed significantly. For the traits that were time-sensitive, either we included block as an explanatory variable (e.g., leaf length, rosette packing) and used analysis of covariance (ANCOVA) to test for a significant effect of elevation or we were forced to analyze only the first two blocks (e.g., rosette shape: the proportion of head leaves was not meaningful after the rosettes had opened out in preparation for bolting). Statistics were done in SAS and Stat-View (SAS Institute, Cary, North Carolina).

In addition to testing for local adaptation, we assessed whether populations were significantly differentiated for the same traits with ANOVA. Individuals were nested within maternal families, which were nested within populations in a random-effects model. Block was also included as a factor.

We also estimated the variance components with the VARCOMP procedure in SAS to determine how much phenotypic variance was due to differences among populations, among maternal genotypes within populations, and among individuals within maternal families. To partition the variance, a design must be balanced at all levels—number of offspring per family, number of families per population—whereas because of mortality and the particulars of our seed collections, we had a slightly unbalanced data set. Therefore, for these analyses we chose a random subsample of the offspring and families for each trait so the design would be balanced. Final sample sizes were different for different traits. We did not partition the variance for leaf reflectance, because only one offspring per parent was included.

## Results and Discussion

### Plant Size and Growth Rates under Cold and Warm Conditions

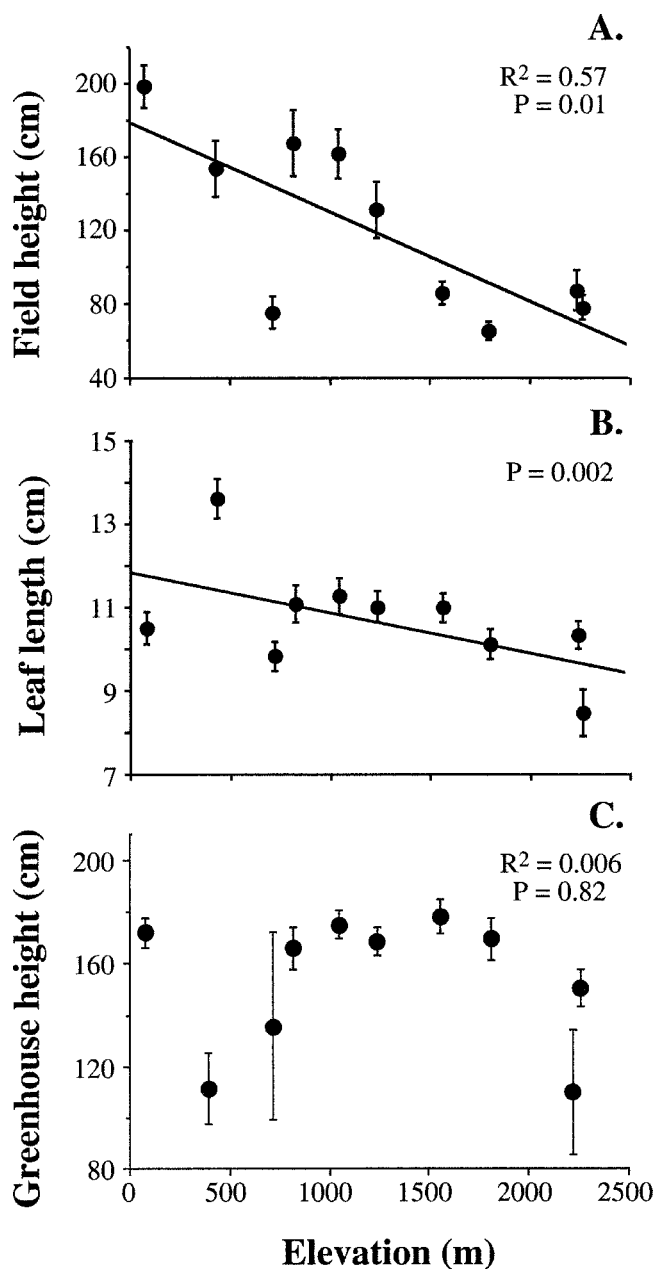
Small plant size is thought to reflect adaptation to harsher conditions and a shorter growing season, and is a common feature of alpine ecotypes in classic studies (e.g., Clausen et al. 1940, 1948; Böcher 1949). Consistent with this expectation, in naturally occurring field-grown plants we found a negative trend in plant height with elevation (Fig. 2a,  $R^2 = 0.57$ ,  $p = 0.01$ ). In our growth-chamber experiments, early growth rates were not dependent on the elevation of source populations in either cold temperatures, where we expected faster growth in high-elevation plants, or in warm temperatures, where we expected faster growth in low-elevation plants (Larcher 1995). Not surprisingly, seedlings from all populations grew much more slowly at cooler day/night air temperatures of 15/5° C than did plants at 25/15° C (Fig. 3). However, growth rate at cooler temperatures was not higher for plants derived from populations at higher elevations than for those from lower elevations (Fig. 3a;  $R^2 = 0.026$ ,  $p = 0.67$ ). Also, there was no corresponding negative association between elevation and growth rate at warmer temperatures (Fig. 3b;  $R^2 = 0.06$ ,  $p = 0.49$ ).

In the common-garden experiments, we measured two aspects of rosette size—number of leaves and leaf length—and expected to see a decrease in size with elevation. Although number of leaves did not covary with elevation ( $R^2 = 0.12$ ,  $p = 0.31$ ), leaf length did decline significantly in populations from higher elevations (Fig. 2b; ANCOVA elevation effect:  $df = 1,32$ ,  $MS = 19.8$ ,  $F = 12.2$ ,  $p < 0.002$ ). Unlike the field populations, however, after bolting these plants did not show a negative trend in height with elevation (Fig 2c;  $R^2 = 0.01$ ,  $p = 0.79$ ).

### Freezing Tolerance

Freezing tolerance, as measured by  $F_V/F_M$  for leaf discs cooled to  $-13^\circ\text{C}$ , showed variation but did not increase with elevation (Fig. 4;  $R^2 = 0.08$ ,  $p = 0.44$ ). One population in the Sierra Foothills (Highway 140, at 390 m) was an outlier with very high freezing tolerance (Fig. 4), but removing this outlier did not result in a significant relationship between freezing tolerance and elevation.

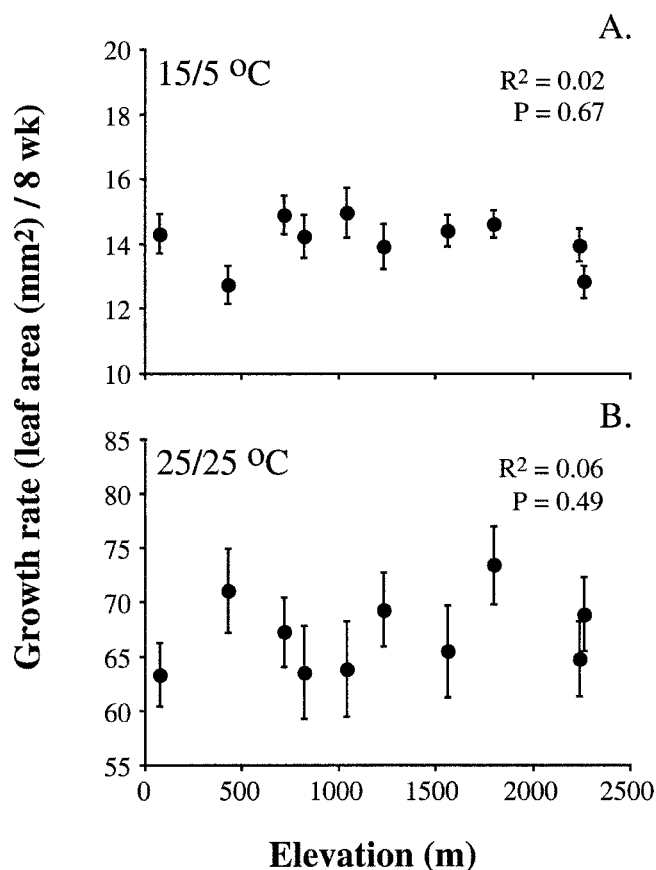
The response of plants to development at cold temperatures resulted in an overall increase in freezing tolerance, showing a high degree of plasticity. That is, offspring grown at 15/5° C generally showed greater freezing tolerance than their siblings grown at 25/15° C. However, maternal families from high-elevation populations did not show a bigger developmental response to cold than families from low-elevation populations ( $R^2 = 0.01$ ,  $p = 0.76$ ), as one might expect if they were adapted to growth in cold conditions.



**Figure 2.** (a) Plant height measured on *V. thapsus* individuals growing in field populations along an elevational gradient (height =  $179.6 - 0.05 \times$  elevation). (b) Rosette size (leaf length) as a function of the elevation from which seeds were collected (analysis of covariance used to include block as an explanatory variable). (c) Plant height in the common garden as a function of the elevation from which seeds were collected. Error bars ( $\pm 1$  SE) represent the variation within populations, but the regression is based on mean values ( $n = 10$ ).

#### Leaf Reflectance (Pubescence) and Rosette Morphology

Reflectance at high elevations may moderate leaf exposure to UV radiation (Körner 1999), and other studies



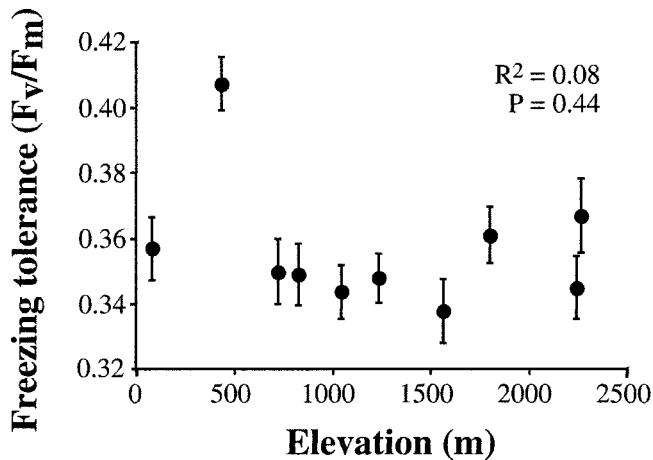
**Figure 3.** Total leaf area (mm<sup>2</sup>) of *V. thapsus* seedlings grown for 8 weeks in growth chambers set at either (a) 15/5 °C or (b) 25/15 °C day/night temperatures as a function of the elevation from which seeds were collected. Error bars ( $\pm 1$  SE) represent the variation within populations, but the regression is based on mean values ( $n = 10$ ).

have found evidence for ecotypic differentiation for UV-absorbing compounds along an elevational gradient (Ziska et al. 1992). Leaf pubescence, closely related to reflectance, can play an important role in the energy budget of plants (Ehleringer & Mooney 1978).

In the complete data set, percent reflectance from leaves did not increase with elevation (Fig. 5;  $R^2 = 0.003$ ,  $p = 0.88$ ). The population from Highway 140 was again an outlier, showing a very high reflectance value despite its relatively low elevation. Without this outlier, there was a marginally significant trend ( $R^2 = 0.35$ ,  $p = 0.096$ ), which became significant when the seven highest populations (from 820 to 2260 m) were considered in isolation (Fig. 5;  $R^2 = 0.82$ ,  $p = 0.005$ ).

Alpine plants often take shapes that are thought to aid in reducing water and heat loss by increasing their boundary layer and protecting inner tissues (Jones 1982; Larcher 1995; Körner 1999). Rosette morphology (i.e., how tightly leaves were packed into heads)





**Figure 4.** Change with elevation in freezing tolerance of *V. thapsus*. Freezing tolerance was assessed by fluorimetry on leaf discs cooled to  $-13^{\circ}\text{C}$ . Error bars ( $\pm 1$  SE) represent the variation within populations, but the regression is based on mean values ( $n = 10$ ). Removing the outlier does not produce a statistically significant relationship.

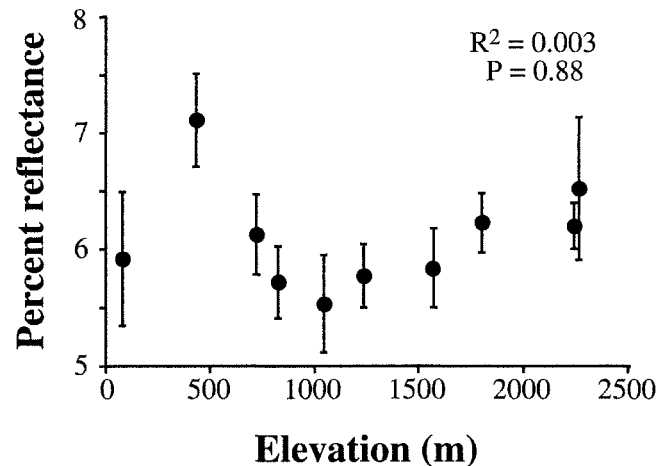
did not vary consistently with elevation (ANCOVA; elevation effect:  $df = 1,32$ ,  $MS = 0.004$ ,  $F = 0.99$ ,  $p = 0.33$ ). Two populations were outliers with much more open rosettes, but only one of these was a low-elevation population.

#### Differentiation among Populations and Estimating the Components of Variance

All traits, with the two exceptions of reflectance and growth rate in warm temperatures, showed significant differentiation among populations (Table 2). Only the two traits associated with rosette size showed significant differentiation among families.

Consistent with these results, when the components of variance in each trait were partitioned among populations, families, and individuals within families, we saw a pattern that was strikingly consistent among traits (Fig. 6). Differences among populations explained a considerable portion of the variance for some of the traits, such as leaf number or freezing tolerance when grown in cold, but the great majority of the variance was explained by differences among individuals, and almost none was explained by differences among families within populations.

There were significant block effects in the experiment, which would inflate phenotypic differences among individual plants in the different blocks. Nevertheless, these plants experienced relatively homogeneous experimental conditions; one would expect the environmental component of variance to be even greater in a natural field setting.



**Figure 5.** Percent plant reflectance at 440 nm as a function of the elevation from which plants were derived. Percent reflectance was extracted from complete spectra measured on 8–11 plants per population (one block). Regression for the full data set is not significant. If considered in isolation, however, the seven highest-elevation populations ( $>800$  m) show a significant increase in reflectance with elevation (reflectance =  $0.05 + 0.000006 * \text{elevation}$ ;  $R^2 = 0.82$ ,  $p = 0.005$ ). Error bars ( $\pm 1$  SE) represent the variation within populations, but the regression is based on mean values ( $n = 10$ ).

#### Implications for the Invasion Biology of Mullein

*Verbascum thapsus* is one of only a few non-native plant species to invade at high elevations in California, and it is thought to be increasing its range in sensitive sites such as Yosemite National Park (P. Moore, personal communication). We sought to determine whether its successful range expansion has been accompanied by adaptation to high-elevation conditions. Aside from leaf length and reflectance above 800 m, we found little evidence for elevational trends in physiological traits, morphological traits, or response to cold conditions. Partitioning the variance revealed little genetic variation among families and high levels of phenotypic plasticity. These experimental results, coupled with information from other studies, suggest that the increasing colonization of higher elevations by *V. thapsus* is unlikely to have been driven by rapid adaptation.

Other researchers have tested for differences among populations of introduced *V. thapsus* and similarly found evidence for slight adaptive differences, but they have concluded that the species is characterized by wide tolerances and high levels of phenotypic flexibility. In a common-garden study, Reinartz (1984) found that plants from Texas are more likely to reproduce as annuals than those from North Carolina or Canada.

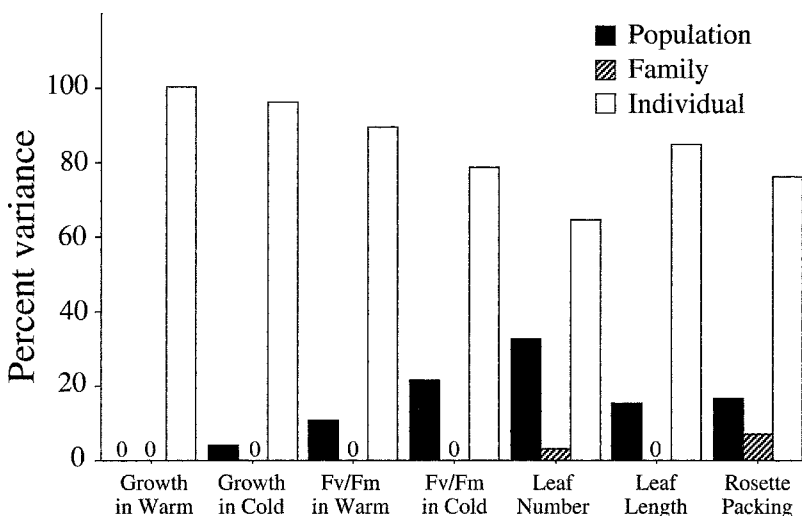
**Table 2.** Nested analysis to test for significant differentiation among populations and among maternal families within populations of *Verbascum thapsus* for physiological traits, growth rates, and morphological traits.

Trait	Population				Maternal family				Block				Error	
	df	SS	F	p	df	SS	F	p	df	SS	F	p	df	SS
Freezing tolerance, plants grown at 25/15° C	9	0.10	5.55	<0.0001	88	0.18	1.10	0.45	2	0.13	30.9	<0.0001	192	0.395
Freezing tolerance, plants grown at 15/5° C	9	0.20	8.65	<0.0001	88	0.20	0.90	0.72	2	0.05	10.0	<0.0001	193	0.495
Leaf reflectance %, one block only	9	0.0018	1.41	0.19	—	—	—	—	—	—	—	—	88	0.12
Growth rate, first 8 weeks, plants grown at 25/15° C	9	3,322	1.16	0.32	88	26,745	0.96	0.58	2	22,970	36.2	<0.0001	193	61,199
Growth rate, first 8 weeks, plants grown at 15/5° C	9	143	2.21	0.02	88	711	1.12	0.26	2	638	44.2	<0.0001	193	1,391
Leaf length (cm)	9	505	22.4	<0.0001	87	298	1.36	0.03	3	1,182	157	<0.0001	275	691
Number of leaves per rosette	9	32,492	19.0	<0.0001	88	23,520	1.41	0.02	3	2,180	3.8	0.01	286	54,286
Proportion of leaves in "head"	9	0.55	5.81	<0.0001	88	0.96	1.04	0.39	3	1.47	46.7	<0.0001	283	2.97

Texas populations also flower earlier and longer, although all populations show great variability in life history. Semenza et al. (1978) found that *V. thapsus* seeds germinate over a broad range of temperatures. They also found few differences in germination response to light or temperature in seeds collected from 11 populations ranging in elevation from 250 to 2190 m. A re-analysis of their data reveals no significant relationship between elevation and optimum germination temperature ( $n = 11$ ,  $r^2 = 0.12$ ,  $p = 0.29$ ). Williams and Kemp (1976) looked at the temperature response of photosynthetic rate in *V. thapsus* and detected slight differences among three latitudes and three elevations. They concluded, however, that all populations show wide tolerances. These results together support the designation of *V. thapsus* as a "general-purpose genotype," although local adaptation appears to have played some role in molding phenotypic traits over larger geographic scales.

Our study and those of others are limited in that it is not yet clear how much apparently small differences among populations in traits such as leaf size and reflectance may affect fitness. Future work should take a reciprocal transplant approach to distinguish conclusively between adaptation and flexible phenotype as the primary driver of the expanding invasion success of *V. thapsus*. Under the adaptation hypothesis, the prediction would be that populations collected from lower elevations would be less invasive at the highest sites than those collected from high elevations. From our results, one would also predict that leaf size, and perhaps leaf reflectance, may be related to fitness at high elevation. But one might also find that the outlier population at Highway 140 may perform better at high-elevation sites than any population currently present at those sites.

Although there was no trend with elevation, we



*Figure 6.* Percentage of total phenotypic variance attributed to differences among populations, among maternal families within populations, and among individuals within maternal families for a range of growth rates, physiological traits, and morphological traits of *V. thapsus* (see text for detailed explanation of traits).

found significant variation among populations in traits such as rosette shape and freezing tolerance. This phenotypic differentiation could reflect various processes. Local adaptation could have occurred in response to a selective agent that was not correlated with elevation. However, a general familiarity with the sites and characteristics of the populations (Table 1) does not suggest any obvious factor that would lead to the observed patterns. Genetic drift could also have produced the differentiation among *V. thapsus* populations in morphological and physiological traits. Drift should be important in invading colonizers, because repeated bottlenecks associated with the founding of new populations result in reduced genetic variation within populations and increased differences among them (Barrett & Husband 1990; Pascual et al. 2001). The populations we found in this region were generally small, which probably reflects both an early colonization phase and eradication efforts by local conservationists. This small population size would increase the importance of drift and should decrease the genetic variance seen among maternal parents within populations.

Initial founder effects may also greatly influence the level and distribution of genetic variance. If *V. thapsus* were introduced multiple times, as some have speculated (Gross & Werner 1978), the characteristics of a population in any given area could be determined primarily by its site of origin and the number of individuals by which it was founded. One population at an elevation of 390 m (Highway 140) was an outlier for several traits, and it may derive from a separate introduction event and a different native region. Preliminary data based on molecular markers are consistent with this interpretation (K. Dlugosch & I.M.P., unpublished data). Although this population had levels of freezing tolerance and reflectance that would seem well adapted to high elevation, it was found at the second-lowest elevation we sampled. Unlike the Santa Cruz Island or Boulder Creek populations, this population was not a geographic outlier, occurring in the Sierra Nevada foothills within 70 km of the highest site. Gene flow between this population and those at higher elevations may be possible within a time frame of years or decades, which could lead to a shift toward more typically alpine-adapted traits.

A remarkably large proportion of the phenotypic variance in the traits we studied was at the level of individuals within families, with relatively little variance among populations and zero variance among maternal families for most traits. The paucity of genetically based variation within populations relative to variation among populations suggests that founder effects and genetic drift may in fact drive genetic structure in this species. It also suggests that there may be little genetic variance on which selection may act within populations, serving to limit local adaptation.

## Relevance of Evolutionary Biology to the Study and Control of Invasions

Our evolutionary studies of mullein in California reveal two striking aspects of this invasive weed. First, there is little evidence of local adaptation to specific environmental conditions. Second, partitioning the variance showed a conspicuous lack of genetic variation among families and a preponderance of phenotypic plasticity. These results support the hypothesis that *V. thapsus* succeeds across a wide environmental gradient because of its ecological flexibility. This is not to say there will be no evolutionary change within this species in the future; in fact, the presence of genetic variation for extremely high freezing tolerance in one low-elevation population suggests that gene flow among populations could potentially lead to increased fitness at high elevation. However, current success and spread do not appear to be driven by marked adaptive changes.

The slow advance of mullein as a problematic environmental weed in Yosemite National Park underscores the idea that our struggle against invasive plants has only just begun. A species that has been present in the region for at least 70 years can still be at a fraction of its potential distribution and abundance, even without range expansion driven by evolution. From a policy and management perspective, it is important to emphasize that the costs of controlling such species will only increase exponentially over time, highlighting the financial benefits of early eradication and vigilant control efforts when populations are small (Zavaleta 2000).

Our results imply that invasive plants may not necessarily rapidly evolve new physiological limits. This lends credence to the use of known environmental tolerances and geographic boundaries to predict invasion potential. Just as the best predictor of whether an introduced plant will be invasive on a new continent is whether it already invades elsewhere (Reichard & Hamilton 1997), we may be able to confidently predict the threat of invaders to particular sensitive habitat types based on their abundance in those habitats at home. Returning to our model species, within its very wide distribution, mullein is known from alpine regions in Europe (Tutin 1964) and is currently invading volcanic peaks on Hawaii and other Pacific islands (Juvik & Juvik 1992; Ansari & C. Daehler, unpublished data). This species should therefore be on the watch list for any protected area in an alpine or sub-alpine region.

Finally, with the introduction of non-native species, as with many other ecological issues with important policy implications (e.g., pesticide use, release of genetically engineered organisms), evolution is our largest source of uncertainty and therefore our largest source of anxiety. Although general-purpose genotype invaders make formidable opponents, we at least have a sense that we know what we are dealing with. When evolutionary

change becomes a consideration, we lose the ability to predict where an introduced species will end up and what sort of impact it will have. Understanding the evolutionary components of an invader's success is key to predicting its long-term ecological success. There is a great need for more studies investigating the role of genetic changes during invasions, so that we can begin to make more robust generalizations about the long-term risks of species introductions.

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