# Evolution of fast-growing and more resistant phenotypes in introduced common mullein (*Verbascum thapsus*)

**Sabrina Kumschick[1,2]\*, Ruth A. Hufbauer[1], Christina Alba[1,3] and Dana M. Blumenthal[4]**

[1]*Department of Bioagricultural Sciences and Pest Management, Colorado State University, 1177 Campus Mail, Fort Collins, CO, 80523-1177, USA;* [2]*Centre for Invasion Biology, Department of Botany and Zoology, Stellenbosch University, Matieland, 7602, South Africa;* [3]*Department of Invasion Ecology, Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, CZ-252 43, Czech Republic; and* [4]*USDA-ARS Rangeland Resource Research Unit, Fort Collins, CO, 80526, USA*

## Summary

**1.** Species introduced into areas outside of their native range face novel biotic and abiotic conditions, which probably impose novel selection pressures. Adaptation to these new conditions may increase the ability of introduced species to establish and spread. Like many other introduced plant populations, introduced genotypes of common mullein (*Verbascum thapsus*) are more successful in their introduced than in their native range, with increased growth and fecundity. These differences appear to be at least partly genetically based. The most successful introduced populations also grow in an environment that is drier and has fewer competitors than native populations. It is not known, however, whether differences between native and introduced mullein populations are related to these environmental differences between ranges.

**2.** We used a common garden experiment with 23 native and 27 introduced populations of common mullein to test whether common mullein in the introduced range exhibits evolutionary shifts with respect to responses to competition, drought stress and nitrogen (N) stress. We also used choice experiments to learn whether introduced mullein is more or less resistant to a generalist herbivore than native mullein.

**3.** Without competition, introduced genotypes grew larger than native genotypes under high resource availability (control) and N stress, but not water stress. Survival, however, was increased in native populations under competition and N stress. The introduced genotypes also had a lower root:shoot ratio than the native genotypes. With competition, introduced genotypes grew larger than native genotypes across all treatments, with that difference being significant under N stress. The introduced genotypes were also more resistant to a generalist herbivore.

**4.** *Synthesis:* Together, high biomass, strong responses to high water availability and low root:shoot ratio suggest that mullein has evolved a fast-growing, weedy phenotype in its introduced range rather than adapting to a low-water environment through increased root growth. Although fast-growing plants can be more palatable to herbivores, in this case there does not appear to be a trade-off between growth and defence against a generalist herbivore. Mullein appears to have evolved to be both faster growing and better defended in the introduced range.

**Key-words:** abiotic, biotic, defence, herbivory, invasion ecology, nitrogen, resource availability, *Trichoplusia ni*, water stress

## Introduction

Upon introduction into a new range, organisms are exposed to different environmental conditions and therefore different selection pressures than in their native range (Mooney & Cleland 2001). Adaptation to these new conditions may facilitate establishment and spread in the introduced range (Cox 2004; Phillips *et al.* 2006). Much research to date has focused on adaptation to novel biotic conditions (Blossey & Nötzold 1995; Atwood & Meyerson 2011), but introduced populations

also experience and adapt to novel abiotic conditions (Mooney & Cleland 2001; Maron, Vilà & Arnason 2004a; Facon *et al.* 2006; Lee *et al.* 2011). Given that traits relevant to invasion, such as growth and defences against herbivores, are strongly influenced by both abiotic and biotic factors, understanding the evolution of such traits will require simultaneous consideration of multiple characteristics of novel environments.

Simply by chance, an introduced population's new environment is likely to differ in temperature, water availability or soil type, from the native environment. Given that many introduced species fail to establish self-sustaining populations (Williamson 1996), adaptation to novel environmental conditions often may be necessary for invasion to proceed (Facon *et al.* 2006). Evidence suggests that introduced species can adapt rapidly to novel abiotic conditions. The evolution of latitudinal clines, for example, is considered to provide strong evidence of adaptation, generally to differences in temperature regimes (Endler 1977; Partridge & French 1996; Gilchrist *et al.* 2000). Several clines have been shown to evolve rapidly in introduced populations. Wing length in the fly *Drosophila subobscura*, a presumed adaptation to latitudinal gradients in temperature, evolved within only two decades following introduction into North America (Huey *et al.* 2000). Body size and feather colour in introduced house sparrows (*Passer domesticus*) rapidly evolved in response to environmental variation in North America, recreating patterns of phenotypic differentiation similar to those found in its native European range (Johnston & Selander 1964, 1971). Introduced populations of plants have also adapted to geographical gradients in abiotic conditions, for example by adjusting size, fecundity or flowering time to differences in climate (Weber & Schmid 1998; Maron *et al.* 2004b).

Where environments are changing, rapid adaptation to novel abiotic conditions can also provide both native and introduced organisms with an advantage over other organisms that adapt less quickly (Barrett 2000; Fussman, Loreau & Abrams 2007). Many current environmental changes, including altered disturbance regimes, N deposition and increased carbon dioxide, lead to environments with high resource availability and would therefore select species and genotypes that can take advantage of available resources through rapid growth and high fecundity (Bossdorf *et al.* 2004; Wright *et al.* 2004; Bradley *et al.* 2010). Additionally, because resource availability often fluctuates (Davis, Grime & Thompson 2000), genotypes with the ability to perform well over a range of resource levels may be well poised to become invasive. Evidence for this can be found in the evolution of increased responsiveness to N or water addition (Caño *et al.* 2008; Qing *et al.* 2011).

An introduced population's biotic environment may be both different from and more benign than its native environment, particularly when introduced populations escape their natural enemies. The possibility that enemy release selects for genotypes with lower levels of defences and increased growth rates (the evolution of increased competitive ability or EICA hypothesis; Blossey & Nötzold 1995) has been examined for numerous species and environments, with mixed results (Atwood & Meyerson 2011). With respect to defence, only a few studies have found evidence for higher susceptibility of introduced plants to herbivores (Blossey & Nötzold 1995; Siemann & Rogers 2003; Maron, Vilà & Arnason 2004a; Wolfe, Elzinga & Biere 2004). Müller-Schärer, Schaffner & Steinger (2004) argue that in the introduced range, only specialized enemies decrease in abundance, which should lead to decreased defence investments against specialists but unchanged or even increased defence against generalists. Indeed, this hypothesis may explain some of the variation in defence results (Joshi & Vrieling 2005; Stastny, Schaffner & Elle 2005; Huang *et al.* 2010).

With respect to growth, the predictions of EICA hypothesis are more often supported, with introduced genotypes typically growing larger than native genotypes of plants in common gardens (Bossdorf *et al.* 2005; Atwood & Meyerson 2011). However, studies that directly evaluate competitive ability are still in the minority (Atwood & Meyerson 2011). Furthermore, among studies that do consider competition explicitly, increased growth is most often observed in the absence of competition (Leger & Rice 2003; Bossdorf *et al.* 2004; Blumenthal & Hufbauer 2007; Atwood & Meyerson 2011). This pattern, together with the evolution of traits such as rapid growth, early germination and flowering and high reproductive biomass (Wolfe, Elzinga & Biere 2004; Erfmeier & Bruelheide 2005) suggests that invasive populations may tend to evolve weedy phenotypes adapted to rapid resource acquisition and growth (Bossdorf *et al.* 2004; Wolfe, Elzinga & Biere 2004; Blumenthal & Hufbauer 2007; Caño *et al.* 2008; Qing *et al.* 2011). Such differences could reflect adaptation to environments with high resource availability, as noted above, or adaptation to enemy release: because fast-growing phenotypes can be particularly susceptible to enemies (Coley, Bryant & Chapin 1985), enemy release may decrease fitness costs associated with such phenotypes and increase investment not just in growth, but in rapid growth in particular (Blumenthal 2006; Zhang & Jiang 2006; Blumenthal *et al.* 2009).

Understanding the role of evolution in invasion will ultimately require understanding the combined effects of adaptation to abiotic and biotic aspects of novel environments. In turn, this requires knowledge of the differences in abiotic and biotic environment between species' native and introduced ranges. Common mullein (*Verbascum thapsus*, Scrophulariaceae) is an excellent system for a simultaneous evaluation of adaptation to abiotic and biotic shifts for several reasons. First, it experiences different abiotic conditions between arid introduced habitats in western North America and more mesic habitats in Europe (Alba & Hufbauer 2012). Secondly, the biotic environment also differs between these regions, with fewer specialist herbivore species present in the introduced populations and lower damage by chewing herbivores overall (Alba & Hufbauer 2012). Thirdly, both individuals and populations are larger in the introduced region than in the native region (Alba & Hufbauer 2012). And fourthly, there is evidence that genetic differentiation between native

and introduced populations contributes to these differences in performance (Alba *et al.* 2011). Here, we report the results of a common garden experiment that, to the best of our knowledge, uses one of the highest levels of replication among populations published in the comparative invasions literature (at least 23 per range). We evaluate performance of plants subjected to six treatments: two levels of competition crossed with three resource manipulations (drought stress, N stress and high resource availability controls). We then test whether introduced mullein populations have evolved differences in (i) drought tolerance, (ii) tolerance to low N availability, (iii) competitive ability, (iv) growth rate and (v) herbivore resistance. This work builds upon a previous common garden study of mullein performance and investment in chemical and structural defence under high resources (Alba *et al.* 2011) but includes substantially higher population replication and explicit consideration of environmental factors known to differ among ranges: resource availability, competition and herbivory (Alba & Hufbauer 2012).

## Materials and methods

### STUDY SPECIES

Common mullein (*Verbascum thapsus*) is a primarily biennial weed native to Eurasia and introduced to the United States by early western European settlers for medicinal purposes (Gross & Werner 1978). It has an extensive distribution in its native range occurring throughout the British Isles and Europe (including Scandinavia), spanning to the east in Russia and China and to the south in the Caucasus Mountains and western Himalayas (Clapham, Tutin & Warburg 1952; Gross & Werner 1978). Its North American distribution includes some Canadian provinces and all 50 states in the United States, where it reaches noxious status in Colorado, South Dakota and Hawaii. It grows in larger, denser populations, with larger individuals, in the western portion of its invaded North American range than in its native European range (Alba & Hufbauer 2012). Several lines of evidence suggest that this increased performance is associated with differences in resource availability, competitive regime and natural enemy communities in the native and introduced ranges. In particular, mullein has experienced a shift in the climatic regimes that characterize much of its introduced range. Precipitation is considerably lower in highly invaded areas of the western United States than in much of the native range (Gross 1980; Gross & Werner 1982; Alba & Hufbauer 2012). In addition to shifts in these abiotic factors, the abundance of co-occurring plant species is limited in mullein's introduced range, creating a sparse canopy that likely reduces neighbourhood competition for light and possibly other resources. Introduced mullein also exhibits partial or complete escape from several herbivore guilds that are important in the native range (Alba & Hufbauer 2012). A previous common garden experiment provided evidence for increased growth in introduced populations, but no evidence for an evolutionary shift in investment in chemical and structural defences (Alba *et al.* 2011).

### EXPERIMENTAL DESIGN

We performed a common garden experiment in glasshouses in Fort Collins, Colorado, USA, to explore how performance of mullein differs between the native and invaded ranges and between different soil

resource and competition treatments. Results of such common garden studies can vary with the location and environmental conditions of the study (Williams, Auge & Maron 2008). One solution to this problem is to conduct studies in multiple common gardens (e.g. Maron *et al.* 2004b; Williams, Auge & Maron 2008). Here, we instead manipulated environmental factors that we hypothesized to influence mullein performance, using a neutral glasshouse environment to avoid results specific to the introduced range. As such, we tested whether plants appear to be more or less adapted to those conditions imposed in the glasshouse, rather than whether they are adapted to the local external environment.

We grew individual plants from two randomly chosen maternal lines from each of 23 native and 27 introduced populations using field-collected seeds (see Table 1 for sample sites). Seed collections were made over several growing seasons beginning in 2008. Following collection, seeds were removed from their capsules and stored in a refrigerator or freezer until use in the experiment. When possible, seeds were collected from at least 10 plants per population, with plants occurring at least 2 m from each other. We focused our replication at the population level to be able to draw general conclusions about the native and introduced regions sampled (Colautti, Maron & Barrett 2009). Given this, we had relatively little power to test for differences among populations within ranges or maternal lines within populations.

It was logistically impossible to acquire seed from across mullein's geographically vast distributions in each range. Instead, we included native populations that provide broad coverage of western and central Europe and thus encompass the likely provenance of introduction to the United States. We focused on introduced populations across an extensive region of the semi-arid western United States where mullein is considered relatively weedy (for example in comparison with mullein populations growing in the eastern United States; Gross & Werner 1978; Gross 1980; Alba & Hufbauer 2012), and the question of how evolution relates to invasion is most pertinent. As a result, our introduced populations come from a significantly more arid environment than our native populations (Alba & Hufbauer 2012), leading us to test explicitly whether introduced populations have adapted to a low-water environment.

Six siblings from each maternal line were grown; half were subjected to intraspecific competition and half without competition. Each of these individuals was randomly assigned to one of the following three treatments: water stress, N stress (no additional N beyond what was in the potting mix) and a high-resource control (with high water and additional N). This resulted in a total of 600 focal plants. Because we did not have strong a priori hypotheses regarding three-way interactions between water, N availability and range, we did not include a treatment with both water and N stress.

In the competition treatment, each focal plant was grown in the centre of the pot, between two competitors, one from the native range and one from the introduced range. The competitors were taken from one maternal source per range, randomly chosen from among those maternal lines that had enough seeds for all seedlings needed. Using one maternal source per range reduced variability and provided focal plants with a reasonably consistent competitive environment. Using plants from both ranges controlled for potential adaptation of native or introduced populations to the specific competitors used.

Seeds of the competitor plants were sown on top of Jiffy-7 peat pellets [with a bit of Sunshine #3 germination mix (DWF Grower Supply, Denver, CO, USA) to fill the hole of the pellets prior to sowing]. Pellets were placed on a mist bench (average daytime temperature: 24.9 °C; average daytime relative humidity: 68.0%; average

**Table 1.** Sampling locations

Native (EU)

| Country | Latitude | Longitude | Altitude (m) |
|---|---|---|---|
| Belgium | 51.03948 | 5.37579 | 31 |
| Belgium | 50.92721 | 4.42469 | 16 |
| Czech Republic | 50.17970 | 13.37999 | 343 |
| Finland | 60.20610 | 25.13333 | 17 |
| Finland | 61.35197 | 24.83544 | 100 |
| Finland | 61.00024 | 24.41610 | 121 |
| France | 45.94939 | 1.01531 | 268 |
| France | 43.67723 | 3.85595 | 67 |
| France | 43.69820 | 3.85372 | 67 |
| Germany | 42.88756 | 7.58136 | 217 |
| Romania | 45.43936 | 27.05447 | 161 |
| Romania | 45.75631 | 27.20297 | 44 |
| Romania | 47.14608 | 27.63928 | 54 |
| Romania | 45.02156 | 26.47103 | 127 |
| Romania | 44.84469 | 25.91742 | 139 |
| Romania | 45.30881 | 26.96517 | 135 |
| Sweden | 66.81880 | 16.03915 | 868 |
| Sweden | 66.83685 | 16.02581 | 1272 |
| Sweden | 66.83593 | 16.01188 | 1075 |
| Sweden | 66.83727 | 16.02247 | 1065 |
| Switzerland | 46.76580 | 7.12290 | 646 |
| Switzerland | 46.84713 | 7.17373 | 646 |
| Switzerland | 46.98717 | 7.14019 | 453 |

Introduced (USA)

| State | Latitude | Longitude | Altitude (m) |
|---|---|---|---|
| Colorado | 40.51064 | −105.09930 | 1551 |
| Colorado | 40.51404 | −107.62123 | 1948 |
| Colorado | 40.69956 | −105.54407 | 2138 |
| Colorado | 39.82549 | −105.31157 | 2388 |
| Colorado | 40.49153 | −107.31525 | 1921 |
| Colorado | 40.68939 | −105.31041 | 1747 |
| Colorado | 40.78176 | −106.47854 | 2238 |
| Colorado | 40.66529 | −105.21944 | 1603 |
| Colorado | 40.68993 | −105.43177 | 1966 |
| Colorado | 40.67129 | −105.23055 | 1608 |
| Colorado | 40.60116 | −105.09297 | 1517 |
| Colorado | 40.38054 | −106.80385 | 2463 |
| Colorado | 40.50110 | −106.92329 | 2009 |
| Colorado | 40.48645 | −107.10521 | 1964 |
| Idaho | 47.55414 | −116.91631 | 777 |
| Maryland | 38.90028 | −76.55556 | 31 |
| Montana | 46.96261 | −110.75556 | 1648 |
| Montana | 47.06978 | −111.97194 | 1078 |
| Montana | 45.28437 | −112.10693 | 1618 |
| Montana | 47.43206 | −111.31994 | 1058 |
| Montana | 46.87412 | −115.01572 | 1644 |
| Montana | 47.40467 | −111.32792 | 1032 |
| Montana | 45.62225 | −109.28355 | 1109 |
| Washington | 47.62621 | −122.52124 | 21 |
| Washington | 47.62621 | −122.52124 | 21 |
| Wyoming | 43.47993 | −110.76243 | 1901 |
| Wyoming | 41.85194 | −109.18801 | 2180 |

night-time temperature: 21.5 °C; average night-time relative humidity: 71.3%; 16-h light, 8-h dark). Excess seed was sown and we thinned seedlings as necessary to avoid competition at that stage. Germination trays were re-randomized every second day to minimize microclimatic effects. Sixteen days after sowing, competitor seedlings were moved to the glasshouse and transplanted into one-gallon pots containing Fafard V-#2 potting soil (American Clay Works and Supply Co., Denver, CO, USA). Glasshouse conditions were as follows: average daytime temperature: 26.6 °C; average daytime relative humidity: 45.2%; average night-time temperature: 15.2 °C; average night-time relative humidity: 49.1%; 16-h day, 8-h nights.

The target plants were sown 3 weeks after the competitor plants following the same protocol as the competitor plants. Ten days after sowing, we measured the length of one cotyledon per seedling with callipers to provide an estimate of maternal provisioning. We took this measurement to be able to correct for maternal effects later on since the seeds were field-collected. After 16 days on the mist bench, target plants were moved to the same glasshouse as the previously trans-planted competitor plants. If assigned to the no-competition treatment, they were transplanted into pots with soil only, and if assigned to the competition treatment, they were planted between the two previously planted competitors. Competitor plants were clipped back before transplanting the focal plants to avoid shading and facilitate initial establishment. We clipped 0–2 leaves off each competitor, and only the leaves pointing towards the middle of the pot. After transplanting, all plants were watered as needed. Pots were completely randomized within the glasshouse and rotated twice a week using a system of roll-ing trays (Hardy & Blumenthal 2008); during the course of the experi-ment, each tray was on each position of the glasshouse benches. Focal seedlings that died within the first 10 days were replaced with another seedling from the same maternal line. We started to impose the resource stress treatments (N and water; see below for details) 21 days after transplanting to give the plants time to establish in the pots. Target plants were harvested 14 weeks after transplanting.

## WATER STRESS TREATMENT

All plants were watered every 2–3 days and control and water stress plants were watered at the same time. To manipulate water availabil-ity, the watering hose was turned to a standard pressure and plants were watered at that constant flow rate for different amounts of time. Control and N-stressed plants were watered for about four seconds each, such that the potting soil was thoroughly wet. They received about 120 mL of water each time. Soil in the pots was still somewhat moist when they were watered the next time and none of the plants started wilting.

Water-stressed plants were watered for about two seconds each time and thus received approximately half the water of control plants, or about 60 mL of water. Potting soil of all water-stressed plants dried out between watering events, and at least 50% of the plants without competition had started wilting before they received water again. Pots with and without competition received the same amount of water according to their assigned treatment (e.g. water-stressed plants received approximately 60 mL per watering event with and without competition). This factorial combination of water and compe-tition meant that focal plants in competition may have experienced more water stress than focal plants without competition (due to water use by competitors) or less water stress than focal plants without competition (due to shading from competitors).

## NITROGEN STRESS TREATMENT

We used regular Hoagland solution as fertilizer for control and water-stressed plants. Plants under N stress received Hoagland solution with no N added and thus had access only to the N present in the potting soil. This allowed us to isolate the effects of N stress from effects of

other nutrients. All pots received 30 mL of the appropriate Hoagland solution per week, regardless of the competition treatment.

## BIOMASS MEASUREMENTS

Rosettes of the target plants were dried in an oven at 50 °C for 5 days or until a constant weight was reached. All target plants either had leaves removed for use in the feeding experiments (see below) or to balance those experiments. These leaves were also dried and weighed. The biomass of leaves used in the feeding experiments was corrected for the mass eaten by the larvae by assuming that mass removed was proportional to the area removed. These data were added to the weight of the respective rosette to obtain above-ground biomass. Roots of plants without competition were gently washed free of potting soil and dried in the same conditions as the rosettes prior to weighing. Rosettes and roots of non-competition plants were weighed to the nearest 0.01 g, and rosettes of plants under competition to the nearest 0.0001 g (the greater precision was necessary due to their smaller size).

In addition to biomass, we measured diameter of the rosettes and counted leaves of all target plants before harvest. Since the overall results are largely similar for leaf number and diameter as for above-ground biomass, these results are not shown in the main manuscript (see Appendix S1 in Supporting Information).

## PREFERENCE OF A GENERALIST HERBIVORE

In the week before harvest, feeding experiments with larvae of the generalist lepidopteran *Trichoplusia ni* (cabbage looper) were performed. We chose a generalist because mullein still incurs substantial attack by generalist leaf chewers (grasshoppers and caterpillars) in the introduced range (Alba & Hufbauer 2012) and therefore its defences may be under selection by this particular insect guild.

We randomly paired control plants from the native and introduced range, resulting in 39 pairs. For each pair of plants, we clipped the oldest leaf that did not show any signs of senescence and put the two leaves into a single square petri dish (28 cm × 28 cm). As this occurred a week prior to harvest, we also clipped a leaf from plants not used in this experiment to impose equivalent damage to those plants. The oldest leaves were chosen since these are the most likely to be fed on by generalist leaf chewers in natural systems, as they are less well defended than young leaves (Alba, Bowers & Hufbauer 2012). Bottom parts of the petri dishes were covered and stems of the leaves wrapped with wet paper towels to prevent leaves from drying out. Three 4th-instar *T. ni* larvae (ordered from Bio-Serv, Frenchtown, NJ, USA) were put in each petri dish and the dishes were sealed with Parafilm. Lights in the room were left on 24 h a day for the duration of the experiment. After 3 days of feeding, the larvae were removed and leaves cleaned of frass. We scanned the leaves with an Epson Expression 1680 color scanner (Epson America Inc., Long Beach, CA, USA) and used PHOTOSHOP ELEMENTS (Adobe Systems Inc., San Jose, CA, USA) to precisely outline non-eaten leaf area and WINFOLIA (Regent Instruments Inc, Quebec City, Canada) to calculate total leaf area and leaf area eaten. From these data, we calculated percentage leaf eaten per leaf and compared these numbers between the pairs in each petri dish.

## STATISTICAL ANALYSES

All analyses were performed with the statistical program SAS version 9.2 (SAS Institute Inc. 2008). We first evaluated differences in survival by range, competition and treatment using a generalized linear mixed model (Proc. Glimmix). We then evaluated other aspects of performance and phenotype with linear mixed models (Proc. Mixed). Fixed factors in the models included range (native/invaded), competition (yes/no), treatment (water, N and control) and their interactions. Additionally, cotyledon size was included in the models as a covariate to take maternal provisioning into account. Population nested within range and maternal plant nested within population were considered random. Response variables were natural log-transformed for the analyses when needed to reduce heteroskedasticity and improve the normality of the residuals (shoot biomass). Root:shoot ratios were also ln-transformed, as suggested by Poorter & Nagel (2000) as ratios naturally have ln-normal distributions. In cases when transformations were done, the results are presented as back-transformed means and standard errors. Random effects were tested with likelihood ratio tests formed by examining the difference in −2 residual log-likelihood values with and without the random effect being included in the model. The test statistics for the random effects are distributed approximately as $\chi^2$ tests with one degree of freedom and are one-sided tests (Littell, Henry & Ammermann 1996). The data on herbivore preference were analysed differently from the plant performance date. Herbivore preference was assessed by using a simple paired t-test to compare feeding damage (measured as the percentage area eaten per leaf) between the native and introduced range on leaves from the control plants. The samples were paired randomly and each leaf pair within a petri dish was treated as a paired sample.

# Results

## SURVIVAL

Of the initial 600 target mullein plants, 78% survived until the end of the experiment. Survival was higher in the no-competition environment (94%) than in the competition environment (54%, $F_{1,489} = 178.8$, $P < 0.0001$, Fig. 1). Additionally, there was a significant interaction between range and treatment ($F_{2,489} = 4.10$, $P < 0.017$) because native plants had highest survival in the N stress treatment (80% on average), while introduced plants had highest survival in the water stress treatment (81%; see Appendix S2 in Supporting Information).

Population within range accounted for significant variation in survival, but the maternal plant within population did not (see Appendix S2).

## BIOMASS

Shoot biomass was influenced by a significant three-way interaction between range, competition and treatment ($F_{1,362} = 4.25$, $P = 0.0150$). To simplify interpretation, we further evaluated data from the two competition treatments separately.
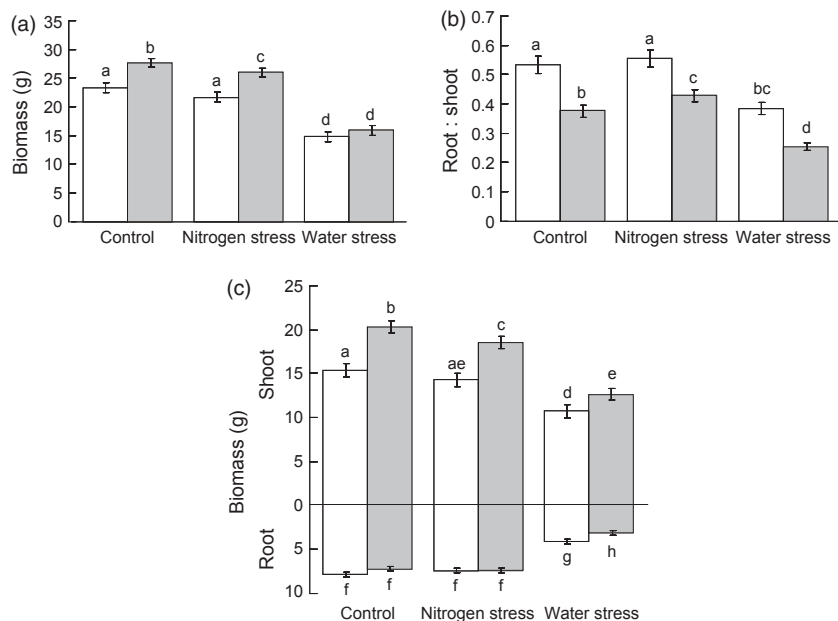
### Without competition

Without competition, there was a significant interaction between range and treatment in determining total biomass ($F_{2,178} = 4.76$, $P = 0.01$, Fig. 2a), such that control and N-stressed plants from the introduced range were larger than
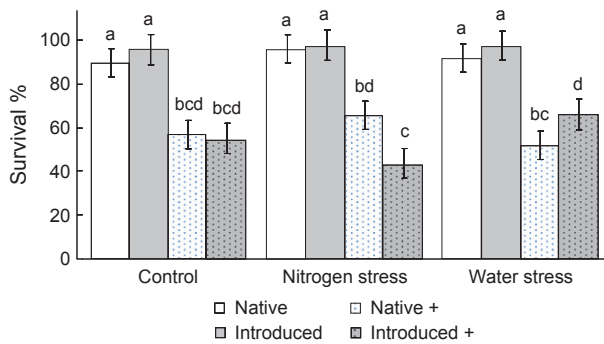
**Fig. 1.** Survival as a function of resource availability and competition (+ stands for plants under competition). Survival was higher in the non-competition treatment than in the competition treatment, and native plants survived best under N stress, while introduced plants had highest survival under water stress. Least-square means are shown, and error bars are standard errors of the mean. Bars with different letters are significantly different at the $P = 0.05$ level. Comparisons were made between and within groups. For statistical details, see main text.

those from the native range ($t_{113} = 3.74$, $P = 0.0003$ and $t_{108} = 3.73$, $P = 0.0003$, respectively), but water-stressed plants showed no difference in total biomass by range ($t_{109} = 0.88$, $P = 0.38$). There were also differences in shoot and root allocation of native and introduced plants. Treatment shaped root:shoot ratio ($F_{2,183} = 78.0$, $P < 0.0001$, Fig. 2b), but did not interact with range. Water-stressed plants had the lowest root:shoot ratio, and N-stressed plants had the highest (Fig. 2b). Introduced plants attained larger shoot biomass than native plants across all treatments ($F_{1,46.6} = 20.7$, $P < 0.0001$, Fig. 2c), with that difference being more pronounced in the control and N stress treatments, leading to a range by treatment interaction ($F_{2,80} = 4.3$, $P = 0.009$, Fig. 2c). Root biomass was influenced by a weaker range by treatment interaction ($F_{2,176} = 2,89$, $P = 0.058$, Fig. 2c). Rather than

being larger, roots were marginally smaller in the introduced control plants relative to native plants ($t_{150} = -1.71$, $P = 0.089$), did not differ in the N-stressed plants ($t_{144} = 0.16$, $P = 0.87$) and were significantly smaller than those of native plants in the introduced water-stressed plants ($t_{145} = -2.75$, $P = 0.007$). These differences in allocation resulted in significantly lower root:shoot ratios in the introduced than in the native plants ($F_{1,46.1} = 31.5$, $P < 0.0001$).
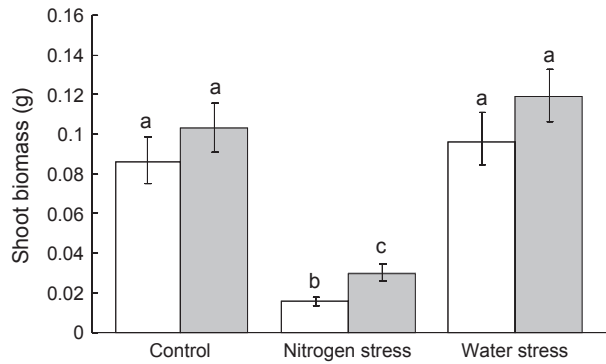
For each of the above response variables, there was evidence for significant variation among populations within ranges (see Appendix S2). Further, for all but root biomass, the family effect (maternal plant nested within population and range) also accounted for significant variation, suggesting that there is substantial genetic variation in these traits both within and among populations.

### With competition

Under intraspecific competition, introduced plants attained higher shoot biomass overall ($F_{1,38.8} = 7.56$, $P = 0.009$, Fig. 3), with a significant interaction between range and treatment ($F_{2,96.8} = 3.10$, $P = 0.0497$) due to larger proportional differences in biomass between ranges in the low-N treatment (Fig. 3). Across ranges, shoot biomass did not differ between control and water-stressed plants ($t_{92.7} = -1.25$, $P = 0.216$), but was significantly lower in N-stressed than in control plants ($t_{103} = 13.48$, $P < 0.0001$). Population within range also accounted for significant variation in shoot biomass under competition, but the family effect was not significant (see Appendix S2).

### HERBIVORY

Leaves from the native range were clearly preferred over leaves from the introduced range by the generalist feeder

**Fig. 2.** (a) Total biomass, (b) root:shoot ratio and (c) above- and below-ground biomass of plants without competition under the different treatments. White = native populations; grey = introduced populations. Introduced genotypes under control and N-stressed treatment are significantly larger than native genotypes (a) & (c). Roots are smaller in introduced plants (c), which leads to significantly lower root:shoot ratios in the introduced range. Least-square means are shown, and error bars are standard errors of the mean. Data are back-transformed where applicable. Bars with different letters are significantly different at the $P = 0.05$ level. Comparisons were made between and within groups. For statistical details, see main text.

**Fig. 3.** Above-ground biomass of plants with competition under the different treatments. White = native populations; grey = introduced populations. Introduced plants are larger, with the biggest difference between ranges in the N treatment. Least-square means are shown, and error bars are standard errors of the mean. Bars with different letters are significantly different at the $P = 0.05$ level. Comparisons were made between and within groups. For more statistical details, see main text.
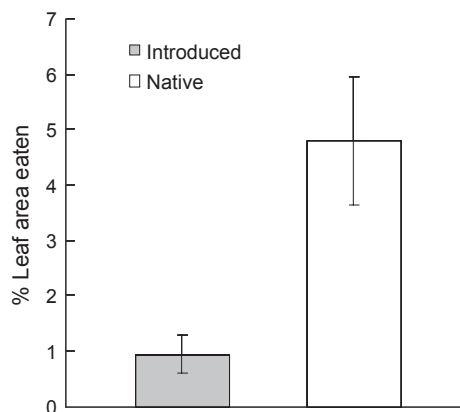


**Fig. 4.** Average of percentage leaf area eaten per range. Error bars indicate standard error of the mean. Significance level: $P = 0.004$.

*T. ni* (Fig. 4). Generally, only low percentages of the leaves were eaten, with maxima of 39.5% damage from the native and 12.7% damage from the invaded range. Only four leaves from the native and one from the invaded range had more than 10% leaf area eaten. These low percentages are not surprising given that the leaves were quite large relative to the larvae.

## Discussion

We found that common mullein from the introduced range performs better across a wide array of environments compared with individuals from the native range, an indication that rapid evolution has taken place. This phenomenon is also known for many other species (Mooney & Cleland 2001; Lee 2002; Bossdorf et al. 2005). Introduced mullein grew larger than native mullein with and without competition, with high and low N and with high water, and was more resistant to a generalist herbivore.

Several of our results – rapid growth, strong responses to higher water availability and low root:shoot ratios in introduced populations – suggest that introduced mullein populations have evolved fast-growing, weedy phenotypes (Wolfe, Elzinga & Biere 2004). These findings match those of previous common garden studies in which introduced plants exhibited stronger responses to N and water addition, and often (but not always) grew larger over the course of an experiment, and thus grew faster than native conspecifics (Blair & Wolfe 2004; Bossdorf et al. 2005; Blumenthal & Hufbauer 2007; Caño et al. 2008; Qing et al. 2011). The faster growth rates observed in many introduced relative to native populations suggest a fundamental shift in their growth strategies that favours rapid resource acquisition and growth over resource conservation and slower growth (Diaz et al. 2004; Wright et al. 2004; Blumenthal & Hufbauer 2007). Such a shift would be expected if environments in the introduced range are relatively benign (Bossdorf et al. 2004; Zhang & Jiang 2006), whether in terms of resource availability or the presence of natural enemies.

For mullein, it might be expected that the semi-arid habitats that comprise a large portion of its introduced range are in fact stressful, especially in terms of water availability, and that increased performance might therefore hinge on adaptation to drought. However, the water-stress no-competition treatment was the only treatment in which introduced plants did not grow larger than native plants. Furthermore, root: shoot ratios were lower in introduced than in native genotypes, rather than higher, as might be expected if these genotypes had evolved to perform well under low water.

This pattern may be explained in part by the fact that low water availability limits photosynthesis as well as growth and can therefore favour above-ground as well as below-ground allocation (Poorter & Nagel 2000). It is also possible that mullein's fast-growing weedy phenotypes themselves are an adaptation to dry environments. Rapid growth is one strategy for coping with variable precipitation (Kimball et al. 2012), and patterns of high responsiveness to water availability in plants from arid regions have been documented previously. Ward, Shrestha & Golan-Goldhirsh (2011) found that *Acacia raddiana* in the Negev desert from drier regions responded more strongly to increased water than individuals from regions with higher precipitation. They concluded that these plants have evolved to be more capable of responding to higher spatial heterogeneity in water availability. This might also be the case with mullein: regions of the western United States where mullein is prevalent are characterized by temporally and spatially heterogeneous summer storms, which could select for the ability to respond rapidly to short-term water availability.

In terms of the biotic environment, evidence suggests that introduced populations of common mullein experience relatively benign conditions that could have selected for fast-growing phenotypes. A sparse plant canopy in the introduced range may reflect a low-competition environment; however, it may also reflect a stressful water-limited environment or, in all likelihood, a combination of the two that varies spatially

and temporally (Alba & Hufbauer 2012). Mullein also has fewer specialized enemies and exhibits less chewing damage in its introduced range (Alba & Hufbauer 2012). Because enemy damage is a cost associated with fast-growing phenotypes (Coley, Bryant & Chapin 1985), release from specialist enemies could have reduced that cost, thereby facilitating evolution of rapid growth (Blumenthal 2006; Zhang & Jiang 2006; Blumenthal *et al.* 2009).

While we found strong evidence for increased growth of mullein, particularly in environments with ample water, we did not find evidence for trade-offs in growth among environments. Low N availability often limits plant growth, even in relatively dry environments (Hooper & Johnson 1999; LeBauer & Treseder 2008), and other successful invaders have been found to exhibit increased responsiveness to N addition in the introduced range compared with the native range (Qing *et al.* 2011). In contrast, we found that introduced mullein plants were more successful than native plants both with and without N fertilization. Similarly, higher growth of introduced than native genotypes is more often observed in the absence than in the presence of competition (Leger & Rice 2003; Bossdorf *et al.* 2004; Blumenthal & Hufbauer 2007; Atwood & Meyerson 2011), while we found that introduced mullein grew larger than native mullein with and without competition (as previously observed in *Silene latifolia*; Blair & Wolfe 2004).

Additionally, we did not observe a trade-off between growth and defence against generalist herbivores, as leaves from the fast-growing introduced genotypes were clearly avoided by *T. ni* larvae. These findings are similar to those of Ridenour *et al.* (2008) who reported that introduced populations of *Centaurea maculosa* have higher performance, compete more strongly and resist herbivores more effectively, than native populations. They are also in concert with the findings of Alba *et al.* (2011), who showed that while introduced mullein populations were larger than native populations, investment in chemical and structural defence was similar between ranges. However, our current findings contrast with Alba *et al.* (2011) in that *T. ni* larvae clearly preferred native genotypes. Our experiment may have captured either a broader spectrum of variation in defence phenotypes (with at least 23 populations per range) or, by using feeding trials, a more complex aggregation of defensive traits that shape palatability to herbivores. Overall, our findings do not match the predictions of the original EICA hypothesis, which rest on the assumption that increased performance must come at the expense of defence (Blossey & Nötzold 1995). Instead, the results suggest that a shift in the herbivore community from specialist- to generalist-dominated may have led to evolution of increased resistance to generalists (Müller-Schärer, Schaffner & Steinger 2004).

A conundrum posed by our results is that introduced mullein performed as well as or better than native mullein in all environments that we tested except when exposed to competition and N stress simultaneously. Introduced mullein genotypes grew faster and had lower root:shoot ratios, a weedy phenotype (Wolfe, Elzinga & Biere 2004) that enables them

to take advantage of high-resource environments; they also performed better in N-poor and competitive environments and were more defended than native mullein. Given the multitude of environments a species encounters in nature, it is likely that there are environments that we did not test in which native mullein genotypes would perform better (e.g. competition for resources other than water and N, or resistance to specialist enemies). However, it may be possible for evolutionary shifts associated with introductions to produce higher performance in introduced genotypes overall via a shift in the selection regime, such as more consistent selection for both rapid and early growth and increased defence against generalists. Additionally, increased genetic variation associated with the invasion could enable faster responses to selection (Ellstrand & Schierenbeck 2000; Verhoeven *et al.* 2011) and/or masking of genetic load (Ellstrand & Schierenbeck 2000), while decreases in genetic variation could lead to purging of genetic load (Glémin 2003; Facon *et al.* 2011). There are no data available yet for mullein on selection regimes *per se* nor on molecular genetic variation. Phenotypic data show strongly reduced rather than increased variation (R.A. Hufbauer, A. Monty and M. Rusin, unpubl. data), supporting both strong selection and purging as possibilities. Purging has recently been documented within an invasive species (Facon *et al.* 2011), and models show that the conditions that allow purging may be found during invasions (Glémin 2003; Facon *et al.* 2011). A next step in the evolutionary ecology of biological invasions will be to link explicitly underlying genetic processes (shifts in selection, purging of genetic load) with population responses to changes in abiotic and biotic environments.

In sum, we found increased performance of introduced mullein across an array of environments, a pattern that appears to match at least some of the novel aspects of mullein's introduced environment. Understanding whether such increased performance carries costs under other environmental conditions, or represents increased performance in general, will be key to understanding the importance of these evolutionary changes to invasion success.

## Acknowledgements

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1.** Results for plant diameter and leaf number.

**Appendix S2.** Table B1: Shoot biomass overall. Table B2: Total biomass without competition. Table B3: Shoot biomass without competition. Table B4: Root biomass without competition. Table B5: Root:shoot ratio without competition. Table B6: Shoot biomass with competition. Table B7: Survival overall.