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Evolution of increased *Medicaco polymorpha* size during invasion does not result in increased competitive ability

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Abstract

Species invading new habitats experience novel selection pressures that can lead to rapid evolution, which may contribute to invasion success and/or increased impact on native community members. Many studies have hypothesized that plants in the introduced range will be larger than those in the native range, leading to increases in competitive ability. There is mixed support for evolution of larger sizes in the introduced range, but few studies have explicitly tested whether evolutionary changes result in decreased competitive responses or increased competitive effects on other species in the community. Here, we show that introduced *Medicago polymorpha* genotypes produced 14% more aboveground and 41% more belowground biomass than genotypes from the native range, suggesting that evolutionary changes in size occurred after introduction. However, these size differences were only observed in the absence of competition. The competitive effects of introduced and native range genotypes on three species that commonly co-occur with *Medicago* in invaded regions were remarkably similar. These results suggest that evolutionary increases in size during biological invasions do not necessarily alter the competitive effects of the invader on other community members, but may increase invasion success in disturbed or low competition environments.

Keywords Competition · Contemporary evolution · EICA · Genetic variation · Invasion ecology · Invasive species

Introduction

Rapid evolution occurs when species experience strong selection that drives evolutionary changes in traits quickly enough to alter the outcome of ecological interactions (Hairston et al. 2005; Strauss et al. 2008). One scenario in which species may evolve rapidly is when they invade new ranges and experience different selection pressures than they experienced in their native range. Comparisons of species in their invasive and native ranges suggest that this is common; 16 of 23 studies of introduced species detected significant evolutionary change in at least one trait after invasion (Buswell et al. 2011).

Although the majority of explanations for invasion success focus on ecological mechanisms (Catford et al. 2009), rapid evolution has the potential to influence invasion success, particularly when evolutionary changes occur in traits known to predict invasive species spread (Dlugosch and Parker 2008; Ebeling et al. 2008; Whitney and Gabler 2008). For example, evolutionary increases in dispersal ability at invasion fronts have accelerated the spread of invasive cane toads in Australia (Phillips et al. 2010). Similarly, a rapid adaptive shift in flowering time is the main contributor to increased reproductive success, and, therefore, potentially the northern spread, of invasive purple loosestrife (*Lythrum salicaria*) in North America (Colautti and Barrett 2013). Evolutionary changes may occur early in the invasion process, because genotypes with particular traits are better colonizers, or at later invasion stages due to the novel environmental conditions that impose selection in the invasive range (Sakai et al. 2001).

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Many studies of evolution during invasion have focused on size as an important trait. The Evolution of Increased Competitive Ability (EICA) hypothesis posits that individuals in the invasive range escape natural enemies, and individuals that reallocate resources from defense to growth and competitive ability are more successful (Harper 1964; Blossey and Notzold 1995). EICA predicts larger and more competitive individuals in the invaded range, relative to individuals from the native range. Conversely, the Evolution of Reduced Competitive Ability Hypothesis (ERCA; Bossdorf et al. 2004) predicts that individuals that invest less in competitive ability, and more in reproduction, will be more successful in the invaded range. Several studies have confirmed the predictions of EICA, finding larger sized individuals in the invasive range (Blossey and Notzold 1995; Colautti et al. 2009; Beaton et al. 2011; Huang et al. 2012; Yang et al. 2014). However, other studies have found no difference in size when comparing invasive and native populations (Meyer and Hull-Sanders 2008; Colautti et al. 2009; Gonzalez-Teuber et al. 2017; reviewed in Felker-Quinn et al. (2013)).

Although several studies have examined differences in size between individuals in the invasive and native ranges, fewer have examined whether evolutionary changes in size translate to differences in competitive ability (e.g., Vila et al. 2003; He et al. 2009; Ridenour et al. 2008; Joshi et al. 2014). Competitive ability can be measured as either competitive response, the ability of a focal species to maintain growth and fitness in response to competition from other species, or competitive effect, the reductions in growth or fitness other species exhibit when growing in the presence of the focal species (Goldberg and Werner 1983; Miller and Werner 1987). Decreased competitive responses of potential invaders to other species could allow invaders to overcome biotic resistance imposed by competition from other species. Increased competitive effects of potential invaders may allow species to overcome biotic resistance, but are also likely to result in negative consequences of invasion for the native community.

Some studies demonstrate that evolutionary changes in size increased the competitive effects of the invaders on other species (Ridenour et al. 2008; Joshi et al. 2014), while others detected no difference in competitive effect (Vila et al. 2003; He et al. 2009). Two studies investigating whether evolutionary changes in size affected the competitive response of invaders to native species found decreased competitive response in invasive range genotypes (Ridenour et al. 2008; Joshi et al. 2014). Relatively few studies have examined whether evolutionary changes in traits affect both competitive effect and response, and most have used only a few populations or genotypes for comparison.

To distinguish between evolutionary changes in plant size, competitive effect, and competitive response, we conducted two experiments on a large number of *Medicago polymorpha* genotypes collected from both the native range and introduced regions on five continents (Table 1). We asked (1) has *Medicago* size evolved in the introduced range? (2) Has the response of *Medicago* to competition evolved in the introduced range? (3) Has the competitive effect of *Medicago* on coexisting plants in the introduced range evolved?

Methods

Study system

Medicago polymorpha (hereafter "*Medicago*") is native to the Mediterranean and Middle East, but is a common invader in grasslands around the world, including Asia, Australia, South America, northern Europe, Africa, and the United States. At the McLaughlin Reserve (Lake County, California), where we have performed field experiments on *Medicago* in the past (Lau and Strauss 2005; Lau 2008; terHorst and Lau 2012; Bayliss et al. 2017), *Medicago* competes primarily with other legumes and grasses, including *Vicia villosa,* introduced in the early 1900s, *Trifolium hirtum*, introduced in the early 1800s, *Bromus hordeaceus*, introduced in the mid-to-late 1800s (Heady 1977), and the native *Acmispon wrangelianus* (formerly *Lotus wrangelianus*). *Acmispon*, in particular, suffers large reductions in fitness as a result of *Medicago* invasion (Lau and Strauss 2005; terHorst and Lau 2012), but it is unclear whether different genotypes affect *Acmispon* differently.

Because *Medicago* is a common invader around the world and is also valued for its potential as a forage crop, the National Plant Germplasm System at the United States Department of Agriculture (USDA) maintains a collection of *Medicago* genotypes. We obtained many genotypes from the USDA and also collected several genotypes from the McLaughlin Natural Reserve, Napa County, California (Table 1). *Medicago* is almost entirely selfing (Vitale et al. 1998), and because strong inbreeding leads to homozygosity, seeds from a single plant are effectively a single genotype. Hereafter, we refer to accessions as genotypes. Each genotype was grown in a common garden greenhouse environment at the W. K. Kellogg Biological Station for at least one generation to reduce maternal effects due to historical environmental differences.

Experiment 1: evolutionary changes in size and competitive response

To test for evolutionary changes in biomass production, fecundity, and competitive response, we compared introduced and native range *Medicago* genotypes grown in three

Table 1 *Medicago* genotypes used in these experiments were obtained from the USDA, ARS, National Genetic Resources Program or from field collections from the McLaughlin Natural Reserve, Napa County, California USA

Experiment	Range	Country	Accession	Seed Origin
2	Introduced	Alabama	566876	USDA GRIN
1, 2	Introduced	Australia	w6 5527	USDA GRIN
1, 2	Introduced	Belgium	566880	USDA GRIN
1, 2	Introduced	Bolivia	478439	USDA GRIN
1, 2	Introduced	Bolivia	478440	USDA GRIN
1	Introduced	Bolivia	478466	USDA GRIN
1, 2	Introduced	Brazil	404356	USDA GRIN
1, 2	Introduced	California	577391	USDA GRIN
1, 2	Introduced	California	577392	USDA GRIN
1	Introduced	California	CVI30	McLaughlin Reserve
1, 2	Introduced	California	CVI50	McLaughlin Reserve
1, 2	Introduced	California	GP1	McLaughlin Reserve
1	Introduced	California	GP5	McLaughlin Reserve
2	Introduced	California	I4	McLaughlin Reserve
1, 2	Introduced	California	I9	McLaughlin Reserve
1	Introduced	California	W1	McLaughlin Reserve
1, 2	Introduced	China	458758	USDA GRIN
1, 2	Introduced	Czech Republic	w6 5524	USDA GRIN
2	Introduced	Hungary	w6 5565	USDA GRIN
1, 2	Introduced	Japan	w6 19003	USDA GRIN
1	Introduced	Japan	w6 19008	USDA GRIN
1	Introduced	Minnesota	w6 5510	USDA GRIN
1, 2	Introduced	Pakistan	577437	USDA GRIN
1, 2	Introduced	Peru	478530	USDA GRIN
2	Introduced	Uruguay	404795	USDA GRIN
1, 2	Native	Algeria	577402	USDA GRIN
1, 2	Native	Bulgaria	w6 19534	USDA GRIN
1, 2	Native	Cyprus	368953	USDA GRIN
1	Native	Egypt	469263	USDA GRIN
1, 2	Native	Ethiopia	517214	USDA GRIN
1, 2	Native	France	566883	USDA GRIN
1, 2	Native	Germany	w6 5533	USDA GRIN
1, 2	Native	Iran	227025	USDA GRIN
1	Native	Iraq	577409	USDA GRIN
1, 2	Native	Italy	566877	USDA GRIN
2	Native	Lebanon	w6 4614	USDA GRIN
1, 2	Native	Malta	577399	USDA GRIN
1, 2	Native	Morocco	577408	USDA GRIN
1, 2	Native	Portugal	493293	USDA GRIN
1, 2	Native	Spain	543039	USDA GRIN
1, 2	Native	Tunisia	535520	USDA GRIN
1, 2	Native	Turkey	459130	USDA GRIN

Column headers are as follows: experiment, in which experiment (1 or 2) the genotype was used; range, "introduced" or "native" based on CABI Invasive Species Compendium (www.cabi.org/isc); Country, nation of collection; accession number, USDA GRIN reference number or accession numbers assigned from our collections from natural populations on the McLaughlin Reserve

interspecific competition treatments (0, 4, or 8 competitors). We used 16 genotypes that were collected from the native range of *Medicago*, and 21 genotypes collected from introduced regions around the world, including six genotypes from different populations within the McLaughlin Reserve (Table 1). Seeds were physically scarified and germinated in water-filled petri dishes. A single *Medicago* seedling was planted into each 620 ml pot filled with potting media

(SunGro LP5: Canadian Sphagnum peat moss, fine perlite, low nutrient charge with Gypsum and dolomitic limestone; SunGro Horticulture Canada Ltd., Alberta, Canada) and watered every 3 days or as needed. We chose a low nutrient substrate to ensure nutrient limitation and competition and to better represent the nutrient poor field conditions. The competitive environment was manipulated by sowing either 0, 4, or 8 *Bromus hordeaceus* seeds into the appropriate pots. *Bromus* spp., are the most common plant species in many sites at the McLaughlin Reserve, where *Medicago* is found. *B. hordeaceus* is also native to the Mediterranean and likely co-occurs with *Medicago* in its native range. *B. hordeaceus* seeds were obtained from L.A. Hearne Company (King City, CA, USA). The experiment initially had 555 replicate pots (37 *Medicago* genotypes × 3 competition treatments × 5 replicates), though 12 plants died shortly after transplant and were removed from the data set. Replicate pots were randomly distributed across the greenhouse. After 70 days, some plants were beginning to show signs of senescence, so all above- and belowground *Medicago* biomasses were harvested, and dried at 65 °C for > 24 h prior to weighing. We used biomass as an estimate of plant performance and also separately examined above- and belowground biomass as estimates of investment in shoots or roots. We counted the number of fruits on each plant at the time of harvest, though this measure is biased against genotypes with later phenologies. We present the analysis of fruit number in the online supplement (Table S1), but do not interpret those results here.

We tested for differences in biomass of *Medicago* from the introduced and native ranges with generalized linear mixed models, using proc glimmix in SAS (version 9.4) and using AIC to find the best error distribution (gamma distribution in all cases; $\Delta\text{AIC} > 175$ for all variables, compared to gaussian and log-normal distributions). We included range, competition treatment, and their interaction as fixed factors, and genotype (nested within range) and the genotype x competition interaction as random factors. We used backwards stepwise model selection to find the model with the lowest AIC value, using $\Delta\text{AIC} > 2$ as the criteria for retaining a model. We used separate models to analyze aboveground, belowground, and total biomass as response variables. We tested the significance of random effects with likelihood ratio tests.

Experiment 2: evolutionary changes in competitive effects

To test for differences in competitive effect between introduced and native range genotypes, we grew one individual of each of 34 genotypes (15 native range, 19 introduced range, Table 1) in interspecific competition with one individual of either *Acmispon wrangelianus, Vicia villosa* or *Trifolium hirtum* (each collected from the McLaughlin Reserve in Napa County, California, USA). These other species are common competitors with *Medicago* in the McLaughlin Reserve. This design allows us to examine relative differences in competitive effect between genotypes, but does not provide an estimate of the absolute competitive effect, because we did not grow each species without competition. The experiment included three replicates per genotype per competition treatment ($N = 306$; 34 genotypes × 3 treatments, $n = 3$). Plants were germinated and grown in 164 ml Containers™ (Stuewe and Sons Inc., Corvallis, OR, USA) filled with the same potting media used in Experiment 1. Replicate pots were randomly distributed across the greenhouse. On day 40, we measured the height of the competitor. After 43 days, the above- and belowground biomass of all *Medicago* and competitor plants were harvested separately and dried at 65 °C for 24 h prior to weighing.

We tested for the effects of introduced and native genotypes of *Medicago* on the biomass and height of competitor species with general linear mixed models (proc glimmix, SAS version 9.4). We log-transformed the dependent variables to meet assumptions of normality and homogeneity of variance. We included *Medicago* range and competitor identity, and their interaction, as fixed factors and *Medicago* genotype (nested within range) and the genotype x competitor interaction as random factors. We used separate models to analyze height and aboveground, belowground, and total biomass as response variables. We tested the significance of random effects with likelihood ratio tests.

Results

Experiment 1: evolutionary changes in size and competitive response

We detected a significant interaction between *Medicago* range (introduced or native) and the *B. hordeaceus* competition treatment on *Medicago* total biomass production ($P = 0.005$, Table 2). This interaction was significant for belowground biomass ($P = 0.007$), but not aboveground biomass ($P = 0.32$) (Table 2). In the absence of competition, the total biomass of introduced range genotypes was 27% larger than native range genotypes; aboveground and belowground biomasses were 14 and 41% greater, respectively (Fig. 1). In the presence of competition, *Medicago* was much smaller and introduced and native range genotypes did not differ significantly in size (Fig. 1).

We detected significant variation among genotypes, beyond that explained by range, in aboveground biomass ($P < 0.001$), belowground biomass ($P < 0.001$), and total biomass ($P < 0.001$) and variation among genotypes in aboveground biomass responses to competition [significant

Table 2 *F* statistics (*G* for random factors) and associated *P* values from generalized linear mixed models testing the effects of competition and range (native vs. invaded) on *Medicago* biomass production (Experiment 1)

Source	Total Biomass		Aboveground biomass		Belowground biomass		Fruit number	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Range	0.01	0.94	< 0.01	0.99	0.01	0.92	0.10	0.75
Competition	**1537**	**< 0.001**	**962**	**< 0.001**	**1093**	**< 0.001**	**4.27**	**0.02**
Range × comp.	**5.77**	**0.005**	1.16	0.32	**5.38**	**0.007**	1.30	0.28
Random effects	Total Biomass		Aboveground biomass		Belowground biomass		Fruit number	
	G	*P*	*G*	*P*	*G*	*P*	*G*	*P*
Genotype	**112**	**<0.001**	**67.3**	**< 0.001**	**114**	**< 0.001**	**44.1**	**< 0.001**
Gen. × comp.	1.28	0.26	**5.95**	**0.015**	3.14	0.076	0.0	0.99

Statistically significant terms ($P < 0.05$) are shown in bold. Degrees of freedom for total, aboveground, and belowground biomass were 1.35 for range and 2.70 for competition and the range × competition interaction. Degrees of freedom for fruit number were 1.35 for range and 2.40 for competition and the range × competition interaction

genotype x competition interaction ($P = 0.015$), Table 2, Fig. S1].

Experiment 2: evolutionary changes in competitive effects

Even though *Medicago* genotypes collected from the introduced range tended to be larger than genotypes from the native range when grown in the absence of competition (Fig. 1), the increased size of introduced range genotypes did not translate into increased competitive effects on co-occurring plants in the invaded range (Fig. 2). Competitors had similar total biomass in the presence of invasive range and native range genotypes ($P = 0.45$, Fig. 2, Table 3). This result was consistent for all three competitors (species × range effect $P = 0.81$, Fig. 2). The effects of different *Medicago* genotypes on competitor biomass were remarkably similar (genotype effect $P = 0.97$). However, genotypes varied in their effects on competitor height, although the magnitude of this effect differed across competing species [significant genotype x competitor species interaction ($P = 0.013$), Table 3, Figs. S2–S4]. We found the most variation in the effect of *Medicago* genotypes on *Trifolium* height (coefficient of variation = 1.2, Fig. S4), followed by *Vicia* (CV = 0.41, Fig. S3) and *Acmispon* (CV = 0.21) (Fig. S2).

Discussion

Medicago polymorpha has evolved increased size in its introduced range, although this evolutionary effect was only evident in the absence of competition. The evolutionary increase in plant size did not translate to decreased competitive response to, or increased competitive effects on, other species. These results suggest that, although evolution occurred during or after invasion, it is unlikely to help *Medicago* overcome the biotic resistance imposed by competitors, or lead to increased negative effects on other community members in the introduced range.

Many invasive species undergo rapid evolution in novel habitats (Buswell et al. 2011). Some have evolved decreased defenses, while others have evolved increased size or height or altered leaf morphologies, or some combination of the four (Buswell et al. 2011). The fact that introduced genotypes outperform native range genotypes only in treatments without competition (Fig. 1) supports the previous studies demonstrating that increases in size are only observed in low stress environments (e.g., Leger and Rice 2003; Blumenthal and Hufbauer 2007). However, our results do not support the predictions of EICA, which presume that increased size should lead to increased competitive ability. Few studies have shown that rapid evolution can increase competitive effects [but see Ridenour et al. (2008), Uesugi and Kessler (2013), Joshi et al. (2014)], and a recent review by Felker-Quinn et al. (2013) suggests that there is little support for the EICA Hypothesis in its entirety, despite strong evidence for rapid evolution of invasive species. Their review and other recent studies (Vila et al. 2003; He et al. 2009) support our conclusion that rapid evolution does not necessarily affect competitive ability despite increases in vegetative growth. Other hypotheses may also explain evolutionary changes in size [reviewed in Atwood and Meyerson (2011)], such as release from natural enemies (Blumenthal 2006; Van Grunsven et al. 2009), plasticity leading to local adaptation (Sexton et al. 2002), and multiple introductions and hybridization (Roman 2006). Therefore, we urge caution in assuming that morphological evolution necessarily leads to increased invasiveness in competitive environments or increased competitive effects on native species.

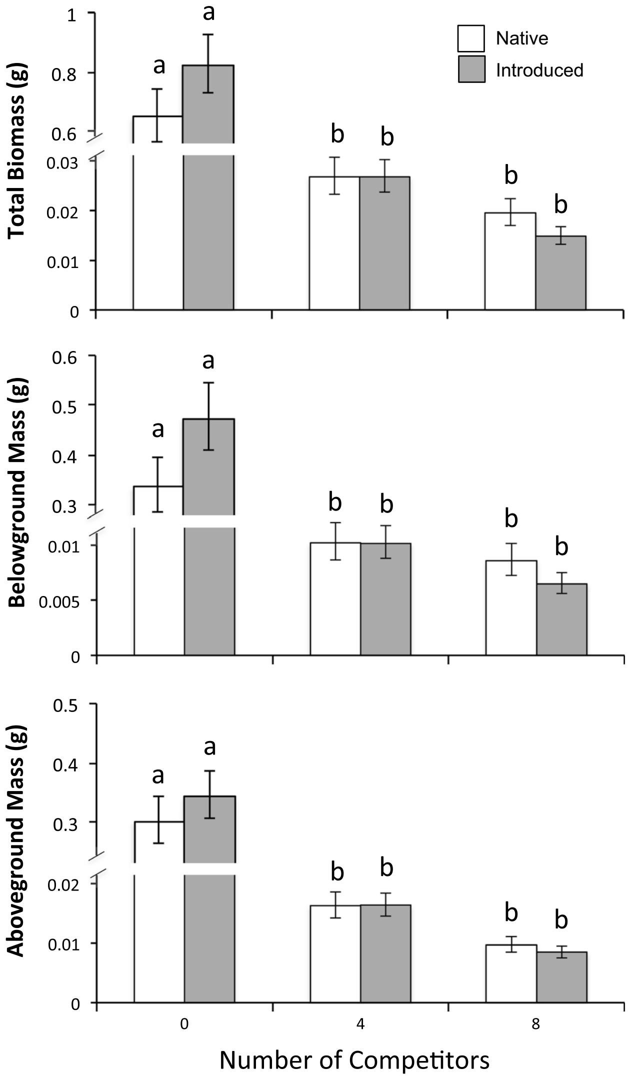

Fig. 1 Mean ± SE total biomass, aboveground biomass, and belowground biomass of native range (open bars) and introduced range (gray bars) *Medicago* genotypes grown in the absence of competition, or in the presence of four or eight *B. hordeaceus* competitors. Note the broken scale of the *y*-axis, as biomass in the presence of competitors was quite low

The fact that we did not observe any evolution of competitive ability was not due to a lack of opportunity for selection. Although we did not observe differences in responses to competition between native and introduced range genotypes, we found significant variation among genotypes within each range. We found variation among genotypes in their response to competition from *B. hordeaceus* (Fig. S1) and in their effect on competitor height (but not biomass), although the extent of this variation was dependent on the competitor species (Figs. S2–S4). This genotypic variation in competitive effect and response likely results from differences in morphology and growth, including plant height, degree of lateral spread, root:shoot ratio, and growth rate. The presence of such genetic variation also indicates that these traits have the capacity to evolve in response to divergent competitive environments between introduced and native ranges. The success of some genotypes may be explained by variation in environmental parameters in their collection range. We tested this hypothesis for genotypes for which climate data was available, but found no effect when regressing plant biomass on the latitude, elevation, or annual precipitation of the collection range (all $P > 0.38$). However, exact geography of collection locations was often limited to broad geographic regions (e.g., large states or countries) and average climate data for the region may not represent the climate at a particular collection site; thus, lack of evidence for associations with climate should be interpreted with caution.

Our experiments collectively measured competitive effect and response in pairwise competition. However, evolution in a community context may differ from evolution in pairwise competition (terHorst et al. 2015), and evolution in response to other selective agents may explain the evolution of increased size (Atwood and Meyerson 2011). For example, invasive range genotypes of *Brassica nigra* are better competitors when surrounded by a natural community, but native range genotypes are better competitors in pairwise scenarios (Oduor et al. 2013). Evolution may also occur in response to selective agents other than competitors or other natural enemies, and the ecological consequences of evolutionary effects may be context dependent (Ellner et al. 2011; terHorst et al. 2014). For example, the observed evolutionary difference in competitive ability between invasive and native range genotypes of *Trifolium* spp. was largely dependent on the soil biota (Shelby et al. 2016). Because most studies investigating evolutionary changes in size and competitive ability are conducted in relatively simplistic greenhouse environments or heavily manipulated (e.g., weeded or tilled) field environments, how evolution and the ecological effects of evolution play out in natural diverse communities requires further exploration.

The evolutionary increase in size in the introduced range was almost entirely due to increases in belowground biomass, indicating the need to study both above- and belowground processes. Had we only examined aboveground biomass, as many studies do, we would have found scant evidence for evolutionary changes in size. This pattern indicates that genotypes in the introduced range have increased investment in roots relative to shoots. Such an investment may increase competitive ability for water or nutrients, although the lack of difference in competitive response and effect between introduced and native range genotypes observed here suggests that this is unlikely. Instead the increased root:shoot ratios observed in introduced genotypes may be a response to direct selection by abiotic conditions, such as the dry conditions, these plants experience in many parts of the introduced range (Schenk and Jackson 2002) or potentially

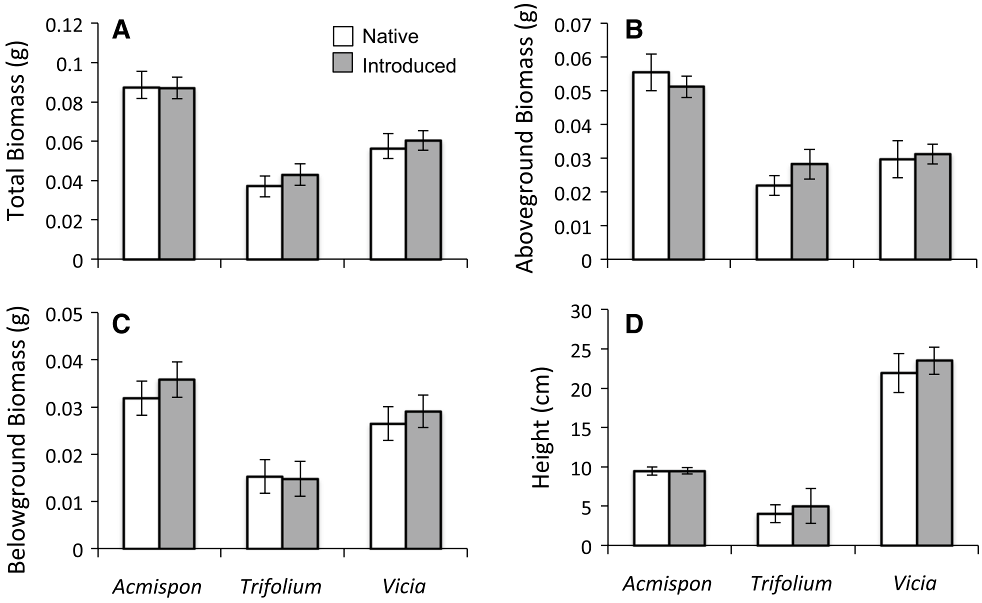

Fig. 2 Mean ± SE **a** total biomass, **b** aboveground biomass, **c** belowground biomass, and **d** height of *Acmispon wrangelianus, Trifolium hirtum*, and *Vicia villosa* individuals growing in competition with *Medicago* genotypes from the native range (open bars) and introduced range (gray bars)

Table 3 *F* statistics (*G* for random factors) and associated *P* values from generalized linear mixed models testing the relative competitive effect of invaded vs. native range *Medicago* genotypes on competitor growth (Experiment 2)

Source	Total Biomass		Aboveground biomass		Belowground biomass		Height	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Range	0.57	0.45	0.52	0.47	0.22	0.64	0.06	0.82
Competitor spp.	**24.2**	**<0.001**	**24.7**	**<0.001**	**12.72**	**<0.001**	**48.0**	**<0.001**
Range × comp spp.	0.21	0.81	0.82	0.44	0.11	0.89	0.14	0.87
Random effects	Total Biomass		Aboveground biomass		Belowground biomass		Height	
	G	*P*	*G*	*P*	*G*	*P*	*G*	*P*
Genotype	0.001	0.97	0.01	0.92	0.001	0.97	2.33	0.127
Gen × comp spp.	0.001	0.99	0.001	0.99	0.001	0.99	**8.76**	**0.013**

Three competing species were included in the experiment ("Competitor spp."). Statistically significant terms ($P < 0.05$) are shown in bold. For height, denominator degrees of freedom were 32 for range and 54 for range and competitor species. For all other variables, denominator degrees of freedom were 224 for all factors. For belowground biomass, denominator degrees of freedom from range, competition, and the range × competition interaction were 114, 85, and 85, respectively

even a lack of compatible rhizobium resource mutualists (terHorst et al., in review). Introduced *Medicago* genotypes receive fewer fitness benefits from rhizobia, potentially favoring increased investment in belowground biomass that allows them to better forage for nutrients directly from the soil (terHorst et al., in review).

This finding is consistent with other hypotheses for biological invasions that predict that invasive species will be those best able to take full advantage of high resource, low competition environments, in part because they are well-adapted to productive or human-altered environments in their native ranges (Gray 1879; Baker 1974; Dostal et al. 2013). While these hypotheses are typically invoked to explain which species are likely to be invasive, similar arguments may be made within species—those genotypes that are best able to invade may be those genotypes best able to take full advantage of disturbed, low competition conditions, possibly because they originate from disturbed or human-altered landscapes in the native range (Hufbauer et al. 2012). Local adaptation to disturbed environments in the native range may more generally lead to exaptation to low competition environments in novel invaded habitats.

Medicago was initially introduced to many locations through both accidental transports of the seeds and deliberately as a cover or fodder crop. It is possible that such agricultural practices resulted in artificial selection before or after introduction. This may account for the success of *Medicago* in disturbed and human-altered landscapes. Alternatively, invading populations may rapidly adapt to low competition environments in the introduced range. Either way, genotypes that are able to produce high biomass in low competition environments are likely to produce more seeds and, therefore, may have higher population growth rates than smaller genotypes. Greater seed production and higher population growth rates would allow these genotypes to more effectively overcome the problems associated with small populations (e.g., demographic stochasticity) that are known to limit the reproduction and spread of invasive species (Firestone and Jasieniuk 2013). Although each of these scenarios seem plausible, the previous work on this system failed to find evidence that disturbance increased the fitness of introduced genotypes more than native genotypes, although substantial genetic variation in response to disturbance was detected (Bayliss et al. 2017).

Caveats and future directions

The densities of the common competitor used in experiment 1 are similar to those experienced by *Medicago* in natural California grasslands. However, not all genotypes may have experienced this competitor in their evolutionary history. Similarly, although we examined competitive effects on three species in experiment 2, competition with these other species may not be relevant in the region in which they were collected. Three of the four competitors in our experiment were also non-native species; because there are so many invasive species in California grasslands, a potential invader is more likely to interact with invasive than native species. These results are worth considering in that context, but also in the context that most ecologists consider biotic resistance to invasion as arising from communities of native species. Future work should consider how *Medicago* genotypes respond to other species and levels of competition in these communities. The strong effects of only a few *B. hordeaceus* individuals on *Medicago* biomass, for example, left little scope for genotypic variation that may have been more obvious in less competitive environments. In addition, we measured competition in terms of biomass production, but ultimately what determines invasion success is fecundity and recruitment.

We demonstrated that the evolution of increased size during biological invasions did not increase competitive effects on other species or minimize the response to competitors. We are, therefore, left to speculate on the origins and advantages of increased size. Measuring natural selection on size in different environmental contexts, ideally in both the introduced and native ranges would help identify the environments in which increased size, and particularly increased belowground biomass, is adaptive. Unfortunately, few studies have measured natural selection on exotic species in their introduced environment and even fewer have done so in both introduced and native regions (Colautti and Lau 2015). Furthermore, all but one of the competitors used in our experiments were other invasive species. Invaders now make up the bulk of plants observed in California grasslands, so this is a realistic contemporary scenario for *Medicago* invasion, but it may not reflect conditions of the past, or those in other geographical regions. Other species that are native to California may respond differently to potential invaders.

Conclusions

Our study, combined with the previous conflicting findings that characterize tests of evolutionary changes in competitive ability, points to the need for further work investigating the selective agents acting on exotic and invasive species. While enemy escape is one potential selective agent causing the evolution of increased size (assuming growth-defense trade-offs), strong selection for increased growth in low competition environments, whether because of disturbance, drought, or other biotic or abiotic stressors, is an alternative hypothesis that warrants exploration and seems to be consistent with the patterns observed in this study. Regardless of when or why the evolution of increased size occurred, the global dispersal of *Medicago* into new locations is leading to introduced populations composed of plants capable of growing to larger sizes. Fortunately, for the communities *Medicago* invades, the evolution of increased size has not been accompanied by evolutionary increases in competitive effect or response that would exacerbate the ecological impacts of this invasive species, although the presence of genetic variation for both competitive response and competitive effect suggests that such evolutionary effects are possible.

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Author contributions ZLGP designed and performed Expt. 2 and wrote the manuscript. CPT designed Expt. 2, analyzed the data, and wrote the manuscript. SMM designed and performed Expt. 1. JAL conceived and designed both experiments and wrote the manuscript.

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