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Biological invasions: Insights into ecology and evolution

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BIOLOGICAL INVASIONS: INSIGHTS INTO ECOLOGY AND EVOLUTION

By

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Biological Invasions: Insight into Ecology and Evolution

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Introductions of exotic species commonly restructure native communities, but the mechanisms driving native species responses are poorly understood. Invasion biology has focused its research on the negative impacts of invaders, yet invaders can also have positive interactions. A holistic understanding of how biological invasions can have a full range of potential responses from negative to neutral to positive. Understanding the mechanics that underlie native species responses to invasions can help elucidate those factors structuring the native communities we see today. I used a combination of experimental and observational studies in the field and laboratory to determine the effects of spotted knapweed (*Centaurea stoebe*) invasion on native web spiders at the species and community levels while also testing key tenets of ecological and evolutionary theory.

I found that the invader trait of plant architecture could be linked to the native spider trait of web construction to explain differences in native web spider species' population responses to *C. stoebe* invasion. Architectural changes associated with *C. stoebe* invasion favor irregular web-spider populations over orb-weaver populations by providing greater release from substrate limitation. Phenotypic plasticity of web size and its relationship to fitness may also influence differences in species responses. The irregular web spider *Dictyna* exhibited substantial web size plasticity with larger webs linking to higher prey captures and increased reproduction, while the orb-weaving spider *Aculepeira*, demonstrated no plasticity in web size and showed no associated fitness benefits. Interestingly, the fitness benefits associated with web size plasticity in early stages of invasion do not persist into later stages of invasion. Plasticity was significantly reduced in later stage populations, suggesting selection for reduced web size plasticity due to increased competition for food. Lastly, I also found that *C. stoebe* invasion was associated with changes in host-parasitoid interactions between *Dictyna* and its egg sac parasitoid. *Dictyna* from invaded grasslands were much more likely to kill parasitoids, compared to individuals from uninvaded, native grasslands. The combination of these results show the diverse effects of invasion on native species and communities and also highlight the value in using biological invasions as natural experiments to study ecology and evolution.

DISSERATION FORMAT

The following chapters were formatted for individual publication in specific peer-reviewed scientific journals. Chapter 1 was submitted in June 2014 to *Ecological Applications*. Chapters 2 and 3 have not been submitted, but are in preparation for submission to *Oecologia* and *Behavioral Ecology*, respectively. I worked on these manuscripts extensively with my co-advisors Drs. Douglas Emlen and Dean Pearson and they are all listed as co-authors on at least one of the chapters. Because these chapters were a collaborative effort I use the collective "we" throughout all three chapters.

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Chapter 1:

Invasive plant's architecture increases native spider populations: linking native and invader traits

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Abstract: Introductions of exotic species commonly restructure native communities, but the mechanisms driving native species responses to invasions, particularly positive responses, are poorly understood. Here, we simulated a large-scale, *in situ* invasion of spotted knapweed (*Centaurea stoebe*) using only dead flowering stems of the invader to determine if the invader trait of plant architecture could be linked to the native spider trait of web construction to explain the dramatic differences in native web-spider species' population responses to *C. stoebe* invasion. After two years, irregular web-spiders were over 30 times more abundant on simulated invasion plots compared with uninvaded controls, while orb-weaver populations showed weaker, marginally significant increases on invasion plots. Irregular web spiders on simulated invasion plots built webs that were 4.4 times larger and 5.0 times more likely to capture prey compared with populations on control plots; changes that led to > two-fold increases in recruitment. Orb weavers showed no significant differences in web size, prey capture rates, or recruitment between treatments. Web-spider responses to simulated invasion matched patterns following natural invasions, suggesting that *C. stoebe*'s architecture is the primary invader attribute driving native web-spider responses to *C. stoebe* invasions. Differences in species-specific responses to *C. stoebe*'s architecture were linked to differences in web construction behavior as it relates to web substrate (plant architecture) constraints in the native system. Orb-weavers construct webs between multiple plant substrates, so these spiders are unconstrained by the architecture of individual native plant species and minimally released from substrate constraints following invasion. Conversely, irregular web spiders build webs within individual plants. The diminutive architecture and relative rarity of viable native plant substrates greatly constrains these spiders. Hence, the architectural changes associated with *C. stoebe* invasions favor irregular web-spider populations over orb weaver populations by providing greater release from substrate limitation as well as more suitable substrates that allowed these spiders to construct larger webs, increase prey captures, and increase reproduction. Linking invader traits with native species traits can help explain native species' responses to invasion and also elucidate the factors structuring native communities.

Key words: architecture, *Dictyna*, ecosystem engineer, facilitation, *Centaurea stoebe*, niche theory, resource limitation, simulated invasion, spiders, substrate limitation, traits

Introduction

The introduction of exotic organisms commonly results in immediate and substantial reorganization of native species within recipient communities (Vila et al. 2011). Yet, the mechanisms driving native species responses to invasions are not well understood. Advances have been made in elucidating how invaders negatively impact native species via competition, consumer interactions, and natural enemies (Mitchell and Power 2003, Parker et al. 2006, Callaway et al. 2011). However, exotic species also generate a range of strong positive responses in native species (Rodriguez 2006, White et al. 2006). The positive effects of invaders have received far less attention, yet positive interactions are critical aspects of community structuring (e.g., Maestre et al. 2009). Understanding how anthropogenic perturbations like biological invasions affect native organisms requires accounting for the full range of potential responses of native species from negative to neutral to positive (Ortega et al. 2013). Moreover, understanding the mechanics that underlie native species responses to invasions can help elucidate those factors structuring native communities.

Niche theory proposes that a species' presence or absence and relative abundance within a community is determined by how its functional traits interact with biotic and abiotic processes to determine its fundamental and realized niches (Hutchinson 1957, Macarthur and Pianka 1966, Weiher and Keddy 1999). This body of theory should be applicable for understanding native species responses to invasions and other anthropogenic perturbations. However, an important caveat is that invasions involve more "community reassembly" than complete community assembly (something more akin to secondary than primary succession). That is to say, the composition of native systems has already been determined by regional and local filters, so the introduction of an exotic organism creates a biotic perturbation that reorganizes native species within an already defined parameter space. Although some introduced organisms certainly alter abiotic conditions (Vitousek and Walker 1989, Sperry et al. 2006) thereby changing the fundamental niche space, much restructuring following plant invasions likely derives from biotic interactions affecting the realized niches of the natives. In either case, it should be possible to understand how native species respond to an invasion by evaluating how the invader's traits alter the system (biotically and/or abiotically) and how native species traits align or fail to align with these new conditions. While functional traits of invaders have been examined extensively in an effort to predict invader success (Pyšek and Richardson 2007), few studies have explored how functional traits of native species might help predict their responses to invasion in the context of how those traits fit with invader-driven change (Ortega et al. 2013).

Native web spiders represent an important guild of predators that are strongly influenced by exotic plant invasions (Pearson 2009, Petillon et al. 2010, Lau 2013, Schirmel and Buchholz 2013); an outcome that can have profound food web ramifications (Pearson 2010). These spiders exhibit a range of web building strategies that represent extended phenotypes or functional traits which are tightly linked to their ecological roles in native communities (Wise 1993, Cardoso et al. 2011). Because different web designs require specific substrate attributes, web spiders are sensitive to habitat modification (Wise 1993, Petillon et al. 2010, Schirmel and Buchholz 2013). Hence, web spider responses to plant invasions should be predictable, at least in part, as a function of how plant invasions alter web substrates in relation to specific web-building strategies.

In the intermountain grasslands of the western United States, invasions by the perennial forb spotted knapweed (*Centaura stoebe* L. formerly *C. maculosa*) have been linked to a dramatic reshuffling of native web spider communities. These invasions result in an approximate 20-fold increase in orb weaver densities and a near 80-fold increase in irregular web spider densities (Pearson 2009). These changes in native spider abundance have been attributed to a shift in plant architecture associated with the fact that *C. stoebe* and other invading forbs generate taller, more expansive, and far more abundant flowering stems than the native forbs commonly used by native spiders (Pearson 2009, Pearson et al. 2012). However, studies to date have only been observational, comparing spider populations between invaded and uninvaded grasslands. Because *C. stoebe* invasions greatly alter native plant, vertebrate, and invertebrate communities (Ortega and Pearson 2005, Ortega et al. 2006, Pearson and Fletcher 2008), all of which could affect native spider populations (Petillon et al. 2010, Lau 2013, Schirmel and Buchholz 2013), the specific mechanism by which *C. stoebe* invasion affects spider populations is not certain.

Here, we set out to disentangle the effects of *C. stoebe* invasion on native spider species in this system. In particular, we wished to determine whether linking the invader's traits with native species traits could explain the natives' responses to invasion. Our objectives were to (1) experimentally determine whether the invader's architecture caused native web spider population responses observed in natural invasions and (2) evaluate whether differences in web construction strategies could help explain the differences in population responses of native spider species to *C. stoebe* invasion. To accomplish this, we simulated large-scale *in situ* invasions by introducing only dead stems of *C. stoebe* into native grasslands in order to isolate the plant's architecture from other effects of invasion. We compared web construction, prey capture rates, reproduction, and population densities of spiders on these simulated invasion plots and adjacent control plots (no stems added), with initial spider densities on all plots standardized by removing native spiders and seeding plots with known spider densities. We predicted that spider population responses to the simulated invasion treatments would mimic those observed in natural invasions if invader architecture was the primary factor driving spider responses. We also predicted that population and demographic responses of different spider species to the treatments should link to differences in web construction strategies if web construction was the key trait determining native species responses to invasion.

Methods

STUDY SYSTEM

We conducted our research in the semi-arid, low-elevation grasslands of the Rocky Mountains in the Blackfoot Valley of western Montana, USA. These grasslands are dominated by one native bunchgrass (*Festuca capestrus* Rydb., formerly *F. scabrella*; Fig. 1.1A), with native forbs comprising much of the plant diversity. Forbs serve as the dominant web substrates for native spiders in this habitat. However, native forbs are highly ephemeral, flowering in the wetter months of May and June and senescing by mid-July, leaving little in terms of residual standing stems for most of the year. They also generate flowering stems that are shorter, less expansive, and less abundant than *C. stoebe* (and other invaders), thereby providing lower quality and less plentiful substrates for web-building spiders (Pearson 2009, Pearson et al. 2012; Fig. 1.1B and 1.1C). Web-building spider communities in this system are fairly simple, being comprised of a few species of cribellate or irregular web spiders (Family Dictynidae: *Dictyna major* Menge and *D. coloradensis* Chamberlin), orb weaving spiders (Family Araneidae: *Aculepeira packardi* Thorell; Family Tetragnathidae: *Tetragnatha laboriosa* Hentz), and funnel web weavers (Family Agelenidae), none of which are abundant (Jensen et al. 2005). Of these groups, *Dictyna* spp., *A. packardi*, and *T. laboriosa* were the most common in our study areas.

D. major and *D. coloradensis* dominate most web-building spider communities in western Montana grasslands (Pearson 2009). These two species are ecologically similar (Jackson 1978) and indistinguishable in the field (J. Slowik, University of Alaska Fairbanks, *pers. comm.*), thus we treat them as a species complex and refer to them as *Dictyna*. However, identification of specimens from our populations indicate about 95% of the spiders are *D. coloradensis*. *Dictyna* are small spiders (female mean total body length approximately 3 mm for *D. major* and 3.8 mm for *D. coloradensis*) that overwinter in the plant litter as sub-adults, emerging in April and May as the temperature warms (Chamberlin and Gertsch 1958, Wheeler et al. 1990). These spiders breed and begin to produce egg sacs (1-5) by the end of June through mid-July. Spiderlings emerge and disperse by ballooning in mid- to late-July. *Dictyna* prey mostly on small insects (e.g. Hymenoptera, Diptera), which they capture and retain in their webs (Chamberlin and Gertsch 1958).

The orb-weavers are the second most abundant group of web-building spiders in these grasslands, with *Aculepeira packardi* being the most abundant of this group. These are larger spiders (female mean total body length 10.77 ± 2.19 mm) (Dondale et al. 2003). Sub-adults overwinter in plant litter, emerging in spring as the weather warms. *A. packardi* construct large, orb webs by attaching their silk to multiple plants, suspending their web between plant substrates. Adults become sexually mature in early August, with females producing 1-3 egg sacs by late August into September (Levi 1977). Spiderlings emerge and immediately disperse from their mother's web via ballooning by mid-September. *Tetragnatha laboriosa* is the second most common orb-weaver present, albeit in much lower abundances. This species has a long, slender body (female mean total body length 6.17 ± 0.43 mm) and long legs (Dondale et al. 2003). Web construction and reproductive timing are similar to *A. packardi* (Levi 1981). Both orb-weavers have a broad spectrum of prey items (i.e. Orthoperta, Homoptera, Diptera, Coleoptera, and Hymenoptera), which can range in size by nearly two orders of magnitude (Levi 1981, Nentwig 1987, Dondale et al. 2003).

EXPERIMENTAL DESIGN AND SAMPLING METHODS

We simulated invasions by introducing dead *C. stoebe* stems into native, uninvaded, grasslands at three sites during early spring 2011. At each site two 0.25 ha (50 x 50 m) paired plots were established; one plot received the simulated invasion of 1250 dead *C. stoebe* stems with seed heads removed (to prevent invasion), the other plot served as a control with no stems introduced. *C. stoebe* stems were collected locally and set out in a grid of 25 rows spaced 2 m apart, with 50 stems placed 1 m apart in each row. Distance between control and treatment plots at each site was 25–50 m. Distance between sites averaged 10.3 km. These stem densities simulate a light invasion compared with heavily invaded areas where stem densities can average 320,000 stems/0.25 ha (Pearson et al. 2012).

In June 2011, all web spiders were removed from both treatment and control plots and each plot was seeded with 20 female *Dictyna* and 10 female *A. packardi*. Spiders were placed on native vegetation throughout each plot. These species were chosen as focal subjects because they represent the two distinct web building groups that show different response to *C. stoebe* invasion. Differences in seeding densities reflect relative abundances of these spiders in the native grasslands of western Montana (J. Smith, unpublished data). *T. laboriosa* was not experimentally added, but naturally colonized and so was quantified where possible.

All sites were sampled three times during the summers of 2011 and 2012 to determine demographic and population responses of each species and to identify potential mechanisms underlying community-level responses to invasion. During each sampling period, abundance of each species was censused by walking 50 transects per plot (spaced 2 m apart) and counting all occupied webs. Additionally, web area (for occupied webs), the number and size of whole prey items (measured to the nearest 0.005 mm), and number of spiderlings in each web were also recorded. The length and width of each web were measured to the nearest 0.5 cm (Pearson 2009). These measurements were used to calculate web area based on the geometry of a triangle (Jackson 1978). The abundance of available prey was assessed on treatment and control plots in mid-July 2012 by sweep net sampling 10 50 m transects 0.5 m above the ground on each plot (Tingle 2002). Abundance of available prey was calculated by counting the number of insects in each sample that were less than 6 mm and that had been previously observed being caught and consumed in webs.

STATISTICAL ANALYSES

The change in abundance of spiders from initial seeding densities ($N_{t+1} - N_t$) in response to simulated invasion treatment (simulated invasion vs. control) were analyzed using MANOVA in R (Venables and Ripley 2002, Fox and Weisberg 2011, R Core Team 2013). This analysis allowed us to address the potential interdependence of the species abundance responses with invasion treatment as a fixed factor. We analyzed abundances at two biologically relevant time periods: (1) final sampling (August 2012 abundance – June 2011 abundance), and (2) overwintering (June 2012 abundance – August 2011 abundance). Analysis of overwintering survival was only conducted for *Dictyna* due to logistical issues that prevented sampling fall *Aculepeira* populations in 2011 after recruitment. This was done using a linear mixed-effects model (LMM) in R (package nlme) with treatment as a fixed effect and site as a random blocking factor (Pinheiro et al. 2011, R Core Team 2013). The effect of invasion treatment on average web area; likelihood of capturing prey; number of prey captured per web; and number of spiderlings per female, were analyzed with a LMM using PROC GLIMMIX in SAS, with treatment and year as fixed effects, and site as a random blocking factor (SAS 2009). To meet assumptions of normality and equal variance, web area was analyzed using a lognormal distribution and all other metrics were analyzed using the negative binomial distribution. Mean number of juveniles per female ($= \frac{\text{\# juveniles September 2012}}{\text{\# reproducing females June 2012}}$) was analyzed using a LMM in R (package nlme) with treatment as a fixed effect and site as a random blocking factor (Pinheiro et al. 2011, R Core Team 2013). We used the same mixed model structure in PROC GLIMMIX in SAS to investigate the probabilities of capturing prey and capturing large prey (≥ 6 mm) using logistic regression with binomial distributions (SAS 2009). Available prey was analyzed using LMM in R (package nlme) with treatment as a fixed effect and site as a random blocking factor (Pinheiro et al. 2011, R Core Team 2013).

Results

Simulated invasion plots supported significantly higher abundances of web-building spiders compared to controls (overall MANOVA: $F_{3,2} = 426.69$, $P = 0.002$; Fig. 1.2). *Dictyna* populations increased dramatically in response to the simulated invasion ($F_{1,4} = 13.76$, $P = 0.021$), while *Aculepeira* showed a marginally significant increase ($F_{1,4} = 4.87$, $P = 0.092$), and *Tetragnatha* showed no difference in abundance ($F_{1,4} = 1.00$, $P = 0.374$).

Spider species responses to the simulated invasion appeared to be related to web building strategy. *Dictyna* constructed significantly larger webs on invaded versus control plots (Table 1.1, Fig. 1.3A). Although *Dictyna* webs were larger in 2012 compared to 2011, the pattern of larger webs on simulated invasion plots held in both years (Table 1.1). *Aculepeira* web size did not differ by invasion treatment, year, or their interaction (Table 1.1, Fig. 1.3B). Because webs of these species do not persist across sampling periods and since web area did not differ within years between sampling periods (2011: *Dictyna* $t_{153} = 1.355$, $P = 0.178$; *Aculepeira* $t_{28} = 0.953$, $P = 0.349$) web area was pooled across sampling periods within years for the previous analysis.

The larger *Dictyna* webs on treatment plots were significantly more likely to capture prey (Table 1.1, Fig. 1.3C). The likelihood of capturing prey was marginally significantly higher in 2011 compared to 2012, but the pattern of *Dictyna* being more likely to capture prey on simulated invasion plots held in both years (Table 1.1, Fig. 1.3C). Mean number of prey captured in *Dictyna* webs trended towards being greater on simulated invasion plots compared to control plots, and although *Dictyna* captured significantly more prey in 2011 compared to 2012, there was no interaction between treatment and year (Table 1.1, Fig. 1.3E). The likelihood of *Aculepeira* capturing prey was not different between control and treatment plots (though there was a trend toward higher captures on the control plots), between years, or treatment by year (Table 1.1, Fig. 1.3D). There was no difference in the mean number of prey captured per web by treatment, year, or their interaction (Table 1.1, Fig. 1.3F) for *Aculepeira*. *Dictyna* on treatment plots were not significantly more likely to capture large prey (≥ 6 mm), there were no differences between years, nor was there an interaction between treatment and year (Table 1.1, Fig. 1.3G). Similarly for *Aculepeira* there were no differences in the proportion of individuals capturing large prey by treatment, year, or their interaction (Table 1.1, Fig. 1.3H). Abundance of available prey did not differ between control and treatment plots ($t_{56} = -1.362$, $P = 0.179$).

Overwintering survival did not differ for *Dictyna* on treatment versus control plots (linear mixed-effects model [LMM]; $t_2 = -0.124$, $P = 0.913$). In 2011, *Dictyna* webs on treatment plots tended to have more spiderlings compared with on control plots (Table 1.1, Fig. 1.4A). The mean number of juveniles per female was higher on treatment compared to control plots for *Dictyna*, but not for *Aculepeira* (Table 1.1, Fig. 1.4B). *T. laboriosa* only colonized simulated invasion plots, so formal statistical analyses were not possible for web size, prey capture rates, and recruitment.

Discussion

Exotic species invasions restructure recipient communities around the world, yet we seldom understand the mechanisms driving native species responses to invasions. Here, we show that native web spider populations can be facilitated by *C. stoebe* invasions due to interactions between key invader attributes and specific functional traits of the native species. By introducing only dead stems of *C. stoebe* into native grasslands, we caused dramatic increases in native web spider populations that paralleled those documented for natural invasions (Pearson 2009). This result suggests that *C. stoebe*'s architecture is the primary trait causing native web spider responses to invasion. We also show that the mechanism underlying the species-specific responses to simulated invasion derived from differences in web-building behavior, in particular as they relate to web substrate constraints in the native system. Stronger spider population responses to invasion were associated with increased web size, higher prey captures, elevated recruitment, and greater release from substrate constraints. As we show below, examining in detail how these web-building behaviors relate to demographic and population responses of native spiders to invasion helps to elucidate not only spider responses to plant invasion, but also key factors constraining and structuring the native web-spider community.

One of the greatest barriers to understanding biological invasions is the challenge of experimentally manipulating invasions at appropriate spatial scales. Large-scale, *in situ* experimental examinations of invasions are rare due to logistical and ethical constraints, but such studies are necessary to fully understand outcomes of species introductions (e.g., Pearson and Fletcher 2008). How effective was our simulated invasion? The *C. stoebe* stem densities we used were two orders of magnitude lower than the densities *C. stoebe* can achieve through natural invasion (Pearson et al. 2012), so our experiment simulated earlier stages of invasion. Nevertheless, even at low stem densities, responses of the different spider species to simulated invasion mimicked results observed following natural *C. stoebe* invasions (Pearson 2009). The irregular web spiders, *Dictyna,* showed strong positive responses to simulated invasion. Final *Dictyna* densities were 841 spiders/0.25 ha on simulated invasion plots versus 27 spiders/0.25 ha on control plots (31 times higher densities on treatment). The orb weaving spiders, *Aculepeira,* showed weaker positive population responses following simulated invasion compared to irregular web spiders. *Aculepeira* densities were 31 spiders/0.25 ha on simulated invasion grids versus 1 spider/0.25 ha on control grids (23 times higher densities on treatment) and *Tetragnatha* naturally colonized only simulated invasion grids. Overall, spider population responses suggested that initial seeding densities were reasonable and that the study duration was sufficient to capture equilibrium responses for *Aculepeira* on both treatment and control plots and for *Dictyna* on controls. On control grids, *Dictyna* densities fluctuated but remained fairly stable, ending at levels slightly higher than seeding densities. This result suggests that initial seeding densities approximated native carrying capacity for *Dictyna*. In contrast, *Aculepeira* declined rapidly on control plots to stabilize at very low densities, suggesting that initial seeding densities were likely high despite attempts to adjust for the lower natural densities of this species when seeding the plots. On experimentally invaded plots, the populations of both species increased substantially, but differed in that *Aculepeira* densities appeared to stabilize by the end of the experiment while *Dictyna* was still increasing (Fig. 1.2). This result suggests that at the termination of the experiment *Aculepeira* densities approximated their maximal response to the treatment, while *Dictyna* populations were still increasing, despite their dramatic response over the first two years.

Identifying the key invader attributes restructuring the recipient community is a prerequisite to linking native species traits to their responses to invasion. Of course, key invader traits will differ as a function of the native species being considered. Invasion by *C. stoebe* affects many community attributes, including plant diversity and productivity, invertebrate abundance and composition, and abundance of birds and small mammals; all of which could affect spider populations (Ridenour and Callaway 2001, Ortega and Pearson 2005, Ortega et al. 2006, Maron and Marler 2008*a*, Maron and Marler 2008*b*, Pearson and Fletcher 2008). However, by experimentally isolating plant architecture from all other aspects of invasion, we established that changes in architecture alone are sufficient to drive the native web spider population increases observed following *C. stoebe* invasions. This outcome confirms that *C.*

stoebe affects native spider populations by serving as an invasive ecosystem engineer in this system (Pearson 2010). In particular, *C. stoebe* invasion in intermountain grasslands alters two important aspects of vegetation architecture that reflect important attributes of spider habitat – substrate quantity and quality. Increased substrate quantity is likely the primary driver of the dramatic increases in web-spider abundance or at least the necessary first step, given the severity of substrate constraints in this system (Pearson 2009, Pearson et al. 2012). Increasing substrate availability for sessile organisms with high reproductive outputs commonly results in population increases due to release from substrate limitation (see Poulsen et al. 2007). Not surprisingly then, other invasive ecosystem engineers like zebra mussels (*Dreissena* spp.) have been shown to dramatically increase the abundance and richness of native species by reducing substrate limitation (Bially and Macisaac 2000). However, within our system, the higher quality (e.g., larger, more expansive, and more persistent architecture; Fig. 1.1B) of *C. stoebe* substrates also allowed *Dictyna* to build 4.4 times larger webs that captured 5.0 times more prey and more than doubled their chances of reproduction, suggesting substrate quality may also feed into this process. *Aculepeira* showed no indication of change in web size, prey captures, or reproductive output on simulated invasion plots, indicating that any increase in their abundance was due solely to increased substrate quantity releasing them from substrate limitation.

An important question is, why do these spider species respond so differently to invasion, and what can such invasions teach us about native community structuring? The substantial differences in the strength of the responses of *Dictyna* compared to *Aculepeira* following release from substrate limitation appeared to be driven by differences in their web building behaviors relative to substrate constraints present in their natural system. Because *Dictyna* construct their webs entirely within individual plants and most native plant species in our system provide diminutive substrates for this species, web building in these irregular web spiders is severely constrained and every new *C. stoebe* stem offers a functionally viable web substrate. This phenomenon may extend to other spiders in this system that use individual plant substrates as well. However, orb weavers like *Aculepeira*, *Tetragnatha*, and others that use multiple plants to suspend their webs are unconstrained by individual plant characteristics, and thus are much less constrained by the quality of native plant architectures.

These findings shed light on the factors determining native spider community structuring in this system. Although spiders can be food- (Miyashita 1991, Wise 1993, Kreiter and Wise 2001, Moya-Larano et al. 2003, Wise 2006, Lau 2013) or substrate-limited (McNett and Rypstra 2000, Bruggisser et al. 2012, Cunha et al. 2012), in this system substrate limitation appears to be severe and may be the primary factor limiting native web spider diversity in these species poor grasslands. Native spiders appear to produce more offspring than can find suitable substrates for establishment. Hence, increases in substrate quantity can have very powerful and immediate effects on the spider community. However, our results also show that species- or guild-specific web construction traits relative to substrate attributes have very important ramifications for how spider communities are ultimately structured. For the irregular web spiders like *Dictyna* and other species exhibiting plasticity in web construction, changes in substrate quality could also increase reproduction by releasing spiders from food limitation. Orb weavers in contrast are commonly more fixed in their web construction behaviors (Vollrath 1992, Krink and Vollrath 1998, Hesselberg 2010) and may be less capable of exploiting this aspect of change in vegetation architecture.

Our work suggests that linking functional traits of native species to invader traits (or invader-driven changes in the recipient community) can improve understandings of community reassembly following invasion. Retrospective studies like ours applied to other invaders may help to identify the mechanisms underlying invasion outcomes. Moreover, applying retrospective understandings and general niche theory to new systems may prove effective for predicting invasion outcomes. For example, exotic forb invasions into grasslands in central Argentina also appear to increase the complexity of plant architecture and local densities of native web spiders (D.E. Pearson pers. obs.). Mechanistic understandings like those we have developed here could be applied to this system and others to test predictions for native web spider responses as well as the responses of their prey. This approach could move invasion ecology to a new level for predicting impacts within native communities as well as advancing community ecology.

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Tables

Table 1.1. Results from linear mixed models testing the effects of simulated invasion treatment and year on web area, proportion capturing prey, number of prey per web, proportion capturing large prey, number of spiderlings per female, and number of juveniles per female.

		Treatment effect			Year effect			Treatment x Year		
Species	Spider species responses	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Dictyna	Web area	1, 4	57.23	**0.002**	1, 4	21.40	**0.010**	1, 4	0.34	0.591
	Proportion capturing prey	1, 4	9.73	**0.036**	1, 4	6.17	0.068	1, 4	0.03	0.877
	# Prey/web	1, 4	4.51	0.101	1, 4	10.07	**0.034**	1, 4	0.07	0.811
	Proportion capturing large prey	1, 4	0.00	0.969	1, 4	0.00	0.974	1, 4	0.00	0.975
	# Spiderlings/female	1, 4	4.27	0.108						
	# Juveniles/female	1, 2	62.96	**0.016**						
Aculepeira	Web area	1, 4	0.39	0.579	1, 4	0.28	0.632	1, 4	1.76	0.276
	Proportion capturing prey	1, 4	7.75	0.069	1, 4	3.88	0.143	1, 4	3.12	0.176
	# Prey/web	1, 4	1.82	0.270	1, 4	1.25	0.345	1, 4	1.35	0.329
	Proportion capturing large prey	1, 4	2.02	0.250	1, 4	0.55	0.512	1, 4	1.01	0.389
	# Spiderlings/female									
	# Juveniles/female	1, 2	1.64	0.329						

Figure Legends

Figure 1.1. Plant architecture changes following *C. stoebe* invasion in Montana. (A) Native, uninvaded grasslands dominated by bunchgrasses; (B) grasslands invaded by *Centaurea stoebe*; and (C) native spiders building webs on *C. stoebe* plant architecture.

Figure 1.2. Spider density (mean ± 1 SE) on plots where *C. stoebe* stems were introduced (simulated invasion) compared to control plots for three dominant grassland spiders over two-years (2011-2012). Overall, spider density was significantly higher on treatment plots compared to control plots. Populations differed significant between treatments only for *Dictyna* (see *Results*).

Figure 1.3. Responses of *Dictyna* (left) and *Aculepeira* (right) spiders to simulated *C. stoebe* invasion in terms of web area (mean + 1 SE, raw data; A, B), proportion of females capturing prey (mean + 1 SE; C, D); number of prey captured per web (mean + 1 SE; E, F), and the proportion capturing large prey (mean + 1 SE; G, H). *Dictyna* on simulated invasion plot constructed larger webs that increased their likelihood of capturing prey compared to web on control plots. We observed no differences in any of the investigated metrics in *Aculepeira* between simulated invasion and control plots (see *Results*).

Figure 1.4. (A) Mean number of spiderlings per female (mean + 1 SE) and mean number of juveniles per female (mean + 1 SE, raw data) for *Dictyna* and *Aculepeira*. There were significantly more *Dictyna* juveniles present and spiderlings trended toward being significantly higher on simulated invasion plots compared to control plots. We saw no differences in reproduction by treatment for *Aculepeira* (see *Results*).

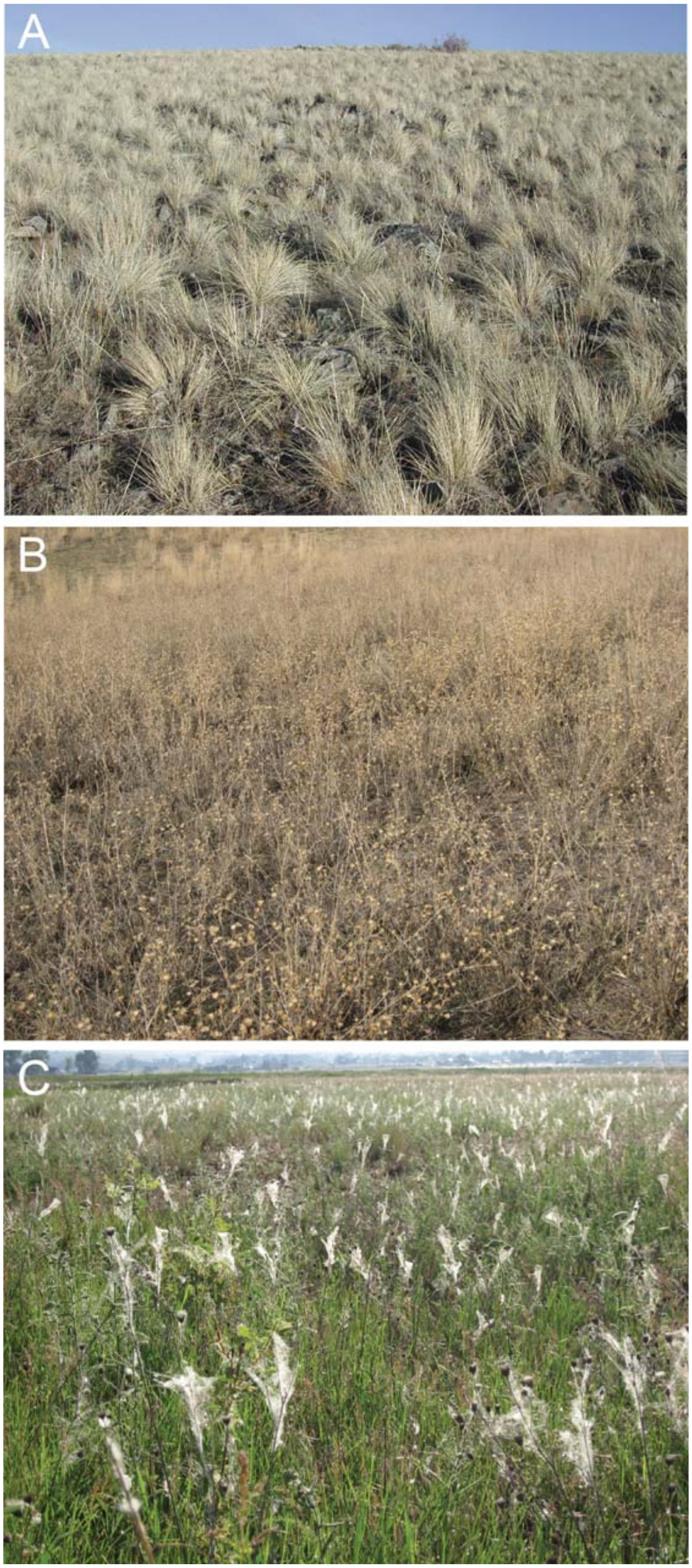

Figure 1.1

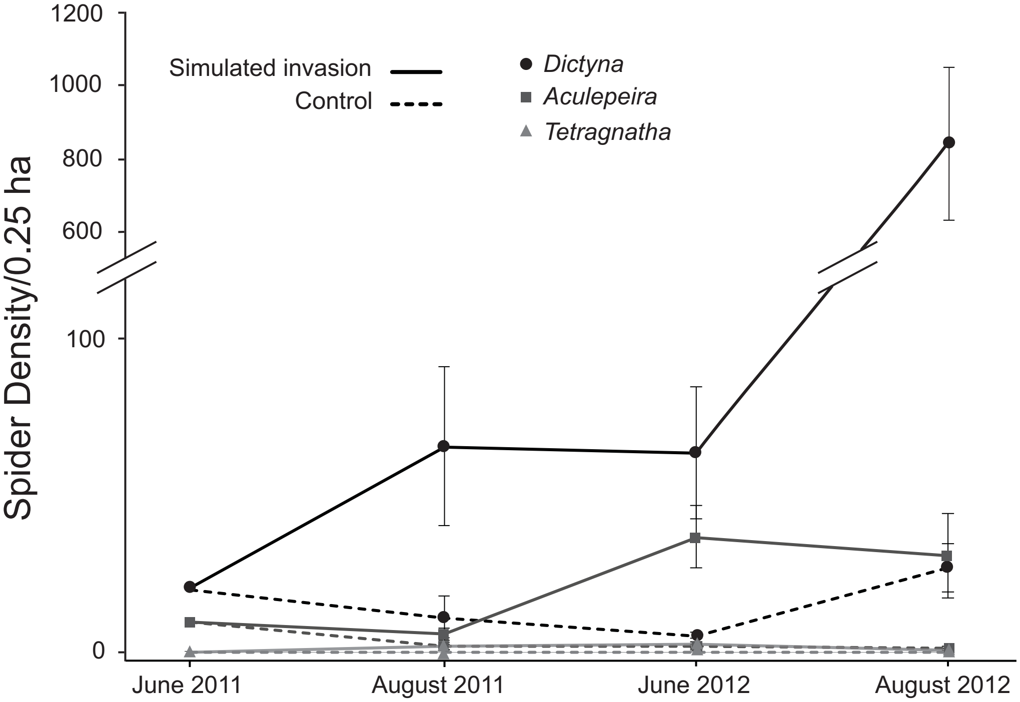
1200
1000
800
600
100
0
Spider Density/0.25 ha
Simulated invasion
Control
Dictyna
Aculepeira
Tetragnatha
June 2011
August 2011
June 2012
August 2012

Figure 1.2

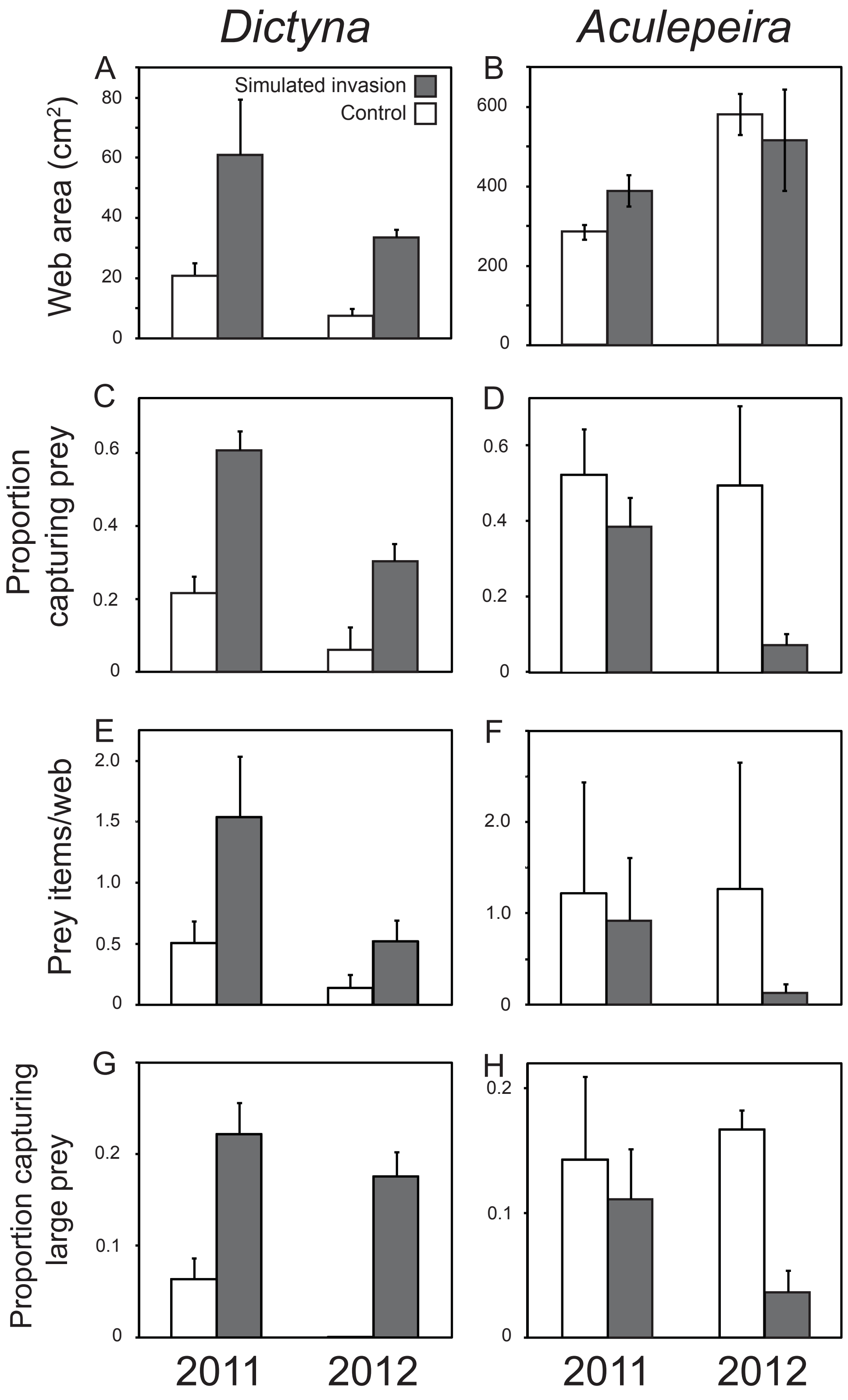
Dictyna
Aculepeira
A
B
C
D
E
F
G
H
Simulated invasion
Control
Web area (cm²)
Proportion capturing prey
Prey items/web
Proportion capturing large prey
0
20
40
60
80
200
400
600
0.2
0.4
0.6
0.5
1.0
1.5
2.0
0.1
2011
2012

Figure 1.3

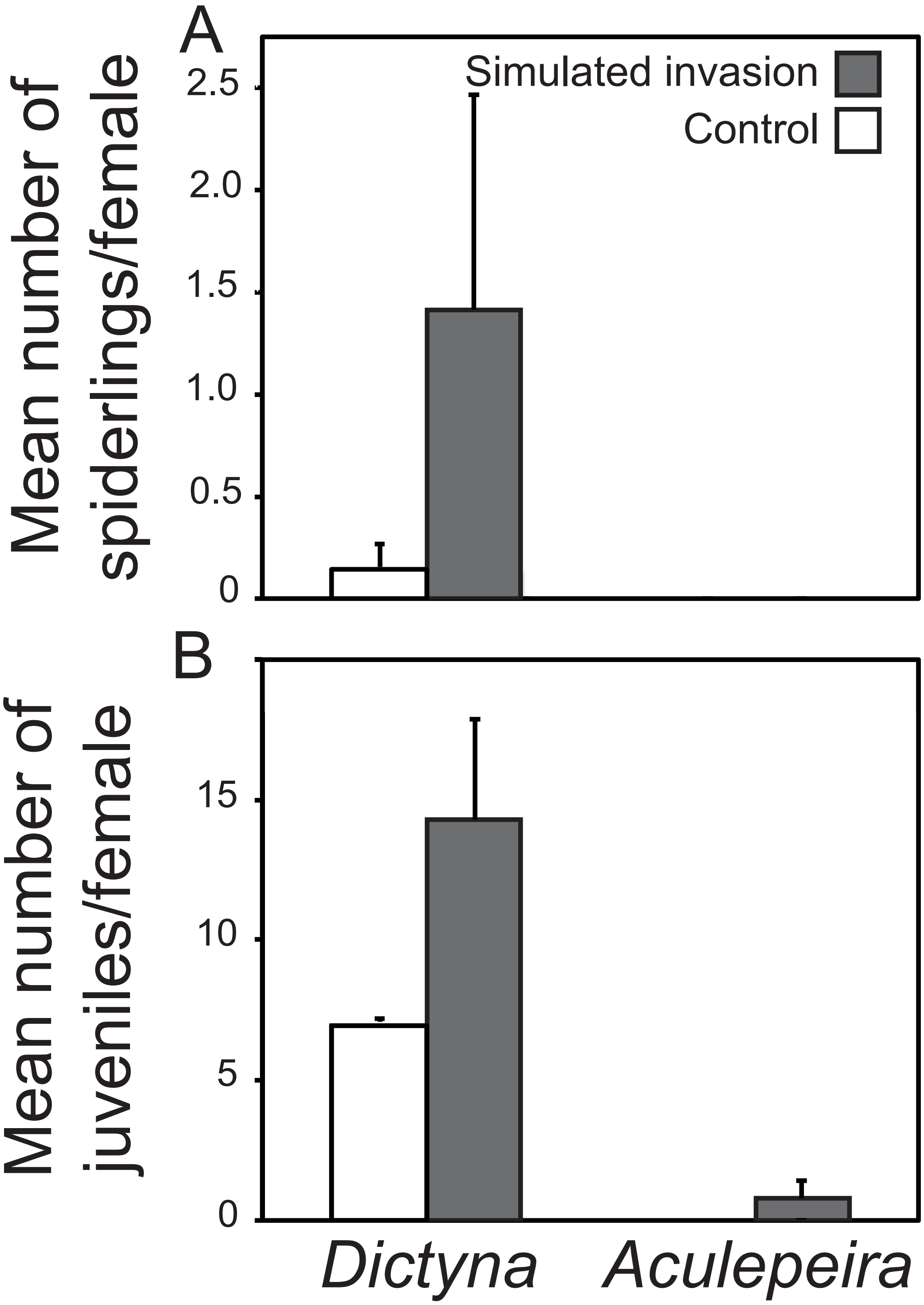
A
Mean number of spiderlings/female
2.5
2.0
1.5
1.0
0.5
0
Simulated invasion
Control
B
Mean number of juveniles/female
15
10
5
0
Dictyna
Aculepeira

Figure 1.4

Chapter 2:

Phenotypic plasticity benefits native spiders in early but not late stages of knapweed invasion

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Abstract: Theory predicts that phenotypic plasticity should play an important role in determining initial responses of native species to rapid environmental changes such as those arising from biological invasions. However, few empirical studies have examined this question and little is known about how plasticity and its relationship to fitness may change for natives over the course of invasion. We examined plasticity and its link to fitness for two native web spider species (*Dictyna coloradensis* and *Aculepeira packardi*) that construct webs on plant substrates in the context of early and late stages of spotted knapweed (*Centaurea stoebe*) invasion. We found that *Dictyna* exhibited substantial plasticity in web size with larger webs linked to higher prey captures and increased reproduction, all leading to strong population increases following invasion. In contrast, *Aculepeira* demonstrated no plasticity in web size and no associated fitness benefits or population increases following invasion. We also found that the fitness benefits associated with web size plasticity found in *Dictyna* populations from early stages of invasion were not evident in populations from later stages of invasion. Moreover, plasticity in web size was significantly reduced in *Dictyna* populations from later stages of invasion, suggesting selection for a reduction in web size plasticity. Simulated invasion and microcosm experiments suggest that the elevated spider densities associated with later stages of invasion may increase competition for food resources, thereby negating the fitness gains normally arising from larger web sizes. This study demonstrates the important but dynamic role that phenotypic plasticity plays in determining the short and long-term responses of native species following invasion.

Key words: *Centaurea stoebe*, facilitation, fitness, invasion, phenotypic plasticity, spiders, web size

Introduction

The earth is experiencing rapid and dramatic environmental changes ranging from climate change to nitrification, land transformation, and biological invasions. These anthropogenic perturbations disrupt natural communities and the ecosystem services they provide. Understanding how anthropogenic disturbances like biological invasions affect native systems requires knowledge of those factors that allow native species to survive the initial invasion onslaught and persist in the post-invasion world. Given the rapid and dramatic changes occurring within recipient communities following biological invasions, plasticity should be an important factor determining native species responses. Studies show higher levels of phenotypic change in anthropogenic contexts compared to natural contexts and that these changes can happen rapidly, both of which support the role of phenotypic plasticity driving species responses (Hendry et al. 2008). Because of this we can predict that high levels of trait plasticity should predispose certain individuals, populations, or species to be better able to cope with the dramatic environmental changes associated with invasion, potentially increasing persistence and even providing a facultative effect following invasion. Furthermore, plasticity has the potential to be adaptive if it places a phenotype close enough to a new phenotypic optimum (Price et al. 2003, Ghalambor et al. 2007). Thus, both the immediate persistence and that of future generations of native species should be highly influenced by the expression and degree of phenotypically plastic traits. While plasticity has been shown to facilitate an exotic organism’s ability to become invasive (Daehler 2003, Richards et al. 2006, Richardson and Pysek 2006, but see Godoy et al. 2011), the role of plasticity in determining native species responses to exotic invaders remains underexplored (Leclaire and Brandl 1994, Solarz and Newman 2001, Berg and Ellers 2010, Garcia-Robledo and Horvitz 2011, 2012).

Biological invasions are powerful phenomena that have been shown to radically alter selective pressures on native species (e.g., Phillips and Shine 2004, Carroll et al. 2005, Sax et al. 2007). But additionally, they are very dynamic processes that change extensively over time. Invasion theory recognizes that invasions transition through stages, and that the prominence of different invader traits commonly changes with each stage of invasion (Catford et al. 2009, Blackburn et al. 2011). For example, selection may favor traits that benefit invasiveness along an invasion front, but act on other traits such as intraspecific competitiveness once the invader has saturated the environment (Phillips and Shine 2006). Under these saturated conditions, one can likely expect abiotic (i.e. microclimate, nutrients) and/or biotic conditions (i.e. abundance, species richness or diversity) or their interactions to drastically change, which likely alters selection pressures on and fitness responses of native species adapting to invasion. However, such changes are not immediate causing selection pressures on native species to additionally change from early to late stages of invasion, in essence creating a moving target. Phenotypic plasticity should promote survival and persistence of native species in the face of new environmental conditions arising from invasions (Ghalambor et al. 2007), however the benefits associated with plasticity as well as the benefits associated with specific traits may change from early to late stages of invasion. Little is known about how changes over the course of invasion might influence native species success or the role of plasticity in this context.

The introduction of invasive plants can dramatically transform the plant architecture of the areas where they invade, which can then dramatically affect native species inhabiting invaded areas (Cheruvelil et al. 2002, Valley and Bremigan 2002, Maerz et al. 2005, Petillon et al. 2006, Pearson 2009, Mattingly and Flory 2011, Pearson et al. 2012). In western Montana, invasion by spotted knapweed (*Centaurea stoebe*) changes vegetation architecture, positively effecting native web-building spiders (Pearson 2009, Smith Chapter 1). In particular, species that construct irregular webs benefit far more than those that construct orb webs (Pearson 2009; Smith Chapter 1). Irregular web spiders in the genus *Dictyna* appear to benefit from knapweed, in part, because they plastically take advantage of the taller, broader, and more persistent stems of knapweed (compared to native plants; Pearson 2009) to build larger webs than they can on native forbs. Larger web sizes allow these spiders to capture more prey, leading to increased fitness (Pearson 2009, 2010; Smith Chapter 1). In contrast, orb weaving spiders such as *Aculepeira packardi* exploit knapweed for web substrates, but are unable to change their web size compared to what they build on native vegetation, and thus do not increase prey captures or fitness (Smith Chapter 1). Hence, the different response of these spiders to invasion appears linked to plasticity in web construction. Additionally, knapweed invasion substantially changes the environment as it outcompetes native vegetation (Pearson 2012), potentially changing the selective environment for spiders and the fitness benefits of plasticity in later stages of invasion.

Here, we examine whether interspecific differences in web size plasticity translates to differences among spider species in numerical responses to invasion. We also evaluated how plasticity and its relationship to fitness changed between knapweed-naïve spider populations (representing early stages of invasion) and spider populations from areas of long-standing invasions to understand how changing conditions driven by invasion may affect fitness and the selection environment.

Methods

Study system

Our research took place in the semi-arid, low-elevation grasslands of the Blackfoot, Bitterroot, and Missoula Valleys of western Montana, USA. These native grasslands are dominated (numerically and commonly in biomass) by the bunchgrasses *Festuca campestris* or *Agropyron spicata*, but native forbs make up most of the plant species richness (Mueggler and Stewart 1980). Taller native forbs like yarrow (*Achillea millefolium*) provide the primary web substrates for native spiders. Exotic forbs such as spotted knapweed (*Centaurea stoebe*) are aggressively invading these grasslands and changing the vegetation architecture (Pearson et al. 2012). The invading forbs generate flowering stems that are taller, more expansive, more persistent, and far more numerous than native forbs, thereby providing more abundant and higher quality substrates for web building spiders compared to native flora (Pearson 2009). Native web building spider communities in these grasslands are comprised of a handful of species that are dominated by irregular web spiders (primarily *Dictyna coloradensis*) and orb weavers (primarily *Aculepeira packardi*). Populations of both of these groups increase in response to exotic plant invasions, but irregular web spiders increase far more than orb weavers (Pearson 2009, Smith Chapter 1), a response that may be related to plasticity in web construction.

Web size plasticity between species in response to initial invasion

To evaluate the relationship between web size plasticity and species responses to invasion, we choose one representative species from both the irregular web spiders (*Dictyna coloradensis*) and orb weaving spiders (*Aculepeira packardi*). Experiments were conducted in the field to compare web size plasticity between these two species by introducing naïve spiders (spiders from areas uninvaded by knapweed) onto plots (0.25 ha) in uninvaded native grasslands containing experimentally introduced knapweed stems (1250 stems laid out in transects; see Smith Chapter

1) and paired control plots with no knapweed stems. Paired plots were located ~50 m apart and replicated at three sites across the Blackfoot Valley. Spiders were classified as using native substrates if the web was only attached to native plants, whereas webs were classified as using knapweed substrates if any part of a web was attached to a knapweed stem. Web sizes of adult females for both species were sampled in June, July, and August of 2011 and 2012 (see Smith Chapter 1). Web size measurements allowed us to construct reaction norms for each species to test for species differences in web size plasticity in response to knapweed invasion. By looking at web size plasticity in naïve spiders, we can infer that observed reaction norms reflect plastic responses and likely represents how these species would respond in the early stages of invasion. These spiders from native uninvaded grasslands that are naïve to knapweed will be referred to as early/native for the rest of the paper.

Web plasticity within species for populations from early/native and late stage invasions

We focused on *Dictyna* to examine whether plasticity changed between early/native and late stages of invasion because it was the only species exhibiting plasticity in web size. We explored this question by introducing *Dictyna* from native uninvaded (representing responses in the early stages of invasion) and knapweed-invaded (late stages of invasion) grassland populations onto native or knapweed sized substrates and observing the web sizes constructed. This method allowed us to construct reaction norms for population (early/native vs. late stages) by substrate type (native vs. knapweed) as a test for differences in plasticity between early/native- and late-stage invasion populations.

Estimates of web size plasticity for *Dictyna* populations from early/native stages of invasion were derived from the field experiment described above where naïve spiders were introduced onto plots where knapweed stems had been introduced or paired plots containing only native substrates (Smith Chapter 1). All web measurements from the invaded plots came from knapweed substrates, while all measurements from the control plots came only from native substrates.

Data for estimating web-size plasticity for *Dictyna* populations associated with late stages of knapweed invasion came from a microcosm study designed to examine the indirect effects of substrate density versus substrate size on *Dictyna* prey (see Pearson 2010). Microcosm plots were located at Deittert Experimental Gardens, The University of Montana campus, Missoula, MT. Each microcosm contained knapweed stems at two knapweed densities (one or 16 stems) that were trimmed to represent native or knapweed substrates (in a 2 x 2 full factorial design). All stems were cut down to 50 cm tall (stem height does not affect prey captures, Pearson 2009). Stems emulating native substrates were trimmed to 4 cm wide to simulate *Dictyna*'s primary native substrate, yarrow. Stems representing knapweed-sized substrates were trimmed to 20 cm wide to accommodate multiple stems in a microcosm. This size still produces an oversized substrate for *Dictyna* webs. These substrate sizes generate web sizes indistinguishable from those of field populations for each substrate type (Pearson 2010). Moreover, in the field experiment above testing naïve spiders, yarrow was the primary native substrate and web sizes on other substrates did not differ significantly from web sizes on yarrow ($t = 0.035$, $P = 0.972$). Hence, the model substrates in the microcosm experiment emulated natural web substrates (see also Pearson 2010). *Dictyna* were collected from 8-10 heavily invaded long-term knapweed populations near Missoula, MT, introduced to the microcosms at one spider per substrate (i.e., one or 16), and allowed to build webs for eight days before being measured.

Plasticity links to fitness

To explore the adaptive benefits associated with plasticity of web size between early/native and late stages of invasion, we examined the relationships between *Dictyna* web size, prey capture rates, and reproduction or recruitment under two scenarios. First, to quantify these patterns at different stages of invasion, we compared *Dictyna* populations between three native grasslands and seven late-stage knapweed invaded grasslands during summer 2010. Second, to try and isolate the causes for differences in the relationships observed in native and late-stage knapweed invasions, we evaluated similar metrics in the simulated invasion experiment described above (Smith Chapter 1). This analysis allows us to compare responses between individuals on native uninvaded plots and individuals on simulated invasion plots representing early stages of invasion (Smith Chapter 1). This experiment controlled for many factors associated with invasion that might alter the fitness benefits of increased web size such as prey communities, abiotic factors, vegetation community, etc. Additionally, using spiders naïve to knapweed controlled for any evolutionary effects that may fix spider behaviors. The experiment controlled for all factors except spider density and web substrate type. Occupied webs in both studies were measured as described above and the number of prey per web counted to estimate prey capture rates (following Pearson 2009). Fitness metrics differed between studies with observational studies looking at the presence/absence of egg sac(s) and experimental studies looking at recruitment by observing the number of juveniles per reproductive female (Smith Chapter 1).

As a further evaluation of how spider density might influence prey capture rates and fitness we examined data from the microcosm experiment outlined above (Pearson 2010). In this study, twenty prey items, gall flies (*Urophora affinis*), were added to each microcosm at the start of the experiment, with 20 more added every 2 days, so that prey densities remained relatively high over the course of the experiment. Webs were observed daily to count new prey captures and daily prey captures were summed to calculate total prey captures per web over the course of the experiment. At the end of the 8-day experiment the presence of egg sacs was noted as a fitness index.

Statistical analyses

We conducted all statistical analyses using R version 3.0.1 (R Core Team 2013), except where otherwise noted. We transformed data to correct for non-normal distributions or unequal variances as noted below. We used the ‘reaction norm’ approach to examine the phenotypic responses of web size across substrates (Via et al. 1995, Pigliucci 2001, West-Eberhard 2003, DeWitt and Scheiner 2004). Assuming linear changes, the reaction norm is usually represented by the regression line of the plot of trait expression against environment. The slope of the reaction norm of the trait is a measurement of the degree of phenotypic plasticity and can be used to compare plasticity between study units, in our case species and populations (Schlichting and Pigliucci 1998). We were interested in the significance of the interaction between study units and substrate type, as a significant interaction indicates that the study units respond differently to substrate type (i.e. show differences in their plasticity). Differences in plasticity between study units may be qualitative [i.e. null plasticity (flat reaction norm) vs. actual plasticity)] or quantitative (i.e. reaction norms of different slope), so *post hoc* tests were used to evaluate the nature of plasticity differences.

To examine if web size plasticity differed between *Dictyna* and *Aculepeira* we conducted a linear mixed-effects model (LMM, package lme4) on log-transformed web size with species

(*Dictyna*, *Aculepeira*) and substrate type (native, knapweed) as fixed factors; and site ($N = 3$) and year (2011, 2012) as random blocking factors. To test for an interaction between fixed factors (species x substrate type) we compared the full model to a null model lacking the interaction using maximum likelihood, followed by parametric bootstrapping of the likelihood ratio statistic to improve our significance estimate (Faraway 2006). If the interaction was significant, differences in web size plasticity between species were evaluated by *post hoc* Tukey's HSD test ($P < 0.05$, package multcomp) of log-transformed web areas within species across substrate types (Hothorn et al. 2008).

To examine if web size plasticity differed between *Dictyna* populations from early/native and late stages of knapweed invasion we conducted a LMM on log-transformed web size with invasion stage (early/native, late) and substrate type (native, knapweed) as fixed factors and study (Pearson 2010, Smith Chapter 1) as a random blocking factor (Bates et al. 2013). 'Study' was included as a random factor to account for any differences between the two studies where the data was obtained. We found no effect of substrate density on web size ($\chi^2 \approx 0$, $P > 0.05$) in the microcosm study, so data were pooled within each substrate type. We were again interested in the significance of the interaction between the fixed factors (invasion stage x substrate type). To test for the significance of the interaction we compared the full model to a null model lacking the interaction using maximum likelihood, followed by parametric bootstrapping of the likelihood ratio statistic to improve our significance estimate (Faraway 2006). If the interaction was significant, differences in web size plasticity between populations from early/native and late stages of invasion were evaluated by *post hoc* Tukey's HSD test ($P < 0.05$) of log-transformed web size within invasion stage across substrate types (Hothorn et al. 2008). Estimates of the differences in web size between substrate types within invasion stage allow us to quantify the degree of plasticity for direct comparison.

Generalized linear mixed-effects models (GLMM) in SAS (PROC GLIMMIX) were used to examine the effect of *Dictyna* web size on the number of prey captured per web and the probability of reproducing, with invasion stage (native, late-stage) as a covariate and site ($N = 9$) as a random blocking factor, using Poisson and binomial distributions, respectively, for the observational data (SAS 2009). The effect of invasion stage (native, late-stage) on web size in *Aculepeira* was analyzed using a one-way ANOVA in R. To meet assumptions of normality and equal variance, web area was analyzed using a lognormal distribution. For the experimental data, the effect of simulated invasion on web size was analyzed with GLMM using SAS (PROC GLIMMIX), with invasion stage (native, early-stage) and year (2011, 2012) as fixed effects, and site ($N = 3$) as a random blocking factor, with a lognormal distribution (SAS 2009). The same mixed model structure was used to investigate the probability of capturing prey using logistic regression with a binomial distribution (SAS 2009). Reproduction (=mean number of juveniles per reproductive female) was analyzed using a GLMM (package nlme) with invasion stage as a fixed factor and site as a random blocking factor (Pinheiro et al. 2011).

A GLMM was used to test for the effect of density (high, low) on the probability of capturing prey with substrate size (large, small) as a covariate, using a negative binomial distribution. A similar model was used to test for the effect of density on the probability of producing an egg sac with web size as a covariate, using a binomial distribution. Both models included block ($N = 18$) and a nested variable (block/density/substrate size) as random blocking factors (SAS 2009). Following models, we examined the significance of the difference in the least-square means at the median web size (= 30 cm^2).

Results

Web size plasticity between species in response to initial invasion

The two native spider species showed different web building responses to native and knapweed substrates (Fig. 2.1). *Dictyna* exhibited a high degree of plasticity to substrate type, while *Aculepeira* displayed a relatively flat reaction norm. Likelihood ratio tests indicated that there was a significant interaction between species and substrate type ($\chi^2_1 = 49.659$, $P < 0.001$). Parametric bootstrapping of the likelihood ratio statistic corroborated these results ($P < 0.001$). *Post hoc* Tukey's HSD contrasts showed that *Dictyna* made significantly different sized webs between native and knapweed substrates ($P < 0.001$), whereas *Aculepeira* webs did not differ between substrate types ($P = 0.896$).

Web size plasticity within species for populations from early and late stage invasions

Analysis of reaction norms showed that *Dictyna* populations from early/native and late stages of invasion responded differently to substrate (Fig. 2.2). Likelihood ratio tests indicated that there was a significant interaction between invasion stage and substrate type ($\chi^2_1 = 23.096$, $P < 0.001$). Parametric bootstrapping of the likelihood ratio statistic supported this result ($P = 0.05$). *Post hoc* Tukey's HSD contrasts ($P < 0.05$) showed that *Dictyna* populations from both early/native- ($P < 0.001$) and late-stage invasions ($P < 0.001$) made significantly larger webs on knapweed substrates compared to native substrates, indicating both populations showed plasticity. However, estimates of the difference in log web size between substrate types within populations showed that populations from early/native-stage invasions (Fig. 2.3, $\bar{x}_{K-N} = 0.751$) had a greater degree of plasticity compared to populations from late-stage invasions (Fig. 3, $\bar{x}_{K-N}$ = 0.373), suggesting a loss of plasticity in later stages of invasion.

Plasticity links to fitness

Observational studies indicated that increasing web size increased *Dictyna* prey captures ($F_{1,854}$ = 78.43, P <0.001), however the strength of this positive relationship differed between invasion stages ($F_{1,854}$ = 15.96, $P < 0.01$). A significant interaction shows that the positive relationship between web size and prey captures was stronger in early/native stages of invasion compared to late stages of invasion (Fig. 2.4A; $F_{1,854}$ = 44.67, $P < 0.001$). Web size was also associated with higher reproduction in *Dictyna* ($F_{1,855}$ = 5.32, $P < 0.05$), but invasion stage was not ($F_{1,855}$ = 0.48, P = 0.510). Web size and reproduction were positively correlated in early/native stages, but negatively correlated in late stages of knapweed invasion ($F_{1,855}$ = 9.73, $P < 0.01$). Observations of *Aculepeira* web size between early/native stages of invasion compared to late stages showed no differences in web sizes constructed ($F_{1,43}$ = 1.149, P = 0.29). Prey capture rates and reproduction metrics were not observed in *Aculepeira*.

Simulated invasion experiments controlling for potentially confounding effects associated with natural knapweed invasions paralleled results from observational studies. Naïve *Dictyna* on simulated invasion plots representing the early stages of invasion constructed significantly larger webs ($F_{1,4}^{\text{substrate}}$ = 57.23, P = 0.002) that exhibited higher probabilities of capturing prey (Fig. 2.4B; $F_{1,4}^{\text{substrate}}$ = 9.73, P = 0.036) and higher reproduction ($F_{1,2}$ = 62.96, P = 0.016) compared to uninvaded control plots. Although webs were larger in 2011 than 2012 ($F_{1,4}^{\text{year}}$ = 21.40, P = 0.010), this did not influence treatment effects on web size between years ($F_{1,4}^{\text{substrate x year}}$ = 0.34, P = 0.591). Nor did the differences in web sizes between years affect differences in prey capture rates ($F_{1,4}^{\text{year}} = 6.17$, $P = 0.068$; $F_{1,4}^{\text{substrate x year}} = 0.03$, $P = 0.877$) between invaded and uninvaded treatments. Between year differences in reproduction could not be investigated because reproduction metrics were only observed in one year. Naïve *Aculepeira* constructed similarly sized webs ($F_{1,4}^{\text{substrate}} = 0.39$, $P = 0.579$) on simulated invasion and uninvaded control plots. Additionally, we found no differences in prey capture rates ($F_{1,4}^{\text{substrate}} = 7.75$, $P = 0.069$) or reproduction ($F_{1,4}^{\text{substrate}} = 1.64$, $P = 0.329$) between simulated invasion and control plots.

The results from microcosm experiments that controlled for spider density and substrate size indicated that individuals on large substrates ($F_{1,48} = 3.98$, $P = 0.052$) and those in low density treatments ($F_{1,48} = 3.32$, $P = 0.075$) tended to capture more prey, but there was no interaction between these main factors ($F_{1,48} = 0.27$, $P = 0.610$). Examination of the difference in least-square means at the median web size (= 30 cm^2) showed that individuals on the low density treatment tended to be more likely to capture prey ($t = 1.74$, $P = 0.088$). Similar models of the probability of producing an egg sac showed that individuals in low densities were significantly more likely to reproduce ($F_{1,49} = 5.92$, $P = 0.019$) and that web size ($F_{1,371} = 3.40$, $P = 0.066$) was marginally significantly positively related to fitness, while the interaction between density and web size ($F_{1,371} = 2.43$, $P = 0.120$) was not significant. The difference in least-square means at the median web size (= 30 cm^2) provided evidence that individuals in lower density treatments tended to be more likely to reproduce ($t = 1.81$, $P = 0.077$).

Discussion

Theory predicts that phenotypic plasticity should play an important role in determining initial responses of native species to rapid environmental changes such as those arising from biological invasions (Bradshaw 1965, Via and Lande 1985, Gomulkiewicz and Kirkpatrick 1992, Scheiner

1993). However, few empirical studies have examined this question and little is known about how plasticity and its relationship to fitness may change for natives over the course of invasion. In comparing two native web spider species that exhibit very different responses to knapweed invasion, we found that: 1) *Dictyna* and *Aculepeira* species differed in how plastic their web sizes were in response to knapweed invasion, 2) greater plasticity in web construction was linked to greater fitness gains and stronger population increases following invasion. We also found that web size plasticity and its relationship to fitness diverged between populations associated with early versus late stages of invasion. Web size plasticity appeared to be adaptive in native grasslands and early stages of invasion where larger webs were strongly correlated with higher capture rates and higher reproduction. In contrast, in heavily invaded grasslands, although web sizes were generally larger, plasticity was reduced and increasing web size was only weakly associated with higher prey captures and no longer linked to increased reproduction. Numerous factors changing between early and late stages of invasion may have influenced the adaptive benefits of web size plasticity, but simulated invasions and microcosm experiments both suggested that increased spider densities associated with later stages of invasion may increase competition for food resources, undermining the fitness benefits of larger web sizes.

At the species-level, *Dictyna* exhibited high levels of plasticity but *Aculepeira* showed no evidence of web size plasticity in response to invasion. An important question then is how might these differences in plasticity effect future adaptation in response to invasion? Our prior work in this system highlights how these differences in we plasticity link to fitness benefits in the context of the traits of the natives and the invader. Naïve populations of *Dictyna* exhibit 14 fold greater population increases than *Aculepeira* in response to simulated knapweed invasion (Smith Chapter 1). These population increases are linked to larger web sizes facilitating a higher prey capture probabilities and increased fecundity in *Dictyna*, but not in *Aculepeira* (Smith Chapter 1), a result mirrored in natural knapweed invasions (Pearson 2009). Orb weavers can adjust many web characteristics including web size in response to biotic and abiotic conditions (Sandoval 1994, Sherman 1994, Vollrath et al. , Herberstein et al. 2000), so presumably a lack of capacity for web size plasticity does not explain *Aculepeira*'s response. *Dictyna* are constrained by native plant architecture because these spiders build their webs within individual plants and most native plant species in this system provide undersized substrates relative to *Dictyna*'s web building capabilities (Pearson 2009). In contrast, orb weaving spiders, such as *Aculepeira*, use multiple plants simultaneously for web substrates and so are not constrained by individual plant traits. As a result *Aculepeira* populations increase to some extent as knapweed invasion increases the abundance of viable web substrates, thereby relieving substrate limitations, but this species does not have the ability to also increase prey captures and reproduction by building larger webs as does *Dictyna*. Spider populations are commonly food limited (Wise 1975, Kreiter and Wise 2001, Wise 2006), so fitness can be greatly increased through adaptations that increase prey captures.

In comparing web size plasticity between early and late stages of invasion we found that *Dictyna* populations from early stages had a much higher degree of web size plasticity compared to populations from long standing invasions. We also found evidence that variation in web size does not generate the same fitness benefits in early- versus late-stages of invasion. Observational studies indicated that increasing web size resulted in higher prey captures and increased reproduction in populations from early-stages of knapweed invasion, while in late stages of invasion increasing web sizes generated moderate increases in prey captures and actually reduced the probability of reproduction. Our experiments introducing naïve *Dictyna* onto simulated invasion plots and paired control plots produced qualitatively similar patterns. Together these studies suggest an explanation for the observed changes in plasticity and its associated fitness benefits over the course of invasion.

Natural web building conditions (i.e. those in uninvaded sites) in western Montana grasslands consist of many small, low quality web substrates and a limited availability of larger, higher quality substrates that are heterogeneously distributed across the landscape. The ability for *Dictyna* to plastically modify web size when given the opportunity is likely adaptive in these native grasslands. In essence, this species is preadapted to invasion by knapweed and similar invasive forbs, and this preadaptation helps to explain the dramatic population increases following invasion. This being the case, why does plasticity in web size and its associated fitness benefits decline for this species as invasion advances? Many biotic and abiotic factors change over the course of invasion. Isolating the primary causes of such change is often difficult because of the multitude of factors and potential interactions. However, our data point to two possible causes behind the observed changes in fitness benefits between native and late-stage invasions. The simulated invasion experiment controlled for many of the potential factors that may be influenced by spotted knapweed invasions, such as abiotic conditions, plant communities, prey communities, the effects that spotted knapweed plants have on altering the system, etc. The only two factors that were not controlled in the experiment were: 1) spider density and 2) substrate architecture. Early and late stages of invasion differ in that spider densities can increase by orders of magnitude and the primary web substrate switches from native plants, primarily yarrow, to spotted knapweed. Hence, spider density and/or substrate architecture may influence the relationship between web size plasticity and fitness.

To examine this question further we re-analyzed data from microcosm experiments that controlled for web substrate architecture (all substrates were knapweed stems) while manipulating spider density and substrate size under saturated prey conditions. These results suggest that the benefits of larger webs for prey captures and fitness may decrease or disappear in high competition environments. Individuals constructing webs in low densities tended to be more likely to capture prey and were significantly more likely to reproduce than at high densities. Although these results do not entirely rule out a role that substrate architecture might play, they suggest that increasing spider densities can result in increased competition that reduces the benefits of larger web sizes even under constant high prey inputs.

How does invasion alter the selective environment? Spider webs are largely passive capture devices, so larger webs tend to intercept more prey which can increase spider survival and reproduction (Rypstra 1982, Venner and Casas 2005, Pearson 2009). However, spider webs represent a significant investment for individual spiders both in silk synthesis and web construction (Tanaka 1989, Herberstein et al. 2000). Hence, as web size increases the relative gains in energetic and fitness returns could theoretically saturate and decline. In a prey-saturated environment, the asymptote in this curve will be driven by the physiology of the spider and the effects that environmental conditions (i.e. temperature) have on spider physiology and energetic demands. However, below this limit, the asymptote is likely driven by prey availability as the gains in captures from increasing web size will be determined by prey inputs. Prey availability can be driven by both absolute prey abundance and competition. Prey abundance is similar between high- and low-level knapweed invasions and between simulated invasion and control conditions (Pearson 2009, Smith Chapter 1), suggesting the differences in fitness benefits associated with increases in web size are not due to differences in absolute prey abundance. Our re-analysis of microcosm data suggests that competition for prey at high spider densities can explain the loss of fitness benefits of larger webs. Hence, the differences we observed in the relationship between web size plasticity and fitness between early and late stages of invasion may be explained as a function of increased competition for prey arising from the dramatic increases in spider densities that follow invasion in this system.

We do not often know the mechanisms that allow native species to respond positively to invasion. Understanding the mechanisms could greatly enhance our ability to predict community-level outcomes of invasions. Phenotypic plasticity is ubiquitous in natural system as a mechanism allowing species to cope with environmental change (Nussey et al. 2007). Our results demonstrate the potentially important role phenotypic plasticity may play in the initial restructuring of native communities following exotic species introductions. It also demonstrates how changes from early to late stages of invasion may alter the selection environment for native species by disrupting processes that determine the adaptive advantages associated with individual traits as well as plasticity in those traits.

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Figure Legends

Figure 2.1. Reaction norms of log web size (Mean ± S.E.) in *Dictyna* and *Aculepeira* constructing webs in native uninvaded grasslands on native or knapweed substrates (data from Smith Chapter 1). The lines have significantly different slopes and hence plasticity of log web area differs between species.

Figure 2.2. Reaction norms of log web size (Mean ± S.E.) to substrate type (native vs. knapweed) for *Dictyna* populations from native (dashed line, data from Smith Chapter 1) and knapweed-invaded (solid line, data from Pearson 2010) grasslands. The lines have significantly different slopes and hence plasticity of web area differs between populations.

Figure 2.3. Model predictions for probability of prey capture and reproduction for *Dictyna* constructing webs in early/native versus late-stage knapweed invasions. Data in left column (A,C) is from observational studies collected in knapweed-invaded and native-uninvaded grasslands, data in right column (B, D) is from experimental studies of simulated invasion in native-uninvaded grasslands (from Smith Chapter 1).

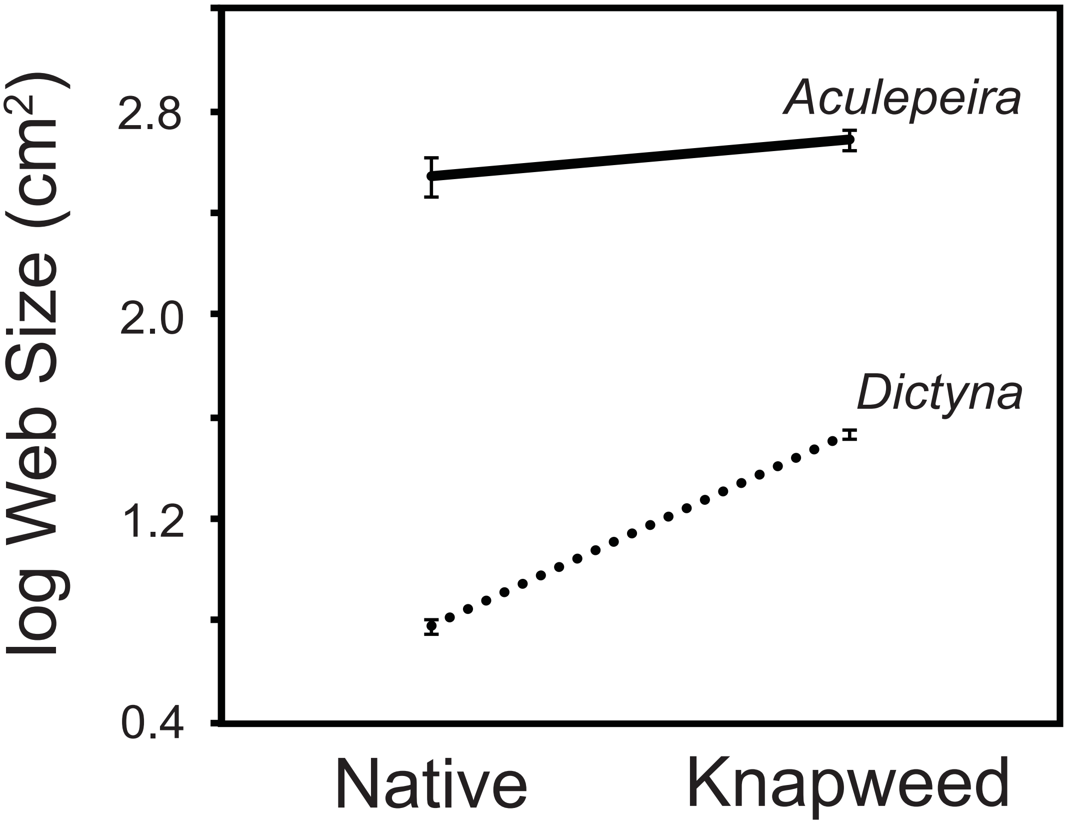
log Web Size (cm2)
2.8
2.0
1.2
0.4
Aculepeira
Dictyna
Native
Knapweed

Fig. 2.1

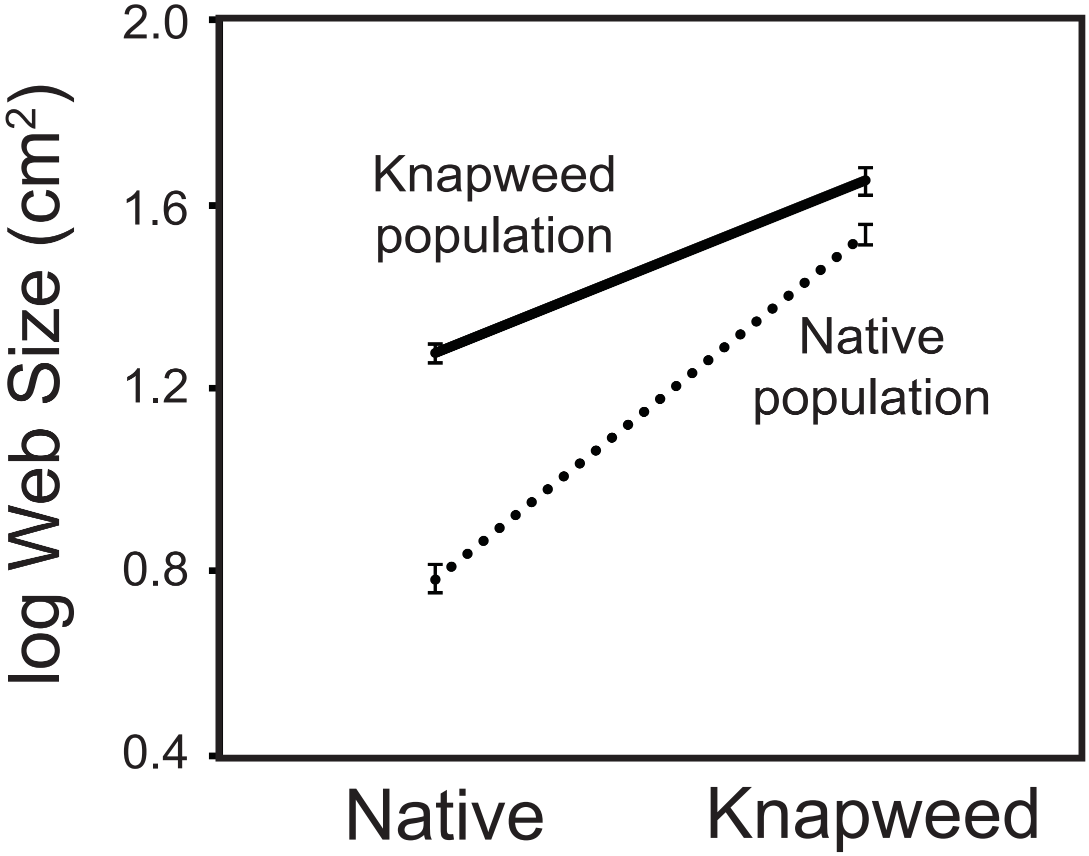
2.0
1.6
1.2
0.8
0.4
log Web Size (cm²)
Knapweed population
Native population
Native
Knapweed

Fig. 2.2

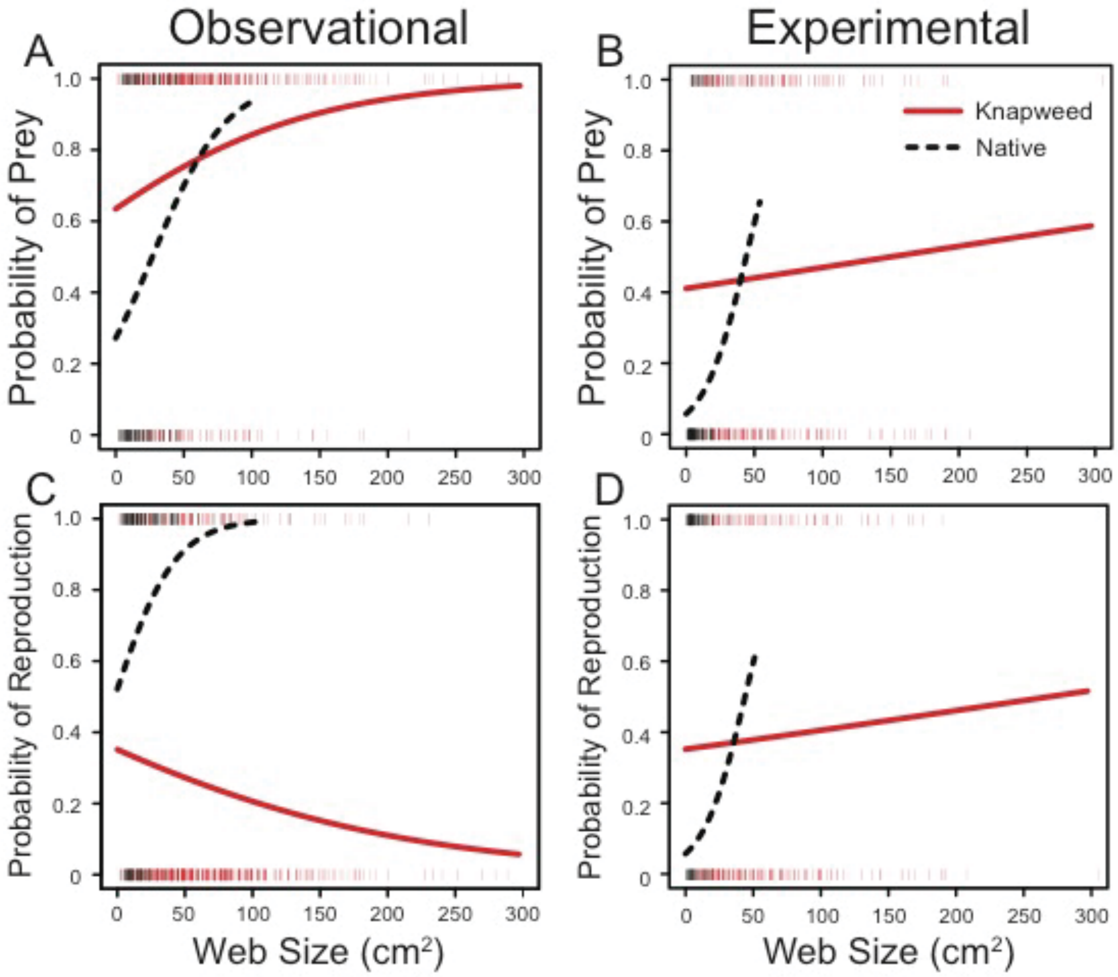

Fig. 2.3

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Chapter 3:

Exotic plant invasion disrupts host-parasitoid interactions: invisible parasitoid wasp becomes visible to spider host

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Abstract: Host-parasitoid interactions commonly form tightly linked interactions. Such coevolved relationships may be particularly susceptible to disruption by exotic species invasions because invasions often happen quickly and at large scales, but the effects of biological invasions on coevolution are poorly understood. In grasslands of the northwestern United States, the wasp (*Pteromalus grisselli* Gibson) parasitizes egg sacs of *Dictyna* spider species. These wasps roam freely over *Dictyna* webs unencumbered by the web and largely unchallenged by the web's host. However, our field observations in grasslands invaded by spotted knapweed (*Centaurea stoebe*), where these spiders reach extraordinarily high densities (up to 74x higher), suggest that *Dictyna* spiders commonly attack their parasitoids. To examine this phenomenon, we conducted laboratory and field studies on *Dictyna* populations from native-uninvaded and knapweed-invaded grasslands. Compared to spiders from native areas, *Dictyna coloradensis* spiders from knapweed-invaded populations were more than three times more likely to kill parasitoids placed in their webs. Moreover, parallel experiments substituting fruit flies as prey items in place of the parasitoids resulted in no difference in kill rates between populations, indicating this response was parasitoid-specific. Field surveys did not detect differences in parasitoid densities between *Dictyna* populations in knapweed-invaded and uninvaded grasslands. In contrast, egg sac parasitism rates tended to be about 33% higher in knapweed-invaded grasslands, though this pattern was marginally significant ($P = 0.092$). Although many details remain to be investigated, our results suggest that knapweed invasion appears to have disrupted this host-parasitoid system and dramatically altered spider behavior. Our results exemplify how tightly linked interactions like parasitoid-host interactions can be sensitive to biological invasions.

Keywords: *Centaurea stoebe*, *Dictyna*, egg sac, coevolution, grasslands, host-parasitoid interactions, invasion, parasitism, *Pteromalus grisselli*, spider

Introduction

Tightly linked interactions (i.e. predator-prey, parasitoid-host) are ubiquitous in nature and is an important process structuring communities and ecosystems (Thompson 1994, Thompson 2009). Host-parasitoid interactions provide striking examples of tightly linked interactions, which commonly develop into strong reciprocal feedbacks wherein each species' effect on the other drives an evolutionary arms race that, over time, becomes highly coevolved (e.g., Brodie and Ridenhour 2002, Benkman et al. 2003, Forde et al. 2004). However, anthropogenic environmental changes, such as the introduction of exotic species, can greatly alter ecosystems (Vitousek et al. 1997, Sala et al. 2000, Lodge et al. 2006) and the effects of exotic species on tightly linked interactions between native resident species are poorly understood (Leimu et al. 2012).

Tightly linked relationships also influence how readily exotic species establish and persist in new habitats (Richardson et al. 2000). For example, many exotic plant species rely on mutualisms (e.g., pollinators, symbiotic microbiota) which allow them to invade. Some of the worst invaders were only successful after the introduction of their symbionts (Richardson et al. 2000). Similarly, new associations between invasive ants and native honeydew-secreting insects facilitate ant invasion, which can have far reaching effects (e.g., O'Dowd et al. 2003, Kaplan and Eubanks 2005, Abbott and Green 2007, Styrsky and Eubanks 2007). However, the introduction of exotic species can also disrupt tightly linked interactions between native species (Callaway et al. 2005, Benkman et al. 2008) and these effects are likely to be more widespread than recognized (Thompson 2009, Leimu et al. 2012).

Spiders have developed a range of remarkable tightly linked relationships. Araneophagy – spiders that eat other spiders – has resulted in intricate predator-prey interactions. Predatory spiders have been shown to mimic the vibratory patterns of prey caught in webs, for example (Jackson 1992). Prey spiders respond by violently shaking their web to drive away or dislodge the predatory spider (Jackson et al. 1993). Sometimes predatory spiders just avoid the prey spider's web entirely, using their own silk to drop in and kill spiders directly, circumventing any anti-predatory behaviors (Jackson and Wilcox 1993). Some araneophagic spiders simulate the courtship behaviors of their potential prey. However, female spiders are extremely effective at recognizing these imposters and driving them away by violently striking, leaping at, charging, and even colliding with them to repel them (Jackson and Wilcox 1990). In Australia, crab spiders manipulate visual flower signals to lure introduced honey bees, while native bees show anti-predatory response and avoid flowers occupied by this predator (Heiling and Herberstein 2004). This last example illustrates how introducing species naïve to a system might substantially alter species interactions.

Parasitoid wasps are among the most important enemies of spiders, parasitizing their eggs sacs as well as being ectoparasitoids of adults (Foelix 2011). Spiders are known to employ several parasitoid-specific defenses, such as detection of cues (i.e., vibration, sound) to identify parasitoids (Hieber et al. 2002, Nakata 2008), and structural thickenings to egg sacs (Austin 1985), which suggest local tightly linked interactions between spiders and their parasitoid predators, presumably in response to stable interactions between these species. Exotic species may suddenly and dramatically alter these types of interactions, and may be especially powerful in situations where the exotic species is a dominant invader.

In the intermountain west of the USA, *Dictyna* (Araneae, Dictynidae) spiders are parasitized by the pteromalid wasp *Pteromalus grisselli* (Hymenoptera, Pteromalidae; formerly *Catolaccus prob. N. spp.*) (Wheeler and McCaffrey 1989, Gibson 2013). These wasps lay eggs in *Dictyna* egg sacs where the developing larvae consume all the spider eggs within parasitized sacs before emerging. Prior studies on the interaction between *D. coloradensis* and *P. grisselli* in knapweed-invaded grasslands reported the parasitoid "alighting and walking freely on…webs…apparently without provoking the spider"; nor were parasitoids ensnared in *Dictyna* webs (Wheeler and McCaffrey 1989). Based on these observations, Wheeler and McCaffrey (1989) concluded that egg sacs were essentially undefended – the wasps had adaptations to walk on webs, and the spiders didn't defend the egg sacs. However, our recent observations in grasslands invaded by spotted knapweed (*Centaurea stoebe*) indicate that female *Dictyna* often fight and kill approaching *P. grisselli* wasps and we have routinely observed parasitoids being caught and consumed by female *Dictyna* (J. Smith *pers. obs.*). Interestingly, we only observed these patterns in *Dictyna* webs constructed in knapweed-invaded grasslands; spiders in native, uninvaded grasslands showed no such aggression, and behaved as reported by Wheeler and McCaffrey (1989), suggesting that parasitoid-spider interactions may have been altered in the wake of knapweed invading these grasslands.

Invasion by spotted knapweed has caused 74-fold increases in *Dictyna* densities, because the spiders are no longer constrained by a lack of scaffolding (= plant stems) for building their webs (Pearson 2009, Smith Chapter 1). We hypothesize that these knapweed-associated changes disrupted parasitoid-host relations between *Dictyna* and *P. grisselli*. Here, we investigate interactions between *D. coloradensis* and *P. grisselli* to see if spider-parasitoid interactions differ between knapweed-invaded and uninvaded grasslands. We compared *D. coloradensis* responses to parasitoids and prey items in the laboratory between populations from knapweed-invaded and native-uninvaded grasslands to examine if parasitoid-specific defenses in these spiders are associated with invasion status. We also examined whether *P. grisselli* abundance and egg sac parasitism rates differed between populations in knapweed-invaded and native-uninvaded grasslands as potential mechanisms for observed behaviors in the field.

Methods

Study system

Our research was based in the semi-arid, low-elevation grasslands of the Rocky Mountains of western Montana. These grasslands are dominated by bunchgrasses *Festuca scabrella* or *Pseudoroegneria spicata*, but native forbs make up most of the plant species richness (Mueggler and Stewart 1980). Native forbs serve as the primary web substrates for native web spiders, with yarrow (*Achillea millefolium*) serving as the primary web substrate for irregular web spiders in the genus *Dictyna*. *Dictyna* spider species are the dominant web-building spiders in these grasslands (Pearson 2009). Within this system, there are two ecologically similar species, *D. coloradensis* (Chamberlin, 1919) and *D. major* (Menge, 1869), which function as a species complex (Pearson 2009) and are indistinguishable in the field (J. Slowik, University of Alaska Fairbanks, *pers. comm.*). *Dictyna* are small spiders (female total body length ranging from 3 mm for *D. major* to 3.8 mm for *D. coloradensis*). In these grasslands, *Dictyna* overwinter as sub-adults in the plant litter, emerge in April and May as the temperature warms, and produce egg sacs (average 2-3, Wheeler 1985) by the end of June through late-July (Chamberlin and Gertsch 1958, Wheeler et al. 1990). We targeted *D. coloradensis* in laboratory experiments where spiders could be sacrificed for identification. Lab results indicated *D. coloradensis* dominated field populations where *Catolaccus* abundance and parasitism rates were estimated, but some *D. major* were likely present.

Pteromalus grisselli (Hymenoptera, Pteromalididae) are small iridescently colored wasps that oviposit in *Dictyna* egg sacs (Wheeler and McCaffrey 1989). Only one other pteromalid species (*Arachnopteromalus dasys* Gordh) has been observed to parasitize the egg sacs of spiders, so this form of parasitism is uncommon, at least in this group (Gordh 1976, 1983, Peaslee and Peck 1983). When *P. grisselli* parasitize an egg sac, the developing wasp larva(e) consumes all eggs in the sac. Although this interaction could be considered *predation* by the developing larva(e), as opposed to *parasitism* (via adults), there is a gradation among these two situations (Melo et al. 2011) and the focus of our study is adult behavior, so for this paper we refer to this interaction as parasitism. Multiple egg sacs in a single web can be simultaneously parasitized, thus parasitism can significantly depress reproductive output.

Behavioral experiments, parasitoid abundance, and parasitism rates

To test under more controlled conditions whether *Dictyna* from knapweed-invaded habitats defend their webs and egg sacs more strongly, we brought female *D. coloradensis* from native and knapweed-invaded habitats into the laboratory and staged interactions between spiders and parasitoids. Female spiders, complete with their webs containing egg sac(s), were collected by breaking off the web substrate and transporting it to the laboratory in a plastic bag (*Dictyna* build their webs entirely on a single plant stem, so moving stems to the lab does not damage the webs; Smith Chapter 1). Individuals were collected from five populations in knapweed-invaded and four populations in native-uninvaded grasslands spanning the Bitterroot, Blackfoot, and Missoula Valleys in western Montana during July 2011. In the laboratory, individual females and their webs were placed inside glass jars (0.47 L, 8.1 width x 11.2 cm height; Specialty Bottle, Seattle, WA) covered with tissue and a lid containing multiple air holes. Jars containing females were placed inside a walk-in incubator (16:8 day night cycle, 26.6° ± 3°C, 25-35% RH) for one week allowing spiders to acclimate to laboratory conditions before experiments. Females were fed five field-collected gall flies (Tephritidae: *Urophora affinis*) every other day, except on the day of the experiments.

Behavioral experiments consisted of two trials conducted in the laboratory at the Field Research Station at Fort Missoula, The University of Montana, Missoula, MT. In the first trial, defensive behaviors toward *P. grisselli* were examined by placing a parasitoid (that freely moved around) in each spider's web approximately one cm from the female and observing whether the female killed the parasitoid or not. To determine whether spider responses to parasitoids reflected a general aggressive response or one that was specific to parasitoids, we repeated the experiments above one week later on a subset of the same spiders by introducing a fruit fly (*Drosophila hydei*) instead of a parasitoid to each web. *Dr. hydei* represents a prey item of the size commonly consumed, but never encountered by these spiders in the wild. Spiders were identified after experiments were completed, and only *D. coloradensis* were included in analyses.

P. grisselli abundance was surveyed via sweep netting at paired knapweed-invaded and native, uninvaded sites ($N = 3$) in the Blackfoot Valley, MT, during July 2011 and 2012 when *Dictyna* were actively reproducing. Each sample was collected by sweeping the net 50 times along a 50 m transect. A total of 10 transects were swept at each site. Insects were killed with ethyl acetate. Rates of egg sac parasitism for *Dictyna* were observed by collecting egg sac(s) during July 2010 from the same paired knapweed-invaded and native, uninvaded sites ($N = 3$) in the Blackfoot Valley, MT, that were surveyed for parasitism abundance. Egg sacs were brought into the laboratory, kept in an incubator (26.6° ± 3° C, 25-35% RH) and observed daily for emergence of parasitoids or spiderlings. If neither emerged, that sac was excluded from estimates of parasitism rates.

Statistical analyses. – We conducted all statistical analyses using R version 3.0.1 (R Core Team 2013). Data was transformed to meet assumptions of normality and equal variances as noted. Differences in defensive behaviors of *D. coloradensis* from knapweed-invaded and native, uninvaded areas were assessed using a chi-square goodness-of-fit test to test for differences in the proportion of trials where spiders killed an introduced insect (*P. grisselli* or *Dr. hydei*) in staged encounters. Differences in *P. grisselli* abundance between knapweed-invaded and native, uninvaded areas were assessed using a generalized linear mixed model (GLMM, package lme4) on parasitoid abundance per sweep sample with invasion status (native, knapweed) and year (2011, 2012) as fixed factors; and site ($N = 6$) as a random blocking factor. To test for the significance of the fixed factors we used maximum likelihood, followed by parametric bootstrapping of the likelihood ratio statistic to improve our significance estimates (Faraway 2006). To examine if rates of egg sac parasitism differed between knapweed-invaded and native-uninvaded grasslands we conducted a GLMM on the proportion of egg sacs parasitized per female with invasion status (native, knapweed) as a fixed factor; and pair ($N = 3$) and site ($N = 6$) as random blocking factors, using a binomial distribution (Bates et al. 2013). Because we knew how many times an event occurred (=egg sac parasitized) and did not occur (=egg sac not parasitized) and to meet assumptions of normality and homogeneity of variance, we constructed a 2-vector response variable representing the proportion of egg sacs parasitized using the number of successes and the number of failures (Crawley 2005). The significance of the fixed factor was analyzed using maximum likelihood.

Results

Female *D. coloradensis* from knapweed-invaded populations killed parasitoids three times more often than did females from native, uninvaded populations (Fig. 3.1A; $\chi^2_1 = 4.868$, $P < 0.005$), but these populations did not differ in their response towards fruit flies (Fig. 3.1B; $\chi^2_1 = 0.448$, $P = 0.503$), suggesting that the difference in behavior was specific to parasitoids. Parasitoid abundance did not differ between sweep samples collected in knapweed-invaded compared to native, uninvaded grasslands (Fig. 3.2A; $P = 0.779$), but tended to be higher in 2010 compared to 2012 ($P = 0.09$). Parasitism rates also tended to be higher on *Dictyna* egg sacs from knapweed-invaded grasslands compared to native-uninvaded grasslands (Fig. 3.2B; $P = 0.092$).

Discussion

How biological invasions influence tightly linked relationships, such as parasitoid-host interactions, in native systems is poorly understood. In examining the associated effects of spotted knapweed invasion on the *Dictyna*-*P. grisselli* host-parasitoid system, we found that in controlled laboratory conditions, female *D. coloradensis* from knapweed-invaded grasslands were far more likely to kill parasitoids than were females from native, uninvaded grasslands. Parallel studies using fruit flies showed no difference in kill rates between these populations, indicating that this behavior was parasitoid-specific. These results provide evidence that knapweed invasion or some aspect associated with this invasion has altered this host-parasitoid interaction by promoting more aggressive *D. coloradensis* defensive behaviors against its primary natural enemy. Furthermore, the fact that spiders in native, uninvaded grasslands still behave as originally reported by Wheeler and McCaffrey 25 years ago, suggest that the aggressive behaviors observed in this study arose rapidly and recently in the two decades following the spread of spotted knapweed.

Interestingly, although spiders in knapweed-invaded habitats were consistently more aggressive than their counterparts from native, uninvaded grasslands, it is not at all clear *why*. Parasitoid abundances were not detectable different between knapweed-invaded and native, uninvaded grasslands. One possibility is that the parasitoids were more effective at finding spiders in knapweed-invaded habitats, and parasitism rates did tend to be higher (by about 33%) in knapweed-invaded grasslands. This pattern is consistent with knapweed invasion having increased parasitoid attack rates on *Dictyna*, thereby increasing selection for spiders exhibiting defensive behaviors against the parasitoids. However, this pattern was only marginally significant, and it's not clear if changes of this magnitude would be sufficient to drive rapid changes in spider defensive behavior. We discuss several possibilities below.

Our laboratory experiments showing that *D. coloradensis* from knapweed-invaded grasslands were over three times more likely than spiders from native areas to kill parasitoids, while being equally likely to kill prey items (Fig. 3.1), indicated that this response was parasitoid-specific. These results differ qualitatively from those of Wheeler and McCaffrey (1989), who reported seeing *P. grisselli* walking freely and uncontested on *Dictyna* webs constructed on knapweed plants early in the invasion process nearly 25 years ago. Although this outcome could be due to rapid evolutionary responses in these spiders to recent changes in selection from parasitoids, we cannot rule out maternal effects since we did not examine the same behaviors in laboratory-reared offspring of the spiders. Additionally, since we brought spiders in from the field with their webs and egg sacs intact, the plant substrates where the webs were constructed were not controlled for in the experiments. It is possible that substrates might have influenced spider behaviors, although this is unlikely under controlled conditions.

Dictyna population densities in knapweed-invaded grasslands in western Montana can be 74-fold higher than in native, uninvaded grasslands (Pearson 2009). Population increases in knapweed-invaded grasslands likely date back 40 years or more to the time when knapweed first became abundant in this system (estimates based on historic photos and Montana Fish, Wildlife, and Parks, *unpublished data*). Such an increase in host populations almost certainly resulted in parallel increases in parasitoid densities. Higher parasitoid densities could result in higher attack rates on *Dictyna* egg sacs, but this result likely depends on whether increases in parasitoids outstrip increases in their prey or whether some other attribute associated with invasion favors higher attack rates even if parasitoid densities remain proportionate to or lower than historic parasitoid-host ratios. Hence, it is important to consider both density and attack rates of the parasitoids to assess possible underlying mechanisms.

We found that parasitoid abundances did not differ between knapweed-invaded and uninvaded-native grasslands in either year (Fig. 3.2A). This lack is surprising given the dramatic differences in host densities between habitats. One explanation is that parasitoid densities at this stage in the invasion process have increased to such an extent over such large areas that their abundance is now elevated across the landscape, even in uninvaded grasslands, due to subsidy effects. These parasitoids are highly vagile and, historically, searched for hosts that were widely spaced at low densities. In contrast, to parasitism densities, parasitism rates on *Dictyna* egg sacs tended to be higher in knapweed-invaded grasslands compared to native-uninvaded grasslands. Although these differences were marginally significant, they were biologically substantial, being about 33% higher in invaded areas. Importantly, collected egg sacs from both native and knapweed-invaded grasslands had female *Dictyna* present. Our experiments showed that

Dictyna females from knapweed-invaded grasslands exhibited parasitoid-specific defensive behaviors. Female egg sac guarding in other spider species can decrease predation and parasitism (Pollard 1984, Fink 1986, Horel and Gundermann 1992, Gundermann et al. 1997). These behaviors may also reduce parasitism rates in knapweed-invaded grasslands leading to the results presented here.

Shifts in *Dictyna* behavior may have important outcomes. Parasitized egg sacs produce no spiderlings, and it is common for parasitoids to attack all 1-3 egg sacs present in a *Dictyna* web. Egg sac guarding may or may not be costly to the spider (Fink 1986, Gundermann et al. 1997). Hence, a 33% increase in parasitism could generate a very strong increase in selective pressures favoring spiders that respond more aggressively towards parasitoids.

If *P. grisselli* densities are relatively similar between habitats, but parasitism rates are higher in knapweed-invaded grasslands, this could mean that webs on knapweed substrates are more susceptible to parasitism than are those on native substrates like yarrow; or that increased host densities favors parasitism in knapweed-invaded habitats. Knapweed stems are more open than yarrow stems (Fig. 3) so it is possible that webs, spiders, and/or egg sacs are more apparent on knapweed plants. However, most parasitoids use olfaction to seek hosts (Vet et al. 1995) so unless *P. grisselli* are visual hunters (see Wackers and Lewis 1999), this seems unlikely. Alternatively, higher host densities could also facilitate higher parasitism rates by creating more efficient systems for parasitoids to find and attack their hosts (Walde and Murdoch 1988). If *P. grisselli* densities have increased to such an extent that they are now much greater across both invaded and native habitats, this may accelerate selection for increased parasitoid defenses across uninvaded habitats as well. *P. grisselli* wasps are believed to be generalist parasitoids (Burks 1954). Hence, if knapweed invasion has increased its abundance via elevated *Dictyna* densities, this could result in wide-ranging indirect subsidy effects paralleling the food web effects documented from knapweed subsidies to *Urophora* gall flies (Pearson and Callaway 2003, 2006, 2008).

Tightly linked interactions like those between hosts and their parasitoids, often develop into balanced coevolutionary relationships. Our results indicate that spotted knapweed invasion has disrupted the *Dictyna*- *P. grisselli* host-parasitoid relationship. *Dictyna* spiders collected from knapweed-invaded grasslands are far more aggressive toward their parasitoids. This suggests that there has been a shift in the selective pressures in this system that renders *P. grisselli* somehow more "visible" or susceptible to *Dictyna* defensive strategies. Our results indicate that tightly linked interactions are susceptible to disruption by biological invasions. They also reveal how these natural experiments can provide fascinating opportunities for studying tightly linked interactions.

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Figure Legends:

Figure 3.1. Behavioral responses of *Dictyna coloradensis* to (A) parasitoid wasp (*Catolaccus* prob. n. spp.) and (B) fruit flies (*Drosophila hydei*) during staged encounters. *D. coloradensis* from knapweed-invaded areas were significantly more likely to kill parasitoids compared to spiders form native-uninvaded areas. There were no invasion-specific responses observed for fruit flies (see *Results*).

Figure 3.2. (A) Parasitoid abundance per site (mean ± SEM) and (B) percent egg sacs parasitized per female *Dictyna* (mean ± SEM) from knapweed-invaded and native-uninvaded grasslands. Parasitoid abundance tended to be higher in knapweed-invaded grasslands compared to native grasslands and to be higher in 2010 compared to 2012. *Dictyna* from knapweed-invaded grasslands tended to have a higher percentage of their egg sacs parasitized compared to spiders from native-uninvaded areas (see *Results*).

Figure 3.3. Examples of typical *Dictyna* webs constructed on (A) yarrow and (B) spotted knapweed.

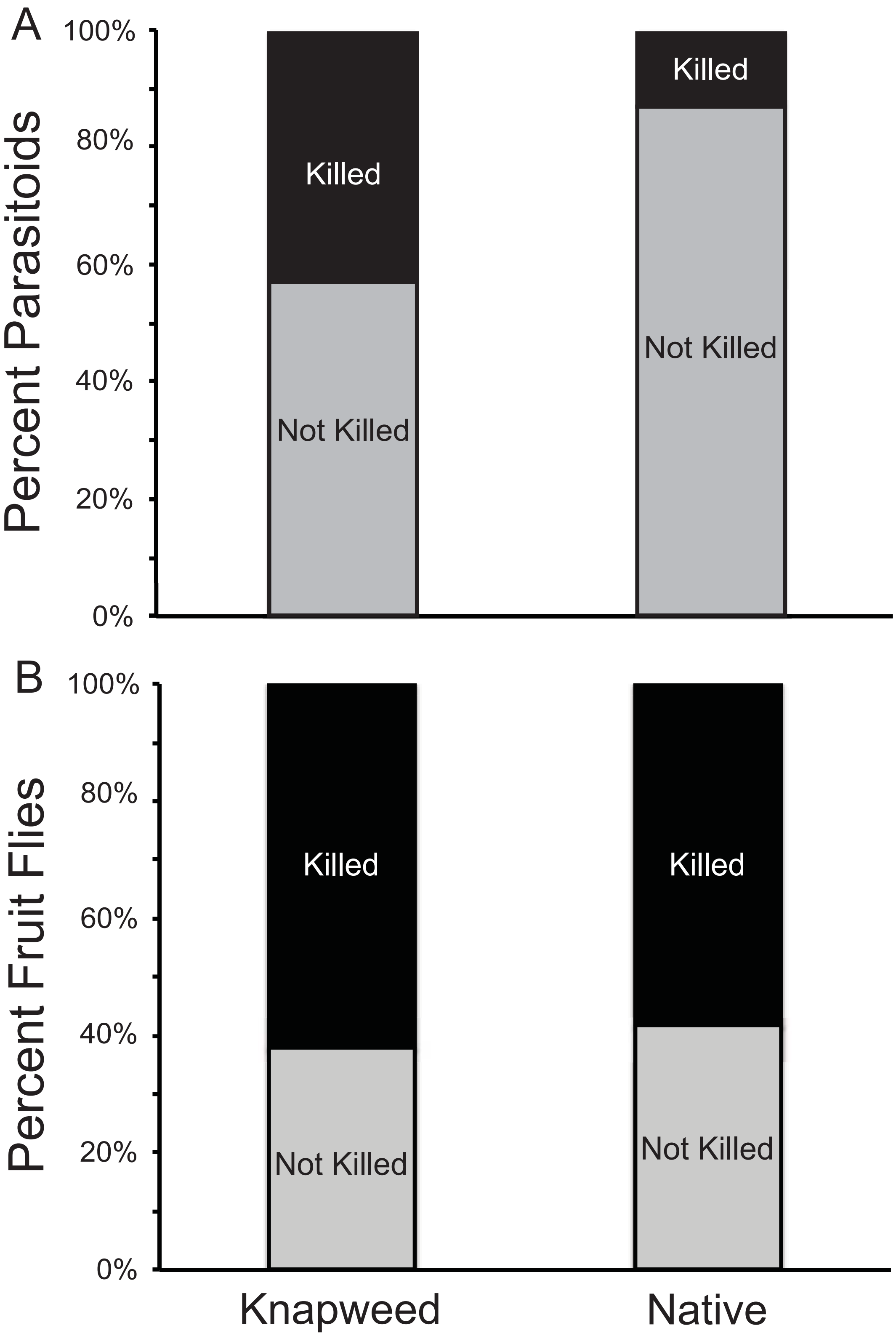
A
Percent Parasitoids
100%
80%
60%
40%
20%
0%
Killed
Not Killed
Killed
Not Killed
B
Percent Fruit Flies
100%
80%
60%
40%
20%
0%
Killed
Not Killed
Killed
Not Killed
Knapweed
Native

Fig. 3.1

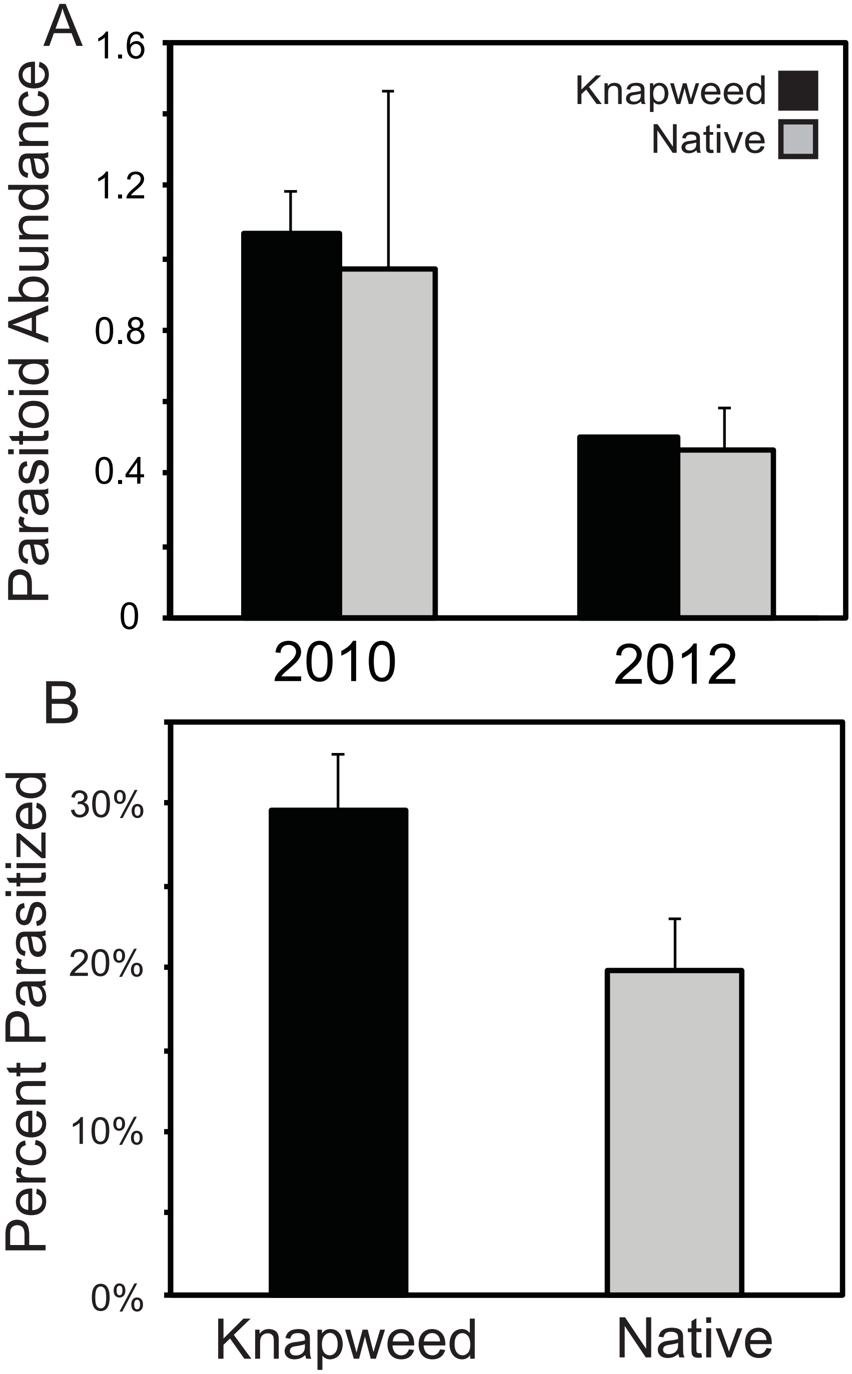
A
1.6
1.2
0.8
0.4
0
Parasitoid Abundance
Knapweed ■
Native ▨
2010
2012
B
30%
20%
10%
0%
Percent Parasitized
Knapweed
Native

Fig. 3.2

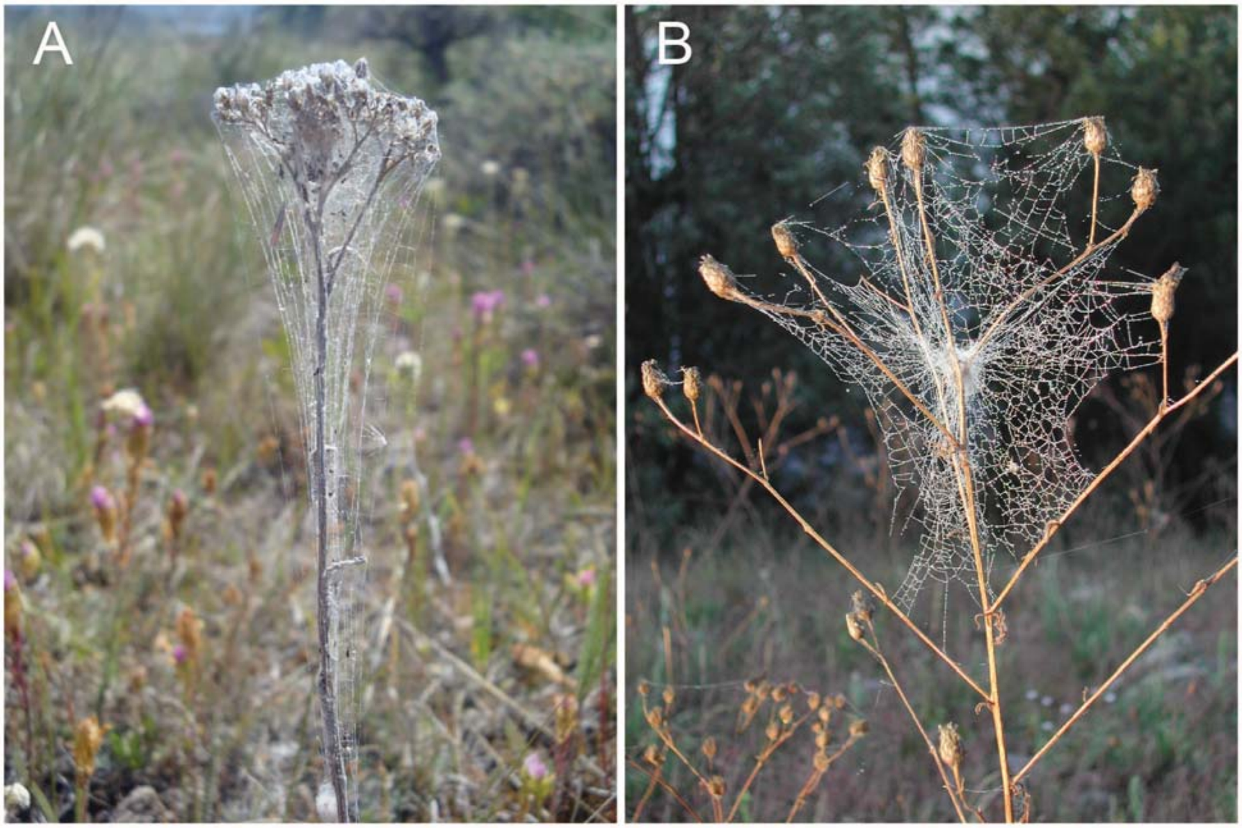

Fig. 3.3

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