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Comparative analysis of phenotypes, phenotypic plasticity, and phenotypic integration of variably invasive *Centaurea* and *Crepis* introductions to North America.

A Dissertation Presented

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Norris Zachary Muth

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Abstract of the Dissertation

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Despite a large amount of research on biological invasions, relatively few generalizations have been supported regarding their causes and consequences. Here I illustrate patterns of interest central to biological invasion research and suggest that better articulated questions and more appropriate experimental designs could significantly improve our understanding. I implement an alternative study design using variably invasive *Crepis* and *Centaurea* (Asteraceae) introductions. In these groups I compared architectural and fitness phenotypes, phenotypic integration, and phenotypic plasticity across phosphorous and water gradients. In single environment univariate analyses we found very few traits that

systematically differed between invasive and non-invasive species. When grown across environmental gradients, invasive species showed greater evidence of tolerance to low phosphorus conditions. Path analysis of integrated phenotypes in a single benign environment suggested that invasive phenotypes may well be less constrained than less invasive species. Across a range of environments, some invasive species demonstrated an ability to relax trait constraints observed under more stressful conditions when they were grown under more favorable conditions.

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Acknowledgments

Toni Lynn Morelli, Sue Natali, Martha Nolan, Peter Park, Iris Roth, Dan Stoebel, Ramona Walls, Eliza Woo and Jeff Yule.

Introduction

Introduced species have long been recognized as posing intriguing ecological and evolutionary puzzles. From Charles Darwin (1859), to various participants of the Modern Synthesis (Ernst Mayr and Theodosius Dobzhansky in Baker & Stebbins 1965), to those of us who have been given a leg up by the scientists standing on their shoulders, the causes and consequences of the success of species introductions remains a vibrant field of inquiry. I hope to add my contributions to this body of work that, before me, has provided practical remedies to environmental problems as well as more fundamental understandings of ecological communities and their evolution.

The work I present here is divided into five chapters. Chapter one outlines three fundamental questions in invasion biology, what kinds of research have been carried out in reference to these patterns, and suggestions for future directions. The historical and contingent nature of invasions is also discussed as are the impacts this has for our research expectations.

Focusing on one pattern described in the first chapter, chapter two presents a largely univariate analysis of variably invasive plant introductions grown in a single common greenhouse environment. The phenologies, architectures and fitnesses of multiple *Centaurea* and *Crepis* (Asteraceae) species introduced to North America are detailed and examined for clues as to how highly invasive phenotypes differ from less invasive ones.

In order to assess the plasticity of my study species I raised a subset of those examined in the previous chapter across two environmental gradients. Drought tolerance and competition for phosphorus have previously been suggested as important factors in the success of *Centaurea* species in their adventive range. Chapter three presents a univariate analysis of the plasticities of species phenology, architecture and fitness in response to variation in these factors.

Chapter four presents the results of a structural equation modeling (SEM) approach with the goal of elucidating differences in phenotypic integration of the variably invasive species grown in a single common environment. A multi-group SEM approach was used to assess biologically meaningful differences between species in overall integration as well as the strength and sign of trait interactions.

In chapter five we applied a similar path modeling approach to the species grown under multiple phosphorus and drought treatments. A multi-group SEM was used to assess biologically meaningful differences in the responses of integrated species phenotypes to a range of conditions from stressful to favorable.

A range of invasiveness among the introduced species used here provides an appropriately controlled context against which to judge successful introductions. The incorporation of environmental variation allows for an explicit assessment of phenotypic plasticity, long thought to play an important role in influencing biological invasions (Baker 1965). SEM brings greater depth to our understanding of these multivariate interactive structures of plant architectures and fitness. The integration of these studies allows for both an in depth assessment of individual invasive species as well as an opportunity to assess general patterns at two taxonomic levels, across congeneric and confamilial species.

I. Explaining Biological Invasions: the Pleasures and Perils of a "Soft" Science

Abstract

Despite a large amount of research on biological invasions, relatively few generalizations have been supported thus far. We illustrate here those patterns of interest central to biological invasion research and suggest that better articulated questions, and consequently more appropriate experimental designs, could significantly improve our understanding. Furthermore, we suggest that invasion biologists can benefit from accepting the discipline as more of a "soft" science in that: (1) generalizations are likely to be few and relatively straightforward; (2) compelling explanations are likely to be pluralistic in character; and (3) adequate studies may feel more like "puzzle solving" than "hard" science. This, it seems to us, diminishes in no way the scientific or practical importance of invasion biology.

Introduction

The increased attention to biological invasions during the last few decades has generated a critical mass of papers and has prompted various reviews, meta-analyses, and critiques (e.g., Kolar & Lodge 2001; Daehler 2003; Levine et al. 2004; Hierro et al. 2005). Here we hope to positively contribute to the critical analysis of practices in invasion biology by examining what invasion biologists do (what questions we address and how we go about finding the answers), and by considering some attempted generalizations and why these often seem unsatisfying.

We begin by parsing out the various biological patterns and mechanisms that invasion biologists study, the experimental designs used to investigate them, and the appropriateness of each. We then examine how the broader discipline of organismal biology (including, but not limited to, invasion biology, ecology, evolutionary biology, etc.) tends to view and generalize the relationships between patterns and mechanisms; we conclude by encouraging a more pluralistic understanding of the goals and practices of organismal biology.

Three patterns in search of an explanation

While the degree of attention focused on biological invasions makes it difficult to exhaustively list all patterns described in the literature, we submit that there are three general empirical claims that are the focus of the majority of published research, or otherwise underpin our current conceptions about introduced invasive species. These are: A) introduced invasives "behave differently" in their new ranges when compared to their native ones (e.g., populations may exhibit larger ranges, different demographics, or individuals may be characterized by different phenotypes); B) some introductions become invasive, while others (the majority) fail or remain restricted in distribution; and C) some habitats or communities are more susceptible to invasion by introduced species than others. Studies examining each of these patterns have of course led to the generation of numerous candidate explanations. We examine the patterns in some detail and attempt to summarize the proposed explanations to set the stage for our conceptual analysis to follow.

Pattern A: Harmless at home, aggressive abroad.

One of the most common mantras of invasion biology is that invasive species are problematic in their introduced ranges, while they are often benign, "well behaved" members of their communities at home (put forth as early as Darwin 1859, and reiterated more recently in Elton 1958 and in Crawley 1987). However, empirical evidence substantiating this phenomenon is largely lacking (Thebaud & Simberloff 2001; Wolfe 2002; Hierro et al. 2005); moreover, attempts to predict potential species invasiveness in the introduced range based on demographic information from the native range would suggest the obvious alternative, that introduced invasives may also be aggressive in their native ranges (Reed 1977; Richardson & Bond 1991). The lack of empirical evidence necessary to substantiate this pattern is likely due in part to the fact that qualitative assessments suggest that the pattern is obvious, and therefore carrying out the necessary multi-continental research projects to verify any particular example is considered a low priority. We agree that the logic of the alleged pattern is persuasive, in fact it is a near-truism that variation between dramatically different locales (as is expected to be the case between introduced and native ranges) must have differential effects on phenotypes and populations. However, whether these differences are biologically meaningful in explaining invasions is a hypothesis that, more often than not, is assumed rather than tested.

Interestingly, the less than robust empirical evidence has not restrained mechanistic attempts to explain this "obvious" pattern (reviewed in detail in Hierro et al. 2005). Among the most commonly invoked explanations are the enemy release hypothesis (Newsome & Noble 1986; Keane & Crawley 2002), the evolution of increased competitive ability (Blossey & Notzold 1995), and founder or bottleneck effects (Tsutsui et al. 2000). These candidate explanations could apply at either (or both) the individual level (e.g., individuals may be larger or more fecund), or the population level (e.g., populations may have higher densities or faster rates of spread). It is interesting to note that most invasion studies focus on characteristics at one level or the other (traits of individuals or traits of populations), and therefore potentially interesting relationships between these levels are often left unaddressed (e.g., increased individual vigor may be associated with decreased population densities, rates of spread, or community effects, while increases in these population parameters may be achieved by less vigorous individuals).

The candidate explanations mentioned above are all logical hypotheses that likely help to account for some of the differences that exist between introduced populations of particular species and corresponding conspecific populations within the native range. The enemy release hypothesis is built on the empirical observation that many introduced species are removed from their usual suites of competitors, predators, pathogens and the like because they fail to accompany the focal species on the journey to the introduced range (Newsome & Noble 1986; Keane & Crawley 2002). Introduced individuals and populations, it is thought, may then thrive under the novel conditions of their new enemy-deprived ranges.

Recently Colautti et al. (2004) noted that the enemy release hypothesis has been used to describe two conceptually distinct phenomena. One is a special case of our pattern A, a species that shows different demographics or phenotypes between conspecific populations in its introduced and native ranges (akin to Colautti et al.'s biogeographical pattern, e.g. Wolfe 2002). However, the same mechanism and terminology ("enemy release") have also been used where introduced species show reduced effects of, or exposure to, herbivory as compared to heterospecific native species co-occurring within the introduced range (Colautti et al.'s community pattern, e.g., Agrawal and Kotanen 2003). It is certainly possible that this second pattern may appear in concert with (because it may be caused in part by) the first one. However, the two do not necessarily entail one another, and if the connection is taken for granted it may lead to confusion while evaluating research findings.

The "evolution of increased competitive ability hypothesis" goes one step further by allowing enemy-release effects to accrue evolutionary interest, so to speak (Blossey & Notzold 1995). That is, natural selection can act to favor genetic variants that adjust allocation strategies to better fit the environmental conditions prevalent in the new range. Specifically predicted is an allocation shift towards decreased enemy defense and increased growth and/or reproduction (Blossey & Notzold 1995). Thus, introduced populations may differ from their native-range conspecifics not only due to environmentally induced "immediate" plastic responses (Pigliucci 2001), but also because of genetic responses to novel selection pressures over several generations.

Another hypothetical explanation of pattern A that involves genetic differentiation between introduced and native ranges is the founder or bottle neck effect. It is logical to think that the severity of reduction in effective population size that almost undoubtedly accompanies most introductions will translate into observed differences in how invasives behave between their introduced and native ranges. This may be a consequence of a conversion of non-additive to additive (i.e., capable of responding to selection) genetic variance that accompany bottlenecks (Cheverud & Routman 1996). This mechanism seems to play a major role in explaining the invasiveness of the Argentine ant, (*Linepithema humile)*, where a loss of genetic variation has apparently led to reduced intraspecific aggression and the formation of dominant supercolonies (Tsutsui et al. 2000).

Pattern B: Differential success of introductions.

Perhaps for reasons of potentially great practical value in addition to the hope for a more predictive science of ecology, the determination of why some species become invasive upon introduction while most fail or remain restricted in range is the holy grail of invasion biology questions. This potential practical and predictive value, as well as the fact that the necessary empirical data can be collected within one continent, is perhaps why this pattern is more generally substantiated than pattern A.

While the focus here tends towards explaining why the successful invaders are good at invading, the important counterpoint of why failing species perform so poorly is left comparatively unexplored. As with the explanatory mechanisms proposed for pattern A, those thought to underlie pattern B are also logically appealing. One of the most common of these explanations is that successful invaders are quite simply good weeds (Baker 1965). That is, successful invaders can be characterized by a host

of "r-selected" traits, including early maturation, high fecundity, rapid growth rates, self-compatibility and non-specialized pollination syndromes in plants, and so on. While this has often been borne out for the successful invaders, it has been less often examined whether failed introductions truly lacked these attributes (but see Rejmanek & Richardson 1996; Prinzing et al. 2002; Pysek et al. 2004; Sutherland 2004): we would not have much of an "explanation" if failures were often "r-selected" species as well.

Also proposed by Baker (1965) is the possibility that what separates introduced invaders from non-invasive introductions is the existence or evolution of broadly tolerant "general purpose genotypes" (similar to "fitness homeostasis" as presented in Hoffman & Parsons 1991). It is logical to think of these general purpose genotypes as characterized by some degree of adaptive phenotypic plasticity. Parker et al. (2003) suggest that the invasion of California by *Verbascum thapsus* could be an example of invasion by a general purpose genotype, and the broader role of phenotypic plasticity in invasions is starting to draw more rigorous empirical attention (Williams et al. 1995; Kaufman & Smouse 2001; Lee & Petersen 2002; Sexton et al. 2002; DeWalt et al. 2004).

It should be further noted that pattern A (and any causal mechanisms underlying it) could also be thought of as a high-level *mechanism* to account for pattern B: the ability to "behave differently" once introduced could allow some introduced species to flourish, while less responsive introductions remain restricted in their distributions.

Pattern C: Differential community susceptibility.

As successful as any invader may be, there is no species that is successful everywhere. Rather, certain communities may be sensitive to invasion by particular kinds of alien taxa, which leads one naturally to the question of why communities are invaded in the first place. Again, the empirical evidence for the differential invasibility of communities is better substantiated than pattern A, in part because all the relevant data can be collected without intercontinental studies. However, for ethical reasons, much research on this pattern relies on observational data or "natural experiments," as opposed to controlled experimental introductions into as yet uninvaded communities or habitats. Despite the fact that ethical constraints make rigorous assessments of certain research questions "out of bounds," it would be difficult to argue that all communities are equally invasible or equally impacted upon invasion.

The explanations advanced for differential community invasibility,

as with many of the previously mentioned mechanisms, are drawn from the greater ecological field of inquiry. The "biotic resistance" hypothesis holds that differential community invasibility may be attributed to variation in native biological diversity, with high diversity inhibiting invaders (Elton 1958; Tilman 1997). It has been noted, however, that the nature of the relationship (whether positive or negative) may depend on scale as well as particulars in experimental design (Tilman 1997; Stohlgren et al. 1999 ; Levine 2000; Hector et al. 2001; Loreau at al. 2001; Weltzin et al. 2003; Byers & Noonburg 2003; Huston 2004; Meiners et al. 2004).

Additional research suggests that community invasibility may be greater in communities that are more "ecologically naive" (functionally-, trophically-, guild- depauperate, though not necessarily low in diversity, e.g., Manne et al. 1999; Fargione et al. 2003; Xu et al. 2004, but see VonHolle & Simberloff 2004), are characterized by greater disturbance (e.g., Crawley 1987), or undergo rapid changes in limiting macronutrients (e.g., Huenneke et al. 1990; LeJeune & Seastedt 2001) or local hydrology (e.g., Hobbs & Mooney 1991). Still others have attempted to formulate more general theories that encompass all or some of the specific hypotheses mentioned above and relate invasibility to fluctuating resource availabilities (Davis et al. 2000) or niche opportunities (Shea & Chesson 2002).

A compelling case can be made that by practice (evidenced by the ease with which one can often categorize a particular study as addressing one or more of these patterns) invasion biologists have identified these patterns as central to the field. While we believe this relatively clear identification of a suite of interesting questions has helped make invasion biology a vibrant field, we feel that greater progress has been hindered, in part, due to systemic problems in the match between research question and experimental approach.

On the selection of proper controls

Invasion biology is no different from any other science, in that the ability to test hypotheses about causal mechanisms is limited by the degree to which one can experimentally or statistically controls for the factor(s) of interest (Shipley 2000). However, it will help to clear the air if we carefully discuss what the appropriate kinds of control should be for each pattern type discussed above, and compare this to the types of controls that are actually often used in practice.

In addressing potential instances of pattern A it should be clear that what is necessary to determine whether a particular species is "behaving

differently" in its introduced range is a study of conspecific populations of the focal species as they exist in both the introduced and native ranges. Simple observational studies of populations in both ranges can provide the necessary information to verify the existence and elucidate the particularities of the pattern (i.e., which individual or population parameters, if any, are actually different between the two regions). Given the extent to which claims of pattern A are made, there are surprisingly few studies that have taken this necessary step (but see Wolfe 2002; Beckstead & Parker 2003). Follow-up studies to address potential mechanisms could include a range of reciprocal transplants (e.g., Willis & Blossey 1999), common garden trials (e.g., Willis & Blossey 1999; Willis et al. 2000; Bossdorf et al. 2004; DeWalt et al. 2004; Maron et al. 2004), and field based environmental manipulation approaches (Twolan-Strutt & Keddy 1996; Meekins & McCarthy 2001; Claridge & Franklin 2002; Cassidy et al. 2004). However, studies of potential mechanisms are conceptually distinct from attempts to validate the existence of the pattern in the first place. In fact, most studies of this type have *assumed* the existence of pattern A and then went on to carry out experiments -- normally just in one region -- to examine potential mechanisms. This seems rather like putting the horse before the cart.

Perhaps the most glaring problems in the design of invasion studies are those that plague efforts to elucidate pattern B. A brief perusal of the invasion biology literature will yield numerous papers that compare "introduced invasive" species to heterospecifics (often congenerics) native to the invader's range of introduction (a recent review of these studies can be found in Daehler 2003). While we believe the use of native heterospecifics can potentially help explain the invasiveness of a particular introduction, we think the circumstances under which they serve as a useful control group are limited to cases in which: 1) the native heterospecifics co-occur locally (and therefore are potentially interacting) with the introduced invasive; or 2) the native heterospecifics are demonstrably unaggressive. Unfortunately, it is often not clear in any particular study whether either of these criteria is met.

Not surprisingly, given that competition is a fundamental explanatory principle in ecology, the value of the first criterion has not been lost on the ecological community, and this is reflected in the numerous studies that use locally co-occurring heterospecifics as controls for the focal introduced invasive species. However, the many studies that use native congenerics (or confamilials, or otherwise closely related heterospecifics), while laudably attempting to control for the phylogenetic non-independence of species, often fail to describe in enough detail the ecology and distribution of the species to know whether or not they coexist

with the focal invader in any meaningful way (criterion 1).

Unfortunately, whether they use congeneric or co-occurring controls, almost all studies fail to describe the range or demographic properties of the heterospecific comparison (criterion 2). It should be clear that if we are trying to determine what attributes make one species invasive, then we need to make certain that our control species aren't similarly aggressive (or that their degree of aggressiveness is somehow accounted for). We believe it does not go without saying that any particular native species is unaggressive. Most obviously highlighting this problem is the possibility that a native "control" is in fact invasive elsewhere (where it has been introduced itself). Moreover, it is also possible that the native heterospecific is actually characterized by very similar rates of spread and other population demographics of interest (propagule pressure, migration rates, gene flow, etc.) when compared to the invader, but that this similarity is masked because of the saturation of the region by the native heterospecific.

Although we think "invasive vs. native" studies can be useful under the criteria mentioned above, we believe their stated goals or conclusions are often inconsistent with the nature of the study. Well designed studies of this type can help us understand if, or explain why, introduced invasive species outcompete (in a broad sense) native species (Sax & Brown 2000). However, if the problem to be addressed is what causes some introduced species to become invasive while the majority of introductions fail or remain restricted in distribution (i.e., we want to assess pattern B in some manner), then native species cannot help us answer this question, as they are not members of the broader pool of introduced species. The necessary comparison groups for any study attempting to assess this pattern are introduced invasive species and similarly introduced unaggressive (or less aggressive) species. The often used native controls are quite clearly not "introduced" in the same way that we mean to describe our focal introduced invasive species (otherwise they would not have been identified as potential native controls). The good news here is that there is rarely a shortage of introduced species that fail to become established or invasive.

With respect to pattern C, there is normally a much better match between experimental design and the stated questions of interest. Although the "invaders" are rarely experimentally introduced to uninvaded sites or habitats, it is possible to perform in-situ experiments that modify the environment of an existing invaded area, potentially providing support for particular mechanistic explanations (e.g., Meekins & McCarthy 2001; Claridge & Franklin 2002; Cassidy et al. 2004). Additionally, it is possible -

- and common -- to bring the introduced invader into the greenhouse, lab, or experimental garden to test causal hypotheses. Finally, it seems to be generally appreciated that in some cases it would be necessary or highly desirable to experimentally introduce a species to an uninvaded site or habitat in order to address some particular question of interest (e.g., Campbell & Echternacht 2003). In these cases it is possible to weigh the risk of extending a species invasion against the potential benefits emerging from a better understanding of the underlying mechanisms of invasion. Such risk-benefit analysis can ethically inform decisions on how to proceed.

Philosophically speaking

The problems we have been outlining so far in the study of invasives are actually one instantiation of a more general malaise that affects a significant number of researchers in ecology and evolutionary biology. The problem lies in a relatively scarce appreciation of the philosophical foundations of the so-called "soft" sciences, of which ecology and evolutionary biology are prime examples (Pigliucci 2002). On the one hand, organismal biologists relish the variety of forms that life on earth takes. That is why we are so interested in biodiversity and spend so much time trying to put some order into our understanding of the bewildering array of living organisms. On the other hand, some of us are constantly tempted by the commonly accepted idea that "real" science is more akin to physics and chemistry (and, in biology, to molecular biology and genetics): the hallmark of a good science is to see past the variety of phenomena and distill things to as few generalizations as possible (as in the quest for a so-called "theory of everything" in physics).

This tendency has produced countless interesting outcomes in biology, from Fisher's (1930) so-called "fundamental" theorem of natural selection (which, however, is neither a theorem nor is it fundamental) to Hubbell's (2001) more recent "unified" theory of biodiversity (which, when examined in detail, appears to be much less "unified" than the title of the book presenting it implies). And yet, few empirical studies have ever benefited from such sweeping generalizations (the jury is still out about Hubbell's attempt, but we are cautiously skeptic), precisely because they throw out the baby with the bath water: in their quest for universal explanations of biological phenomena, these theories eliminate much of what makes biology interesting, i.e., the astounding variation presented by life on earth. For the purpose of our discussion, it is useful to briefly present some relatively recent insights into the nature of "soft" sciences as emerging from the work of Dupré (1993) and Cleland (2001; 2002). They

should help clarify what is, and is not, likely to work, both in the particular field of invasive biology and in the broader ones of ecology and evolutionary biology.

Dupré's book, *The Disorder of Things* (1993), covers a variety of topics, and we do not actually agree with some of the author's conclusions. However, Dupré makes particularly clear one reason why organismal biology is a soft science: reductionism is a one-way street. A research program of reductive explanations (say, seeking the molecular basis of some phenotypic trait) can be extremely successful if one wishes to understand *how* certain high-level characteristics (phenotypes) are possible given some low-level characteristics (genes). However, the multi-layered complexity of the low-level => high-level mapping (what some evolutionary biologists refer to as the "genotype-phenotype mapping problem," see Oster & Alberch 1982) makes it very much unlikely that one can reverse the mapping and identify what *specific* low-level causes produced a particular high-level outcome in any individual case. This can be understood more easily in a couple of ways: from a statistical viewpoint, the problem Dupré is pinpointing is the well-known difficulty of "descending" from some observed patterns to the underlying causes (Shipley 2000), because several alternative combinations of causes can generate (account for) the same pattern. From a biological point of view, this is a result of the well-known redundancy (e.g., Pickett & Meeks-Wagner 1995; Wagner 1999) of the low-level genetic/developmental machinery, by which the same adult phenotype can be produced by a variety of combinations of molecular and ontogenetic processes. The reason this matters to organismal, and in particular invasive, biologists is that Dupré's principle guarantees that for any complex enough system (such as evolving or invading populations) there isn't going to be a single, relatively simple, set of explanations accounting for the observed patterns. Rather, the organismal/invasive biologist has to get dirty and carry out fairly case-specific detective work, using multiple lines of evidence, to figure out what particular combination of phenomena likely yielded the pattern of current interest.

Which brings us to the second insight into these matters, articulated in a couple of papers by Cleland (2001; 2002). She suggests that the hard/soft science divide is actually one that runs along a parallel division between a-historical and historical science (we all realize, of course, that this isn't a dichotomy, but rather a continuum -- indeed, ecology and evolutionary biology are perfect examples of sciences that are a mix of the two components, unlike, say, paleontology, which is by definition completely historical). The queen of the a-historical sciences is, obviously, physics, or rather that part of fundamental physics that is concerned with

the universal behavior of the particles making up ordinary matter. (Largely) a-historical sciences, according to Cleland, tend to be very good at predicting the future, because their objects of study tend to behave in the same manner regardless of the circumstances. Historical sciences are notoriously poor at the same sort of predictions for the opposite reason, which has been a source of much frustration among biologists and social scientists, so much so that we are often accused of suffering from "physics envy" (e.g., Simberloff 1980). However, Cleland argues -- we think persuasively -- that historical research is very good at a different kind of activity:, inferring what happened in the past based on multiple (but rarely if ever just one) lines of evidence. In fact, the more lines of evidence available, the more such inference becomes strong and the conclusions are as reliable as those of the hard sciences. We need not go into the philosophical details of Cleland's reasoning (though we urge interested readers to check her papers), but a simple example will make the point nicely and combine both Dupré's and Cleland's reasoning in the particular case of invasive biology.

Suppose we are interested in the invasion biology of a group of plants, several species of which have made it to a novel geographical area; imagine, as it is commonly the case, that the success at invading varied among species, and sometimes even among populations of the same species. If all we have said so far is approximately correct, a research program focused on the *prediction* of which species or populations of those taxa will become invasive, and which will not, is not likely to succeed. This is because that sort of outcome depends on many contingencies, including the life history and other phenotypic characteristics of the taxa in question, the degree and type of genetic variation available, the specific environmental circumstances under which the potential invader thrives or not, the frequency distribution of such environmental circumstances, and so on (notice that most of this information would be missing from most papers addressing the problem). On the other hand, a research program built around multiple lines of evidence gathered in order to *explain* why certain taxa made it and others didn't, has a much fairer chance of success (though, of course, by no means a certainty to do so).

This may seem like a comparatively small accomplishment, resulting in a series of "just-so-stories" about the invasions (or their failures). But that would be an unwarranted harsh judgment. First, as Sober (1984) wrote while discussing the nature of selection, "It is not the scientist's fault that nature has made some of its secrets relatively opaque to human scrutiny." Second, and more positively, explanations can be tested just as well as predictions, because they themselves are in fact a

form of (backward) prediction. What the historical scientist is after is not as much the ability to predict the future, but to predict the past, so to speak. If the available evidence points toward a certain set of explanations for a given phenomenon, such explanation is likely to "predict" additional pieces of evidence that should be found if the explanatory framework being considered is largely correct.

By example, if certain lines of evidence suggest that an introduced species is spread primarily by farm equipment, then we can "predict" a phylogeographic pattern consistent with this mode, and not for, example, one more consistent with wind dispersal. This sort of conclusion can, within limits, then be turned around to predict (in the strict sense of the term) the occurrence of similar events in the near future, as long as relevantly similar circumstances hold. Furthermore, scientific investigations of this sort seem poised to contribute equally well along the continuum of basic (e.g., modeling spatio-temporal patterns of range expansion) to applied (e.g., suggesting particular management techniques involving the use of farm machinery) scientific questions.

Once we understand that historical sciences are not "limited," just different, and that prediction of future events isn't the only acceptable acid test of good science, the effect is actually liberating. Organismal biologists can then go on about their business with a better idea of what their priorities should be in the field.

A few practical proposals

An endless debate among both biologists and philosophers of science revolves around what counts for "real" science, whether it has to be predictive, and whether it has to include a search for universal laws (Dupre` 1993; Cleland 2001; 2002). At one extreme, particle physics has always been a matter of prediction and ultimate, general, explanations. At the opposite extreme, paleontology and history seem not to be able to produce much in the way of either prediction or general causal explanations. Here we propose that invasive biology, as a special case of organismal biology, falls somewhere in between such extremes, with a more pronounced similarity to the historical sciences (Pigliucci 2002). If invasion biologists were to take seriously the sort of science their discipline embodies, we suggest that we would then see a significant shift in attitude and research programs, along the lines of the following proposals.

1. There will be very few generalizations emerging from invasion biology, and these will be fairly obvious.

For example, it is almost a truism to "predict" that species with higher reproductive output, long-range dispersal, or a capacity to switch from out- to inbreeding will be successful invasives. At an even more abstract level of generalization, to say that species that are capable of rapid spread in a new environment are the best invasives becomes essentially circular.

This does not mean that we should give up efforts to build a general framework for invasion biology, just as no such defeatist attitude has spread among ecologists in general (despite the common saying that ecology is "the elucidation of the obvious"). However, it would be healthy to realize the strict *limits* on this research program imposed by nature and by the historicity of the biological sciences. We would then spend much less intellectual energy and financial resources in the pursuit of unreachable holy grails, not to mention of sparing ourselves the continuous frustration arising from a more or less subconscious inferiority complex with respect to the "hard" sciences. In the closely related field of evolutionary biology, for example, there are few – but very powerful – explanatory principles (e.g., natural selection, genetic drift), and these are by and large used to account for (explain) a variety of instances of evolution, rarely if ever to make long-term predictions about where evolution will go next.

2. Explanations will often be complex.

We should take seriously the proposition that in most (if not all) cases *the* explanation for why a species is invasive (or the equally interesting, but much neglected, question of why so many species are *not* good invasives) is not going to be along the lines of one or even very few major causal factor(s). It will often turn out that the enemy release hypothesis will not "win" to the exclusion of, say, the idea of the evolution in situ of increased competitive ability. Both, as well as other factors, may turn out to have played a crucial and not mutually exclusive role.

This sort of multi-causal explanatory frame goes, again, against the rather mythical image of the hard sciences: it simply sounds too "wishy washy." We could point out that it has been exceedingly successful in the social sciences (e.g., the explanation of the "birth order effect" on people's personalities: Sulloway 1997), but of course the social sciences have an even worse reputation as "soft" disciplines than organismal biology does. This, we maintain, is pure epistemological chauvinism, and it is not

warranted by the realities on the ground (see Simberloff 2004 for a similar take on community ecology). Multiple causal explanations, and complex interactions among such explanations, are here to stay, and represent the bread and butter of a serious discipline of evolutionary ecology (Schlichting & Pigliucci 1998). Indeed, one can reasonably argue that good organismal biologists are precisely those people who are inherently interested in complex problems with multiple interacting causes.

3. Invasion biology is "natural history" -- and there is nothing wrong with that.

A corollary of the above discussion is that much invasion biology (and, we maintain, ecology and evolutionary biology more generally) will turn out to be "natural history" (Stearns & Magwene 2003), in the positive sense of what Thomas Kuhn (1970) called "puzzle solving" science. Most science, according to Kuhn, is in fact a matter of solving specific puzzles within the overarching paradigm accepted by practitioners in the field at any given historical period. While only very few scientists are lucky enough to trigger a paradigm shift, there is a vast amount of good science to be done by the rest of us that falls into the more modest, but endlessly fascinating, category of puzzle solving. Especially in a field such as invasion and conservation biology, where the emphasis on applied science is obvious, we shouldn't fall into the trap of demeaning our own work because the "only" thing that it accomplishes is to provide a complex account of how certain taxa happen to be rare or invasive.

Our call, therefore, is at the same time for a more modest and realistic outlook on what the goals of invasion biology research ought to be, and for a more bold assertion of the value of doing science this way. As philosopher Alfred North Whitehead once stated, "It requires a very unusual mind to undertake the analysis of the obvious."

Traits of invasives reconsidered: phenotypic comparisons of introduced invasive and introduced noninvasive plant species within two closely related clades[1]

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In attempting to determine the traits associated with invasive plant species, ecologists have often used species native to the invaded range as "control species." Because many native species themselves are aggressive colonizers, comparisons using this type of control do not necessarily yield information relevant to distinctions between invasive and noninvasive species. Here we implement an alternative study design that compares phenological, architectural, size, and fitness traits of several introduced invasive species to introduced noninvasive species within two genera of Asteraceae (*Crepis* and *Centaurea*). While there were many significant differences between the genera, there were few shared attributes among invasive or noninvasive congeners, even for traits as seemingly important as the number of inflorescences produced and the size of seed heads. Instead, the results suggest that differences in invasiveness between closely related species is better explained as the result of complex trait interactions and specific introduction histories.

Key words: Asteraceae; *Centaurea*; comparative method; *Crepis*; exotic species; invasion success; invasive species; nonindigenous species.

Although the introduction of some species into novel geographical areas can be viewed as beneficial (e.g., crops, horticultural varieties, biological control agents) and by most measures the majority of introductions are in fact benign (Williamson, 1996), there is a great deal of concern regarding the detrimental effects that certain introduced species may cause (Pimentel et al., 2000). Unfortunately, to date there are few identifiable indicators that allow us to proactively weed out the occasional problematic invaders from the crowd of benign and beneficial prospects (Enserink, 1999; Mack et al., 2000; but see Rejmanek and Richardson, 1996; Reichard and Hamilton, 1997; Rejmanek, 2000; Kolar and Lodge, 2001, 2002).

With respect to the traits common to introduced invasive species, Kolar and Lodge (2002) describe a potential paradox in invasion biology, noting that some researchers are content to view this problem as identified and solved, while others believe that invasive traits are largely idiosyncratic and, therefore, predicting which species will be invasive is impossible. Taking

the position that both of these claims are too extreme and in want of support, Kolar and Lodge (2002) suggest, we think correctly, that there may be a more realistic and fruitful middle ground where the prediction of invasiveness is feasible. Finding this middle ground requires the recognition that "invasive species" is not a homogeneous class of natural objects and that progress in this field may be better achieved by focusing our efforts on meaningfully circumscribed groups, be they taxonomic, ecological, biogeographical, or otherwise. The successful identification of local trends regarding invasive species (in lieu of general laws or an endless collection of anecdotes) could be quite valuable to both scientists and public policymakers.

With this mindset of tackling invasion biology in tractable allotments, the importance of delineating appropriate groups for comparison becomes paramount. If identifying appropriate groups of organisms for recognizing local trends among invasive species is the first step, then identifying the proper groups for comparison is the second. With some exceptions (e.g., Rejmanek and Richardson, 1996; Grotkopp et al., 2002; Kolar and Lodge, 2002; Gerlach and Rice, 2003; Mandak, 2003; Bellingham et al., 2004; Burns, 2004; Sutherland, 2004; Lloret et al., 2005), controls for studies of introduced invasives have been selected from among the closely related species (often congeners) native to the region of invasion. The use of closely related species is helpful on two scores: first, it allows one to directly address the issue of the lack of phylogenetic independence (because species are all related to each other at some level, they have a shared history and therefore are not statistically independent data points; Harvey and Pagel, 1991); second, it insures that the differences between study species are not so great as to be essentially uninformative.

On the other hand, the common practice of using native species as a control can lead to misleading comparisons, considering that there are many aggressively colonizing native

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species. A logical alternative can be arrived at when one appreciates that one of the primary goals of invasion biology is to sort out what makes introduced invasive species different from the host of noninvasive *introductions*. To the extent that this is the case, a well-informed study of the issue requires representatives of both groups of interest, that is, introduced invasives and introduced noninvasives. Fortunately, there is often quite a large number of introduced noninvasives from which to choose, though this does not make the task trivial when one begins to focus on particular geographic regions, ecosystems, or taxonomic groups.

In this study we examine phenological, architectural, and fitness trait differences between populations of introduced invasive and introduced noninvasive plant species within two closely related genera of Asteraceae, hypothesizing that variable modes of invasiveness may exist between different evolutionary lineages. Our premises led to the following questions and expectations: (1) Are there differences in traits between introduced invasive and introduced noninvasive species that are consistent across evolutionary lineages? We predicted that differences between introduced invasives and introduced noninvasives that hold up across genera would be largely restricted to fitness or fitness proxy traits (e.g., onset of reproductive maturity, quantity of offspring), due to the likely existence of numerous different multivariate phenotypes that may yield similar fitness values. (2) Are there differences in traits between introduced invasive and introduced noninvasive species that are found only within one clade? We predicted that genus-specific differences between introduced invasives and introduced noninvasives would most likely be found among the phenological (e.g., time to bolting) and architectural (e.g., branching pattern) traits because these trait patterns are expected to be similar between clades. (3) Are there major differences in traits among introduced invasive species within each clade? We predicted that there would be a detectable amount of variability among introduced invasive species within each genus—even in the presence of clade-specific patterns, as the result of idiosyncratic ecological and evolutionary histories.

MATERIALS AND METHODS

Plant material—We chose species of the genera *Centaurea* and *Crepis* (both Asteraceae) for our study based, in part, on our ability to collect multiple invasive and noninvasive introductions to North America within both genera (Table 1). To assess differential rates of a species' range expansion, which we feel is ecologically consistent with the concept of invasiveness (Richardson et al., 2000; Colautti and MacIsaac, 2004), we characterized each species based on the number of U.S. states with a record of its presence in The PLANTS Database (USDA, NRCS, 2002) (invasive species, present in ≥24 of the 48 continental U.S. states; noninvasive species, in ≤16 states). Intrastate level (county by county) presence/absence records confirm our characterization of the invasive status at this smaller spatial scale (i.e., for the species used in this study, invasives are largely widespread both across and within states, while noninvasives are largely restricted at both scales; Appendix S1, see Supplemental Data accompanying online version of this article). Estimates of the dates of introduction to North America from native Eurasian ranges are given as the earliest found herbarium record compiled from various North American herbaria and available floras (Table 1). Some of these dates no doubt underestimate the actual age of the introduction, and it is also possible that some dates represent populations that did not naturalize and, in some sense, overestimate the relevant age of the introduction.

In every study such as this one, the problem arises of agreeing on what criteria to use to designate certain species as "invasive" and others as "noninvasive." While we applaud explicit justifications in characterizing the

invasiveness of study species, we intentionally avoided the use of "noxious weed" lists, which use a variety of criteria for inclusion, often stressing (quite reasonably from a policy perspective) impacts of human interest over other ecological properties or demographic patterns. While our reliance on databases that use herbaria records could possibly exaggerate or underestimate the distribution of various species (Wu et al., 2005), the same is true of noxious weed lists where, depending on the specific case, a species can be listed without any evidence of it occurring in a given state or be absent from a list despite being quite common.

Additional problems that hamper attempts to characterize invasiveness (including the current study and the vast majority of previous studies) include (1) the reality that invasiveness is more accurately a continuous variable, by no means intrinsically categorical; (2) the fact that "invasiveness" may change through time, as indicated by the "lag time" that is often noted for invasive species; and (3) the difficulty in estimating the rate of spread due to the absence of information on the frequency and localities of a species' introduction. These problems are worth noting and deserve further investigation when possible. However, the inability of most invasion studies to address these problems does not invalidate the utility of such studies, nor does it place invasion biology outside the norm of biology in the degree to which we must logistically treat various aspects of our systems as "all else being equal."

While the intraspecific phylogenetic relationships within *Crepis* and *Centaurea* are poorly known (Babcock, 1947; Susanna et al., 1995; Whitton et al., 1995; Garcia-Jacas et al., 2001), there is little doubt that the genera themselves represent separate clades (at the level of tribe or subfamily; Bremer, 1994; Bayer and Starr, 1998; Panero and Funk, 2002).

All study species are short-lived, predominantly annual, herbaceous plants occurring in a variety of disturbed habitats. The degree of phenotypic similarity between genera allowed us to directly compare a large number of traits potentially relevant to invasion. Twenty-six different seed accessions of the nine study species were obtained from wild-collected populations, European botanical gardens, and North American collaborators (see Table 1 for details).

Plant handling and experimental setup—For all 26 populations, 2–4 seeds (based on availability) were planted into each of ten 4 × 4 × 4.5 cm starter pots with standard autoclaved Pro-mix potting soil (Premier Horticulture, Red Hill, Pennsylvania, USA) and placed in the University of Tennessee White Avenue greenhouse. Ambient lighting and photoperiod were augmented with greenhouse lights for 16 h per day. Once established, individual seedlings were transplanted into 13 × 13 × 13.5 cm pots and distributed in a randomized block design comprising 10 complete blocks. Planting was carried out in October and November 2001.

All traits were measured at the individual level on plants transplanted to larger pots. These traits included (1) days to bolting, a measure of time spent in the vegetative stage; (2) days from bolting to initial flowering (anthesis), a measure of time until reproductive maturity; (3) days from flowering to initial dehiscence, an estimate of time until seed dispersal; (4) number of rosette leaves at bolting, an estimate of investment in the vegetative phase of growth; (5) number of basal stems, a component of plant architecture; (6) stem diameter (size); (7) stem length (size); (8) branch order (number of branch nodes encountered in tracing the longest stem backwards to the rosette), another characterization of plant architecture during the reproductive phase; (9) above ground biomass (dry mass), a measure of overall growth; (10) involucre diameter, a component of reproductive fitness; and (11) number of inflorescences, another component of reproductive fitness.

Data analysis—Data were checked for violations of assumptions of normality and homoscedasticity (days to initial flowering and number of basal stems were log transformed, and a power transformation was applied to number of inflorescences) and analyzed with a two-way analysis of variance (ANOVA). Calculation of sums of squares and significance tests were carried out using JMP (SAS Institute, Cary, North Carolina, USA), version 5.1. For each trait, the full model included the following main effects and interactions: genus (overall differences between *Centaurea* and *Crepis*, treated as a fixed effect), provenance (to account for overall differences between European and North American collected populations, fixed effect), invasive status (overall differences between invasive and noninvasive groups, fixed effect), block (microenvironmental effects, random), genus × invasive status (i.e., differences between invasive and noninvasive species that are specific to individual genera, fixed), species nested within genus × invasive status (i.e., differences between species within each genus/invasive status combination, random), population

TABLE 1. Species, invasive status (as characterized for this study, see Materials and Methods for details), life history characterization (compiled from The PLANTS Database [USDA, NRCS, 2002]) as annual (a), biennial (b), or perennial (p), earliest known record for North America, and population accession source data for the 26 populations of *Centaurea* and *Crepis* used in this study.

Species	Invasive status	Life history	Date of introduction	Population source
Centaurea calcitrapa L.	Noninvasive	a,b,p	1827	Wild collected by J. DiTomaso (CA, USA)
				Botanical Garden, University of Göttingen, Germany
				Civico Orto Botanico, Trieste, Italy
				Kew Royal Botanical Gardens, UK
Centaurea cyanus L.	Invasive	a	1880	Wild collected by NZM (Blount Co., TN, USA)
				Wild collected by NZM (Knox Co., TN, USA)
				Botanical Garden, University of Göttingen, Germany
				Kew Royal Botanical Gardens, UK
				National Botanical Garden of Belgium
Centaurea diluta Aiton	Noninvasive	a	1952	Kew Royal Botanical Gardens, UK
Centaurea solstitialis L.	Invasive	a	1879	Wild collected by J. DiTomaso (CA, USA)
				Civico Orto Botanico, Trieste, Italy
Centaurea sulphurea Willd.	Noninvasive	a	1923	Wild collected by J. DiTomaso (CA, USA)
Crepis capillaris (L.) Wallr.	Invasive	a,b	1903	Wild collected by NZM (Haywood Co., NC, USA)
				Wild collected by NZM (Johnson Co., TN, USA)
				National Botanical Garden of Belgium
				University Botanical Garden, Marburg, Germany
Crepis setosa Haller f.	Noninvasive	a	1904	Wild collected by NZM (Polk Co., OR, USA)
				Wild collected by A. Liston (Benton Co., OR, USA)
				Botanical Garden, University of Göttingen, Germany
				National Botanical Garden of Belgium
Crepis tectorum L.	Invasive	a	1891	Wild collected by NZM (Dane Co., WI, USA)
				Wild collected by NZM (Marquette Co., WI, USA)
				Botanical Garden, University of Göttingen, Germany
				Friedrich-Schiller-Universitat, Jena, Germany
Crepis zacintha (L.) Babcock	Noninvasive	a	1993	Botanical Garden, University of Göttingen, Germany

nested within species (i.e., differences between populations of the same species, random), and error (residual variance).

To reduce the likelihood of our not detecting significant effects (Type II error), we have chosen to highlight all tests where the associated *P* value is less than the typical 0.05. After Moran (2003), in lieu of using the Bonferroni correction (normally applied to maintain the overall probability of committing a Type I error), we report the likelihood (*p*) of finding a particular number of significant test results below our α value (*K*), given the total number of tests performed (*N*), by the following formula:

$$p = [N!/(N - K)!K!]\alpha^K (1 - \alpha)^N - K$$

In addition to the formal *p* values, we report effect sizes (in the form of the sums of squares) and the power of the tests (Cohen, 1992) and discuss the results from a statistically conservative perspective.

Power analyses were carried out using G·Power (Buchner et al., 1997) on all ANOVA tests. We followed the conventions of Cohen (1992) and evaluated whether we had the statistical power to detect "medium" and "small" effects caused by our treatments. "Medium" effect size (ES) means that treatment differences are "visible to the naked eye of a careful observer" (e.g., the invasive plants have visibly more fruits than the noninvasive plants), whereas "small" ES means that the treatment differences are "noticeably smaller than the medium but not so small as to be trivial" (e.g., the invasive plants have more fruits than the noninvasive plants, but it is not as obvious) (Cohen, 1992). This analysis accounts for the possibility that an effect may be statistically nonsignificant, not because the effect is actually biologically insignificant, but rather is due to limited sample size. Power values range from 0 to 1 and was calculated for each effect in the model, based upon the degrees of freedom and the ES (small, medium, or large) of interest. Power values of 0.8 and higher are considered to be sufficient to conclude that there was enough statistical power to detect an effect of the size of interest (Cohen, 1992).

To examine the multivariate relationships among traits, we performed a discriminant analysis on the characters for which we had data for all species (days to bolting, days from bolting to flowering, number of leaves, number of basal stems, stem length, stem diameter, branch order, biomass, involucre diameter, and number of inflorescences). Different plots in canonical

components space allowed us to identify which characters most contributed to identifying plants based on the combined criteria of genus and invasive status. These analyses were also carried out using JMP, version 5.1.

RESULTS

Phenological traits—An analysis of variance showed that the main effect of genus was significant for one of three phenological traits (days from bolting to flowering; Table 2). While there were no significant differences in time to bolting, upon bolting *Crepis* development was, in general, more rapid (Fig. 1).

The nested species effect was significant for both days from bolting to flowering and days from flowering to dehiscence (Table 2). While there were significant differences among congeners in both genera for these two traits, these differences were not related to the invasive status of the species (Fig. 1). The involucres of *Centaurea calcitrapa* and *Crepis zacintha* remained closed throughout the experiment and were therefore excluded from analysis for days to initial dehiscence.

The main effect of provenance explained relatively little of the variance in the phenological traits (Table 2), although it was significant for days to flowering (on average introduced plants flowered 2 days earlier than those collected from the range of origin) and days from flowering to seed set (on average introduced plants set seed 6 days later than those collected from the range of origin). The nested population effect was significant for days to bolting, indicating a moderate degree of variation for this trait within some of the study species (Table 2). There were no significant invasive status,

TABLE 2.   ANOVAs for each phenological trait (columns) and factor (rows) in the experiment with *Centaurea* and *Crepis*. For each factor, we report the degrees of freedom (df), sums of squares (SS), P values, and power (for medium [0.15] and small [0.02] effect sizes). Statistically significant effects at the $P < 0.05$ value are in boldface. R^2 refers to the amount of variance explained by the entire model for a particular trait.

Model effect	Statistic	Days to bolting	Days from bolting to flowering	Days from flowering to dehiscence
R^2		0.58	0.82	0.63
Genus	SS	19045	**7090**	3110
df = 1, 3–5	P	0.1546	**0.0164**	0.1187
	Power med., small	0.96, 0.28	**0.96, 0.28**	0.78, 0.19
Provenance	SS	~0	176	**782**
df = 1, 8	P	0.9938	**0.0058**	**<0.0001**
	Power med., small	0.98, 0.32	**0.98, 0.32**	**0.83, 0.21**
Invasive status	SS	11204	3	1104
df = 1, 3–5	P	0.2550	0.9420	0.3133
	Power med., small	0.96, 0.28	0.96, 0.28	0.78, 0.19
Genus × invasive status	SS	2590	171	314
df = 1, 3–5	P	0.5636	0.6053	0.5760
	Power med., small	0.96, 0.28	0.96, 0.28	0.78, 0.19
Species [genus, invasive status]	SS	33907	2813	**2636**
df = 3–5, 8	P	0.1503	**0.0006**	**<0.0001**
	Power med., small	0.87, 0.18	**0.87, 0.18**	**0.78, 0.15**
Population [species, genus, invasive status, provenance]	SS	**24362**	286	257
df = 8, 113–144	P	**<0.0001**	0.1325	0.5235
	Power med., small	**0.92, 0.17**	0.92, 0.17	0.82, 0.14
Block	SS	2547	180	264
df = 8, 113–144	P	0.8153	0.4373	0.5014
	power med., small	0.92, 0.17	0.92, 0.17	0.82, 0.14
Residual, df = 113–144	SS	83023	3235	4050

genus × invasive status, and block effects for any of the phenological traits.

Architectural and size traits—The main effect of genus was significant for stem diameter and number of rosette leaves and marginally significant for number of basal stems (Table 3). *Crepis* species had more rosette leaves, and more, but thinner basal stems than *Centaurea* species (Fig. 2).

The nested species effect was significant for number and diameter of basal stems and for branch order and marginally significant for stem length (Table 3). While there were large differences among congeners for both the number of basal stems and branch order, the nature of these differences did not correspond to the invasive status of the species (Fig. 2).

The main effect of provenance was significant for the number of leaves at bolting (on average, introduced plants had 11 fewer rosette leaves than those collected from the range of origin, Table 3). The nested population effect was significant for number and length of basal stems, number of rosette leaves, and biomass, indicating a moderate degree of variation for this trait within some of the study species (Table 3).

The significant block effect on biomass (Table 3) was most likely the result of mortality of some of the smallest species in some blocks coupled with a loss of some of the larger species in others, although it may also have been due to microenvironmental heterogeneity in the greenhouse setting. There were no significant invasive status or genus × invasive status effects for any of the architectural or size traits.

Fitness traits—Analyses of variance of the fitness traits showed that the main effect of genus was marginally significant for involucre diameter (Table 4). *Centaurea* species generally had larger seed heads than *Crepis* species (Fig. 3).

The nested species effect was significant for both involucre diameter and number of inflorescences (Table 4). While there were significant differences among congeners for both involucre diameter and number of inflorescences, the nature of these differences did not correspond to the invasive status of the species (Fig. 3).

The nested population effect also was significant for involucre diameter, indicating a moderate degree of variation for this trait within some of the study species (Table 4). There were no significant provenance, invasive status, genus × invasive status, or block effects for any of the fitness proxy traits.

Given that for the least consistently significant of the model effects (excepting the block and the provenance effects for which we had no a priori expectations), we found three significant tests ($K = 3$) for a specific effect (as for the genus main effect) of 11 total tests ($N = 11$) (Tables 2–4), the probability that this would happen by chance is $p = 0.01$ (Moran, 2003). Because we found more than three significant results for all other effects showing significance, we feel confident that our analyses are robust against Type I errors.

Multivariate analysis—A discriminant analysis performed on the genus × invasive status combinations (i.e., invasive *Centaurea*, noninvasive *Centaurea*, invasive *Crepis*, and noninvasive *Crepis*) yielded a first discriminant function that primarily separated the groups according to genus (accounting for 88.7% of the variance; Fig. 4a, b). A second discriminant function largely separated invasive *Crepis* from noninvasive congeners (6.8% of explained variance; Fig. 4a), and a third discriminant function partially distinguished invasive *Centaurea* from noninvasive congeners (4.5% of total explained variance; Fig. 4b).

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Fig. 1. Least squared means for phenological traits measured for established, potted seedlings of invasive (filled circles) and noninvasive (open circles) species of *Centaurea* and *Crepis*. Bars represent 95% confidence intervals. Symbols sharing a letter are not statistically different from one another (overall α = 0.05).

The first function discriminated primarily by a linear combination of number of leaves at bolting (standardized coefficient = −1.21), days from bolting to flowering (0.74), number of inflorescences (−0.68), involucre diameter (0.54), and stem diameter (0.50). The second function discriminated by a combination of the number of inflorescences (1.66), days from bolting to flowering (0.79), involucre diameter (0.54), number of basal stems (−0.52), and stem length (−0.50). The third function discriminated by involucre diameter (−0.95),

number of leaves at bolting (−0.73), branch order (0.65), days from bolting to flowering (0.47), and stem diameter (0.46).

DISCUSSION

Overall, our results suggest that while there are different characteristic phenotypes within *Centaurea* and *Crepis*, there are few phenotypic traits that distinguish invasive and noninvasive introduced species in these genera. This lack of a distinguishable invasive phenotype was apparent across genera (i.e., phenotypes shared by invasive species at the subfamily or tribe level) and within them (i.e., genus-specific invasive species patterns). Despite the fact that much of the variation in traits occurred between the two genera, there was substantial variation among congeners for many traits. Given this, any causal explanation of variation in invasion success related to "traits of invasives" must either reside in traits not examined in this study or as more complex interactions of the traits assessed here. Of course, these possibilities are not mutually exclusive.

Phenological traits—In his list of the traits of the hypothetical ideal weed, Baker (1965) suggests that, all else being equal, invasive species might be expected to have rapid seedling growth and spend less time in the vegetative condition. This is quite reasonable because shortened development times might increase the number of generations per year (in annuals), reduce the likelihood of mortality before reproductive maturity, or both. While it has been claimed that a reduced juvenile period is widespread among invasive species (Rejmanek and Reichard, 2001), appropriately controlled and replicated studies have been largely restricted to long-lived species (Richardson et al., 1990; Rejmanek and Richardson, 1996; Grotkopp et al., 2002; Bellingham et al., 2004) or measures of relative growth rate (Burns, 2004).

In the current study we found no detectable differences in the phenologies of introduced invasive species when compared to introduced noninvasives, across or within the two genera examined. On average, the development of an invasive *Crepis* or *Centaurea* was no more rapid than that of a noninvasive congener (Fig. 1).

Architectural and size traits—Despite the aforementioned lack of shorter developmental schedules of invasive species in our study system, it is possible to imagine that invasives could still grow larger, more robust plants than noninvasives in the same growing season. With respect to the height of invasive species, a review of empirical studies by Kolar and Lodge (2001) provides limited evidence that, in some cases, invasive species are taller than native or noninvasive species. However, in the recent analysis of Lloret et al. (2005) there is no observed relationship between invasiveness and growth form or stem height within the alien flora of the Mediterranean islands.

Our results are similar to those of Lloret et al. (2005); we found no detectable correlations with introduced species invasiveness in any of the size or architecture traits examined. There was very little variation among congeners with respect to stem length or diameter, and where there was appreciable variation among congeners for architectural traits (number of

TABLE 3.   ANOVAs for each architectural and size trait (columns) and factor (rows) in the experiment with *Centaurea* and *Crepis*. For each factor, we report the degrees of freedom (df), sums of squares (SS), *P* values, and power (for medium [0.15] and small [0.02] effect sizes). Statistically significant effects at the $P < 0.05$ value are in boldface. R^2 refers to the amount of variance explained by the entire model for a particular trait.

Model effect	Statistic	No. of basal stems	Stem diameter	Stem length	No. of leaves	Branch order	Biomass
R^2		0.64	0.66	0.60	0.79	0.45	0.49
Genus	SS	36	**112**	12671	**33633**	37	33
df = 1, 5, power med., small (0.96, 0.28)	P	0.0621	**0.0081**	0.1014	**0.0170**	0.5502	0.5686
Provenance	SS	~0	2	76	**2966**	3	2
df = 1, 7–8, power med., small (0.98, 0.31)	P	0.9937	0.1517	0.5237	**0.0002**	0.5034	0.7352
Invasive status	SS	~0	~0	8	10843	94	127
df = 1, 5, power med., small (0.96, 0.28)	P	0.8962	0.9454	0.9612	0.1026	0.3550	0.2866
Genus × invasive status	SS	1	~0	202	544	3	2
df = 1, 5, power med., small (0.96, 0.28)	P	0.7003	0.9817	0.8104	0.6736	0.8620	0.8993
Species [genus, invasive status]	SS	**32**	31	15770	13626	**451**	448
df = 5, 7–8, power med., small (0.70, 0.13)	P	**<0.0001**	**0.0248**	0.0771	0.1743	**0.0009**	0.1337
Population [species, genus, invasive status, provenance]	SS	**7**	10	**8222**	**8905**	52	**303**
df = 7–8, 145–146, power med., small (0.94, 0.18)	P	**0.0077**	0.1747	**<0.0001**	**<0.0001**	0.4338	**0.0054**
Block	SS	2	5	1293	1523	42	**992**
df = 8, 145–146, power med., small (0.94, 0.18)	P	0.7361	0.6717	0.5403	0.4797	0.5854	**<0.0001**
Residual, df = 145–146	SS	54	129	27016	29140	944	1924

basal stems and branches), no apparent trends distinguished invasive species from noninvasive ones (Fig. 2).

Fitness traits—It seems logical that invasive species would differ significantly from noninvasive species with respect to fitness traits, particularly if they are similar in many other respects, as is largely the case among our congeners here. Indeed, Baker (1965) included this on the list of traits of the ideal weed (partitioned into high seed output, seed output under a wide range of environmental conditions, and seed and vegetative components of reproduction). While some researchers have examined seed mass in similarly controlled studies (Rejmanek and Richardson, 1996; Grotkopp et al., 2002; Lloret et al., 2005) and others have considered survival (Gerlach and Rice, 2003; Bellingham et al., 2004) or germination rate (Gerlach and Rice, 2003; Mandak, 2003), only Gerlach and Rice (2003) were able to measure lifetime reproductive output.

Gerlach and Rice (2003) found appreciable differences in the number and mass of inflorescences in their field study (including both *C. solstitialis* and *C. sulphurea*, also used here), and these differences corresponded, to a degree, with the invasive status of the species in question (the most invasive species, given as *C. solstitialis,* had both more numerous and massive inflorescences than less or noninvasive species; however, the moderately invasive *C. melitensis* did not differ from the noninvasive *C. sulphurea* with respect to the number of inflorescences and had less massive seed heads). Our results, while indicating significant variability among congeners in involucre diameter and number of inflorescences, revealed no general pattern with respect to invasive status (Fig. 3). Although the species in each genus that produced the most inflorescences was an invasive, there were also invasive species that did not differ significantly from the congener with the lowest average reproductive output. In the particular case of *C. solstitialis* and *C. sulphurea*, we did not detect a significant difference in the number of inflorescences (Fig. 3), as Gerlach and Rice (2003) observed in their study of these species. The

substantial differences in growing conditions between our study (largely benign greenhouse conditions) and that of Gerlach and Rice (multiple field environments) may explain these discrepancies.

Limitations—Our common garden study differs significantly from most previous assessments of the traits of invasive species in that the inclusion and replication of introduced noninvasive species allowed us to explicitly address the differences between successful and unsuccessful invasions. While this is important, our methods were not without drawbacks. The common garden design and directed taxonomic focus left us with a suite of species that largely had similar life histories, necessarily reducing our ability to comment on the likely importance of these traits. We recognize that variation in life history traits not examined here (e.g., perennation, reproductive biology, vegetative habit, dispersal mechanisms) may often trump variation in quantitative traits when trying to explain range or habitat expansion (see, for example, Gerlach and Rice, 2003; Mandak, 2003; Sutherland, 2004; Lloret et al., 2005). However, that the suite of species used here largely shares a common reproductive biology, growth form, and habitat, yet includes both invasives and noninvasives, suggests that more than life history variation contributes to differential invasiveness.

Another consequence of our common garden approach is that we necessarily included fewer species than broader comparative analyses of data compiled from multiple field, garden, greenhouse, herbaria, floras, or other accounts (e.g., Sutherland, 2004; Lloret at al., 2005). While this limits our ability to generalize, it helps us avoid the confounding effects resulting from variation in the environments, localities, or studies from which more comprehensive species accounts may be assembled.

The current study is also limited in its characterization and comparison of species because all plants were grown in a single, largely benign, greenhouse environment. Our understanding of the role of phenotypic plasticity in the differential success of plant invasions, addressed as early as 40
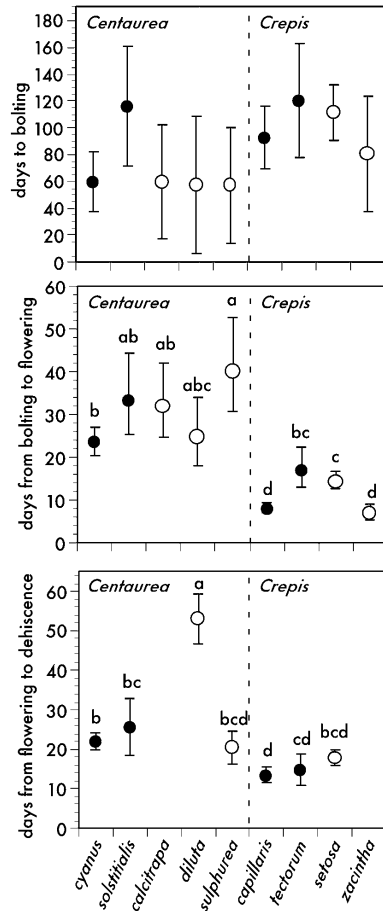
Fig. 2. Least squared means for architectural and size traits measured for established, potted seedlings of invasive (filled circles) and noninvasive (open circles) species of *Centaurea* and *Crepis*. Bars represent 95% confidence intervals. Symbols sharing a letter are not statistically different from one another (overall α = 0.05). Letters are omitted for stem diameter, stem length, number of leaves, and biomass; differences were statistically significant only between members of different genera.

years ago by Baker (1965), has recently been expanded by various empirical studies (Williams and Black, 1994; Pattison et al., 1998; Milberg et al., 1999; Schweitzer and Larson, 1999; Kaufman and Smouse, 2001; Gerlach and Rice, 2003; Burns, 2004; DeWalt et al., 2004; Gleason and Ares, 2004; Suding et al., 2004; Wilson et al., 2004; Brock et al., 2005; Hastwell and Panetta, 2005; Leishman and Thomson, 2005). We plan to broaden our own efforts by exploring the sensitivities of the current findings to relevant environmental variability.

Conclusions—That we did not detect any general relationships between the invasive status and individual quantitative traits (fitness or otherwise) is surprising given how similar (and hence comparable) the study species are with respect to life history and growth form. Insofar as this study successfully maximized the degree of "all else being equal" among our

invasive and noninvasive introductions, these results suggest the following: (1) The differences between closely related invasive and noninvasive introductions may not be properties of the species, as much as they are the results of specific and contingent introduction histories (Colautti and MacIsaac, 2004; Puth and Post, 2005). (2) To the extent that causally relevant phenotypic differences exist between closely related invasive and noninvasive, introduced species, these differences are still likely to be the result of multiple trait interactions and do not necessarily result from straightforward fitness differences (Rejmanek, 2000; Grotkopp et al., 2002).

While the study of species invasions will continue to benefit from more in-depth case studies, the appropriateness of "control species" will be a critical factor determining how much any analysis contributes to our understanding of specific cases and general patterns.

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TABLE 4. ANOVAs for each fitness proxy trait (columns) and factor (rows) in the experiment with *Centaurea* and *Crepis*. For each factor, we report the degrees of freedom (df), sums of squares (SS), P values, and power (for medium [0.15] and small [0.02] effect sizes). Statistically significant effects at the $P < 0.05$ value are in boldface. R^2 refers to the amount of variance explained by the entire model for a particular trait.

Model effect	Statistic	Involucre diameter	No. of inflorescences
R^2		0.96	0.76
Genus	SS	705	48781
df = 1, 5, power med., small (0.96, 0.28)	P	0.0850	0.2409
Provenance	SS	1	2155
df = 1, 5, power med., small (0.98, 0.31)	P	0.2388	0.0683
Invasive status	SS	245	6836
df = 1, 5, power med., small (0.96, 0.28)	P	0.2623	0.6396
Genus × invasive status	SS	5	2333
df = 1, 5, power med., small (0.96, 0.28)	P	0.8599	0.7828
Species [genus, invasive status]	SS	**768**	**137826**
df = 5, 8, power med., small (0.70, 0.13)	P	**<0.0001**	**<0.0001**
Population [species, genus, invasive status, provenance]	SS	**15**	8118
df = 8, 146, power med., small (0.94, 0.18)	P	**0.0012**	0.1706
Block	SS	8	2338
df = 8, 146, power med., small (0.94, 0.18)	P	0.0866	0.5877
Residual, df = 146	SS	80	639
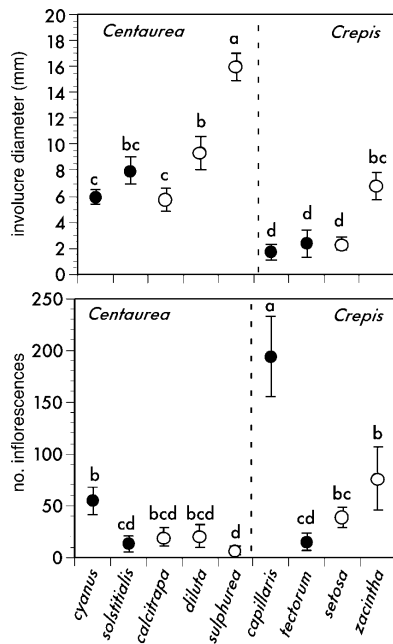
Fig. 3. Least squared means for fitness traits measured for established, potted seedlings of invasive (filled circles) and noninvasive (open circles) species of *Centaurea* and *Crepis*. Bars represent 95% confidence intervals. Symbols sharing a letter are not statistically different from one another (overall α = 0.05).
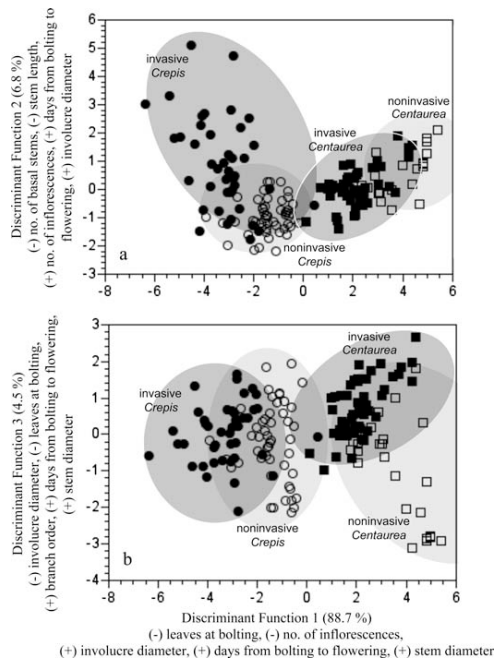
Fig. 4. Plots for analysis of the first and second discriminant functions (top panel), and first and third discriminant functions (bottom) to distinguish genus × invasive status combinations for *Centaurea* (squares) and *Crepis* (circles). Invasives are indicated by closed symbols, noninvasives are indicated by open symbols. Percentage of variance explained by each discriminant function follows parenthetically. Axes symbols indicate positive (+) or negative (-) weights of the variable on the discriminant functions.

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Implementation of a novel framework for assessing species plasticity in biological invasions: responses of *Centaurea* and *Crepis* to phosphorus and water availability

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Summary

1 It is widely considered that phenotypic plasticity is important to species invasiveness. However, few empirical studies have expressly assessed the relationship between species invasiveness and their responses to environmental variability. Thoughtfully incorporating phenotypic plasticity into studies of invasiveness requires explicit links among appropriate environmental variables, traits relevant to invasion success, and comparison groups of species, populations or genotypes (competitors or close relatives) that place focal species in context.

2 We examine trait responsiveness in introduced species of *Crepis* and *Centaurea* (Asteraceae) that have been characterized by different degrees of success. Specifically, we assess the extent to which species are robust in the face of harsh environments and opportunistically responsive to favourable conditions.

3 We exposed all species to water and phosphorus availability treatments in a common glasshouse experiment and report the responses of phenological, architectural, size and fitness traits. We predicted that, compared with less invasive congeners, invasive species would more often display robust fitness in harsh environments (here, drought and no phosphorus addition) and would also be more responsive to favourable conditions (no drought and phosphorus additions).

4 In both *Crepis* and *Centaurea* we found evidence of greater stress tolerance to low phosphorous among the more invasive congeners, albeit for different components of fitness. Contrary to expectations, we observed no relationship between invasiveness and opportunism. Overall, trait responses were highly variable and largely idiosyncratic with respect to invasive categorization.

5 Consistent with basic expectations, across-environment species performance largely corresponded to degree of invasiveness.

6 Our results suggest that, even among closely related species, relationships between invasiveness and phenotypic plasticity do not necessarily reveal consistent patterns, nor do they conform to simple theoretical expectations. We suspect that phenotypic plasticity may indeed play an important role in many species invasions, but the breadth of relevant factors (which genotypes, which populations, which traits, which environments) reduces the likelihood of detecting robust general patterns.

Key-words: biological invasions, *Centaurea*, *Crepis*, drought, environmental variation, invasiveness, jack-of-all-trades, non-indigenous species, phenotypic plasticity, phosphorous

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Introduction

The current prevalence of introduced species provides biologists with an abundance of study systems that are both scientifically interesting and practically important.

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While the potential costs of some introductions is frequently cited as justification for their study (Wilcove *et al.* 1998; Pimentel *et al.* 2000), many more introductions are seemingly benign (Williamson 1996), yet still of great biological interest in terms of our understanding of the general phenomenon of range expansion and rapid evolution under novel conditions. These cases present dramatic 'natural experiments', which can be used to study the dynamics of dispersal, competition, community assembly, hybridization, natural selection, phenotypic plasticity (the focus of the current study), and many other aspects of ecology and evolution.

Although considerable attention has been devoted to determining what traits may make introduced invasive species successful, the vast majority of these studies have compared established introductions to native species. While this can certainly be an interesting comparison (particularly when the introduced and native species are either closely related, recently sympatric or both), more recently studies have begun to systematically compare species or population introductions of differential success (e.g. rate or extent of spread, degree of ecological impact); this potentially yields insight into what separates the relatively few invasive aliens from the host of failed or restricted introductions (Rejmanek & Richardson 1996; Grotkopp *et al.* 2002; Kolar & Lodge 2002; Gerlach & Rice 2003; Mandak 2003; Bellingham *et al.* 2004; Burns 2004; Sutherland 2004; Hastwell & Panetta 2005; Lloret *et al.* 2005; Muth & Pigliucci 2006).

Phenotypic plasticity, the capacity of a genotype to express different phenotypes in response to varied environmental conditions (reviewed in Pigliucci 2001; Miner *et al.* 2005; Bradshaw 2006), is thought to be a type of trait particularly relevant to the differential success of introductions. While the theoretical importance for the role of phenotypic plasticity in species introductions was proposed as early as Baker (1965), and has since been widely supposed to play a role in numerous cases, comparative empirical studies remain relatively few (Williams & Black 1994; Pattison *et al.* 1998; Milberg *et al.* 1999; Schweitzer & Larson 1999; Kaufman & Smouse 2001; Gerlach & Rice 2003; Burns 2004; DeWalt *et al.* 2004; Gleason & Ares 2004; Suding *et al.* 2004; Wilson *et al.* 2004; Brock *et al.* 2005; Hastwell & Panetta 2005; Leishman & Thomson 2005; reviewed in Richards *et al.* 2006). Furthermore, general hypotheses regarding the expectations of phenotypic plasticity among differentially successful introductions are not often well articulated (Richards *et al.* 2006).

A common sentiment seems to be that greater plasticity should contribute to the success of introduced invasive species (e.g. Kaufman & Smouse 2001; DeWalt *et al.* 2004). However, as particular trait plasticities may be adaptive, maladaptive, or neutral in their effects, a general expectation of 'greater plasticity' is not sufficiently specific, and in fact may be misleading. Furthermore, because plasticity is the property of specific traits in response to specific environmental variation, not a general property of a genotype, a more useful framework should provide a means to establish relevant contexts and expectations. A general method to assess differential phenotypic plasticity among introduced species was recently introduced by Richards *et al.* (2006). The important elements of this framework can be illustrated in the form of two crucial distinctions regarding the plasticity of certain types of traits.

First, drawing on ideas presented by Baker (1965), Richards *et al.* (2006) make explicit that there are two aspects to the response of fitness traits to environmental variation that might lead to the success of a particular introduction: (i) the ability to maintain fitness across a broad range of environmental conditions, also thought of as fitness homeostasis (Hoffman & Parsons 1991; Rejmanek 2000) or robustness; and (ii) the ability to increase fitness in favourable environments, or fitness opportunism.

For purposes of distinguishing possible ecological responses, Richards *et al.* (2006) present three idealized scenarios: (i) a fitness response or norm of reaction that exhibits less reduced fitness under harsh conditions, a 'Jack-of-all-trades'; (ii) one that exhibits a more plastic increase in fitness in favourable environments, a 'Master-of-some'; and (iii) one that exhibits aspects of both (in response to the same environmental variable), a 'Jack-and-Master'. While some previous studies have framed their analyses of plasticity around expectations of stress tolerance (Williams & Black 1994; Schweitzer & Larson 1999; Suding *et al.* 2004; Brock *et al.* 2005), and others have expressly looked for aspects of opportunistic responses (Burns 2004; Hastwell & Panetta 2005; Leishman & Thomson 2005), only a handful of studies have discussed the potential for both stress tolerance and opportunistic responses (Pattison *et al.* 1998; Milberg *et al.* 1999; Gerlach & Rice 2003).

Another relevant contribution present in Baker (1965) and revisited by Richards *et al.* (2006) is the distinction between fitness plasticity and plasticities of underlying traits. As there is no necessary relationship between fitness norms of reaction and those of underlying traits, only appropriately designed empirical studies will be able to discern whether certain trait plasticities are adaptive or causally relevant to a successful introduction.

In this study we employ the framework introduced by Richards *et al.* (2006) by comparing the plasticities of phenological, architectural and reproductive (fitness proxy) traits of two sets of introduced congenerics to environmental gradients thought to contribute to their differential success. In order to strike a productive balance between precision and generality we report the average response of species (using multiple populations of each) to estimate plasticity. While this necessarily results in averaged norms of reactions from an unknown combination of potentially variable individual genotypic responses, it allows us to look at a much broader survey of responses than would have been possible if we restricted ourselves to replicating genotypes.

Table 1 Species list, invasive status (as characterized for this study, see text for details); life-history characterization as annual (a), biennial (b), or perennial (p); earliest known record for North America; and population accession source data for the 17 populations used in this study

Species	Invasive status	Life history	Date of introduction	Population source
Centaurea calcitrapa L.	Low	a, b, p	1827	Wild collected by J. DiTomaso (CA, USA)
				Wild collected by E. Grotkopp (Solano Co., CA, USA)
Centaurea cyanus L.	High	a	1880	Wild collected by NZM (Blount Co., TN, USA)
				Wild collected by NZM (Knox Co., TN, USA)
Centaurea melitensis L.	Moderate	a, b	1881	Wild collected by J. DiTomaso (CA, USA)
				Wild collected by M. Pitcairn (Solano Co., CA, USA)
Centaurea solstitialis L.	High	a	1879	Wild collected by J. DiTomaso (CA, USA)
				Wild collected by E. Grotkopp (Solano Co., CA, USA)
Crepis capillaris (L.) Wallr.	High	a, b	1903	Wild collected by NZM (Johnson Co., TN, USA)
				Wild collected by J. DiTomaso (Humboldt Co., CA, USA)
Crepis rubra L.	Low	a	1960	Botanical Garden of the University of Copenhagen, Denmark
				Botanical Garden of the University of Gottingen, Germany
Crepis setosa Haller f.	Low	a	1904	Wild collected by NZM (Polk Co., OR, USA)
				Wild collected by NZM (Benton Co., OR, USA)
Crepis tectorum L.	Moderate	a	1891	Wild collected by NZM (Marquette Co., WI, USA)
				Botanical Garden of the University of Copenhagen, Denmark
Crepis vesicaria L.	Low	a, b	1932	Wild collected by N. Chiariello (San Mateo Co., CA, USA)

It has been proposed that the success of certain *Centaurea* species (Asteraceae) may be the result of their ability to exploit phosphorus (P) rich environments (LeJeune & Seastedt 2001; Suding *et al.* 2004) and their ability to tolerate drought conditions (for *C. solstitialis*, Dukes 2002). Accordingly, we experimentally manipulated P levels and drought conditions for four introduced *Centaurea* species of varied invasion success, as well as for five introduced *Crepis* species (also Asteraceae), which broadly share many ecologically relevant traits and much of their evolutionary history (Table 1).

Our premises lead to the following questions and expectations. (i) Do invasive introductions exhibit any of the proposed fitness responses? We predicted that, within our environmental context, the more invasive *Centaurea* species would be more likely to fit one of the proposed scenarios than either the less successful *Centaurea* introductions or any of the *Crepis* species (as neither environmental variable has previously been suggested to account for the success of *Crepis* introductions). (ii) Do any of the species conform to different scenarios for different fitness components in the same environmental gradient? As this might require more flexibility in phenotypic development, we predicted that this would be restricted to the most successful of introduced species. (iii) Do any of the species exhibit different aspects of different scenarios for the same fitness component in different environmental gradients? Again, we predicted that this would be restricted to the most successful of introduced species. (iv) Are there any consistent relationships between the response of fitness components and underlying phenological or architectural traits? We predicted that traits developmentally more closely linked to fitness components would display qualitatively similar responses to those of fitness components in all species (e.g. in our system,

number of basal stems and branch order), while traits less directly tied to the development of measured fitness components (e.g. phenological characters, stem diameter, stem length, number of leaves, rosette diameter, and biomass) would exhibit more variation in their relationships to fitness. (v) Are any of the response scenarios shared across *Centaurea* and *Crepis*? We expected that there would be many different ways to be invasive, and therefore the likelihood of sharing a particular response between genera would be low.

Methods

PLANT MATERIAL

We chose species of the genera *Centaurea* and *Crepis* for our study based, in part, on our ability to collect multiple invasive and non-invasive introductions to North America within both genera (Table 1). In an effort to assess differential rates of range expansion, which we feel is ecologically consistent with the concept of invasiveness (Richardson *et al.* 2000; Colautti & MacIsaac 2004; Muth & Pigliucci 2006), we ranked invasive status based upon species distributions across US states (USDA PLANTS Database, USDA 2002). Intra-state level (county by county) presence/absence records confirm our characterization of the invasive status at the smaller spatial scale (i.e. for the species used in this study, invasiveness rank within particular states is largely consistent with the national scale ranking).

While we understand the need for explicit justifications in characterizing the invasiveness of study species, we intentionally avoided the use of 'noxious weed' lists that use a variety of criteria for inclusion, often stressing (quite reasonably from a policy perspective) impacts of human interest over other ecological properties or demographic patterns. While it is possible that

our reliance on data bases that make use of herbaria records may either exaggerate or underestimate the distribution of various species (Wu *et al*. 2005), the same is true of noxious weed lists where a species can be listed without any evidence of it occurring in the state, or may be absent from a list despite being quite common.

While the intraspecific phylogenetic relationships within each genus are poorly known (Babcock 1947; Susanna *et al*. 1995; Whitton *et al*. 1995; Garcia-Jacas *et al*. 2001), there is little doubt that the genera themselves represent separate clades (at the level of tribe or subfamily, Bremer 1994; Bayer & Starr 1998; Panero & Funk 2002). All species were introduced to North America (most prior to 1900), presumably from native Eurasian ranges. While we lack adequate introduction histories for many of the species used here, a comparison of available herbaria records suggests that there is no significant association between time since introduction and invasiveness (see Table 1 for earliest known records).

All study species are short-lived, predominantly annual, herbaceous plants occurring in a variety of disturbed habitats. The degree of phenotypic similarity between genera allowed us to directly compare a large number of traits potentially relevant to invasion. Seventeen different seed accessions were obtained of the study species from wild collected populations, European botanical gardens, and North American collaborators (see Table 1 for details).

PLANT HANDLING AND EXPERIMENTAL DESIGN

For all 17 populations, two to four seeds (based on availability) were planted into each of 30 11.5 × 11.5 × 12.7 cm pots of autoclaved sand and placed in the University of Tennessee White Avenue glasshouse. Ambient lighting and photoperiod were augmented with glasshouse lights (400 watt metal halide at 1 m intervals) set at 16 h per day. Planting was carried out in April and May of 2003. Glasshouse temperatures during this period ranged from approximately 20 to 32 C.

Upon initial germination, pots were maintained by hand thinning to one individual throughout the course of the experiment. Following successful establishment and development to the point of true leaf production (seedlings were approximately 3 to 5 weeks old), half of all pots within each block were exposed to a single week-long drought. Controls were watered twice weekly, as were drought-exposed plants following the drought treatment. Concurrent with the drought treatment, three phosphorus (P) treatments were established and administered bi-weekly (at 250 mL per administration) throughout the duration of the experiment. The drought interval fell within this bi-weekly schedule such that drought-exposed plants received nutrient addition prior to and after, but not during, drought exposure. All levels of P treatment received a background of modified Hoagland's solution of

5 mmol $Ca(NO_3)_2 \times 4H_2O$, 5 mmol KNO_3, 2 mmol $MgSO_4 \times 7H_2O$, 1 mL/L 1% Fe-EDTA, and 1 mL/L micronutrient solution (Boron 0.5 mg/L, Manganese 0.5 mg/L, Zinc 0.05 mg/L, Copper 0.02 mg/L, Molybdenum 0.01 mg/L). The P levels varied as follows: a No-P treatment contained only the background modified Hoagland's solution (although trace amounts of P were likely to be present in our potting material, pots and watering equipment); a Field-P treatment included 0.025 mmol $Ca(H_2PO_4)_2 \times H_2O$; and a High-P treatment contained 0.5 mmol $Ca(H_2PO_4)_2 \times H_2O$.

All traits were measured on individual plants except average per cent survivorship, which was assessed per species using blocks as replicate populations. Traits included: (i) days to third true leaf, a measure of seedling development rate; (ii) days to bolting, a measure of time spent in the vegetative stage; (ii) days to initial flowering (anthesis), a measure of time until reproductive maturity; (iv) days to initial dehiscence, an estimate of time until seed dispersal; (v) number of rosette leaves at bolting, an estimate of investment in the vegetative phase of growth; (vi) diameter of rosette at bolting, a second estimate of investment in the vegetative phase of growth; (vii) number of basal stems, a component of plant architecture; (viii) stem diameter, a component of size; (ix) stem length, a component of size; (x) branch order (number of branch nodes encountered in tracing the longest stem backwards to the rosette), another characterization of plant architecture during the reproductive phase; (xi) above-ground biomass (dry weight), a measure of overall growth; (xii) below ground biomass (dry weight), a measure of investment in root tissue; (xiii) involucre diameter (seed head diameter), a component of reproductive fitness; (xiv) number of inflorescences, another component of reproductive fitness; and (xv) per cent survival to flowering, a measure of the likelihood of survival to reproductive maturity.

DATA ANALYSIS

Data were checked for violations of assumptions of normality and homoscedasticity (above- and below-ground biomass and number of inflorescences were log transformed as a result), and analysed with a two-way analysis of variance (ANOVA). Sample size and degrees of freedom varied by trait, depending upon individual mortality and whether particular species had individuals surviving to a particular developmental stage. Analyses were carried out using JMP (SAS) version 5.1. For each trait, the full model included the following effects: genus (overall differences between *Centaurea* and *Crepis*, treated as a fixed effect); drought (overall differences between drought-exposed and control treatments, fixed); phosphorus (overall differences between phosphorus treatments, fixed); block (micro-environmental effects, random); species nested within genus (differences between congeners, random); species by drought (differences among congeners with

respect to plasticity to drought treatment); species by phosphorus (differences among congeners with respect to plasticity to phosphorus treatment); and error (residual variance). Analysis of days to third true leaf did not include either the drought or P-treatment effects, as these treatments were not established at the time these data were recorded. Analysis of per cent survival to flowering used experimental blocks as independent replicate populations of species.

Due to poor survivorship in *Centaurea calcitrapa*, *Crepis rubra* and *Crepis vesicaria*, these species were included only in the analysis of days to third true leaf, and excluded from analysis of later stage phenotypic traits.

To reduce the likelihood of disregarding the results of truly significant tests (type II error), we have chosen not to use the sequential Bonferroni correction (normally employed to maintain the overall probability of committing a type I error), following Moran's (2003) criticism of this method; rather, we highlighted all tests where the associated *P*-value was less than the typical 0.05. After Moran (2003), we report the likelihood of finding a particular number of significant test results below our α-value (K), given the total number of tests performed (N), by the following formula:

$$P = [N!/(N - K)!K!]\alpha^K 1 - \alpha^{N-K}.$$

In addition to the formal *P*-values, we report effect sizes (in the form of mean sums of squares) and the power of the tests (Cohen 1992), and discuss the results from a statistically conservative perspective.

We performed power analyses using G-Power (Buchner *et al*. 1997) on all ANOVA tests. We followed the conventions of Cohen (1992) and evaluated whether we had the statistical power to detect 'medium' and 'small' effects caused by our treatments. 'Medium' effect size (ES) means that treatment differences are 'visible to the naked eye of a careful observer' (for example, the invasive plants have visibly more fruits than the non-invasive plants), whereas 'small' ES means that the treatment differences are 'noticeably smaller than the medium but not so small as to be trivial' (for example, the invasive plants have more fruits than the non-invasive plants, but it is not as obvious; Cohen 1992). This analysis accounts for the possibility that we found an effect to be statistically non-significant, not because the effect is actually biologically negligible, but rather because of a limited sample size. Power values range from 0 to 1, and are calculated for each effect in the model based upon the degrees of freedom and the ES (small, medium or large) of interest. Power values of 0.8 and higher are considered to be sufficient in order to conclude that there was enough statistical power to detect an effect of the size of interest (Cohen 1992). Despite the fact that the size of an effect is a somewhat subjective variable, the power of a statistical analysis is often cited as one of the crucial measures of assessment missing from most published papers (Cohen 1992).
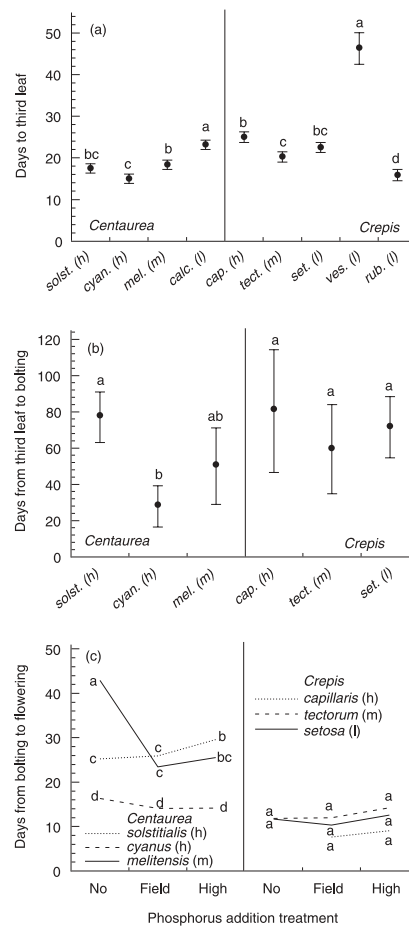
Fig. 1 Least squared means of phenological traits, (a) days to third true leaf, (b) days from third true leaf to bolting and (c) days from bolting to flowering. Within each figure *Centaurea* is on the left, *Crepis* on the right. Invasive designation follows species name as (h)igh, (m)oderate or (l)ow. Bars represent 95% confidence intervals. Among congeners, values sharing a letter do not differ significantly (α = 0.05).

Results

PHENOLOGICAL TRAITS

An analysis of variance showed the only effect of environmental manipulations on phenological characters was a significant delay in flowering time of the moderately invasive *C. melitensis* in the no-addition phosphorus treatment (Table 2, Fig. 1c).

The species effect was significant for all three phenological traits (days to third true leaf, days from third true leaf to bolting, and days from bolting to

Table 2 ANOVAs for each phenological trait (columns) and factor (rows) in the experiment. For each factor we report the degrees of freedom (d.f.), sums of squares (SS) and *P*-values. Statistically significant effects at the *P* < 0.05 value are highlighted in bold (see 'Materials and methods' for discussion of Bonferroni correction). R^2 refers to the amount of variance explained by the entire model for a particular trait. Drought and phosphorus treatment factors are not applicable (NA) for traits that were measured before these environmental treatments were established

		Days to third leaf	Days from third leaf to bolting	Days from bolting to flowering
R^2		0.52	0.61	0.83
Genus	SS	**3491**	9834	1745
d.f. = 1, 4	P	**0.0506**	0.4064	0.1312
	Power (medium, small)	**0.99, 0.53**	0.90, 0.26	0.90, 0.26
Species (Genus)	SS	**6944**	**57751**	**3361**
d.f. = 4, 6	P	**< 0.0001**	**0.0346**	**0.0039**
	Power (medium, small)	**0.99, 0.37**	**0.70, 0.14**	**0.70, 0.14**
Drought	SS	NA	121	33
d.f. = 1, 4	P	NA	0.2814	0.1377
	Power (medium, small)	NA	0.90, 0.26	0.90, 0.26
Phosphorus	SS	NA	423	445
d.f. = 2, 8	P	NA	0.2814	0.1377
	Power (medium, small)	NA	0.84, 0.20	0.84, 0.20
Drought × Species (Genus)	SS	NA	183	45
d.f. = 4, 134–400	P	NA	0.9743	0.5974
	Power (medium, small)	NA	0.43, 0.09	0.43, 0.09
Phosphorus × Species (Genus)	SS	NA	4104	**773**
d.f. = 8, 134–400	P	NA	0.2122	**< 0.0001**
	Power (medium, small)	NA	0.34, 0.08	**0.34, 0.08**
Block	SS	139	593	131
d.f. = 4, 134–400	P	0.1139	0.8105	0.1003
	Power (medium, small)	0.99, 0.33	0.74, 0.15	0.74, 0.15
Residual, d.f. = 134–400	SS	7429	55251	1802

flowering), indicating moderate across-environment variation among congeners (Table 2, Fig. 1). The highly invasive *C. cyanus* was the most rapidly developing *Centaurea* congener for all phenological characters, while there was little appreciable variability among *Crepis* congeners for the same traits (the only exceptions being the less invasive *C. vesicaria* and *C. rubra*, which had extreme values for days to third true leaf, in the opposite direction, but both failed to develop any further, Fig. 1a). *Crepis* and *Centaurea* differed significantly from one another for days to third true leaf (mean *Centaurea* = 18.5 days, *Crepis* = 26.0 days, Table 2, Fig. 1a).

ARCHITECTURAL AND SIZE TRAITS

The main effect of phosphorus addition treatment was significant for several architectural and size traits. High phosphorus addition resulted in larger plants in all cases. However, there was no significant variation among species responses to the treatment (Table 3).

In contrast, responses to the drought treatment were quite variable among species (Table 3). Among the *Centaurea*, *C. cyanus* and *C. melitensis* tended to differ in the direction of their response to drought conditions, with *C. cyanus* being shorter and having less above-ground biomass in the drought conditions, while *C. melitensis* was taller and had greater above-ground biomass under similar conditions (Fig. 2c,e,f). *C. solstitialis* responded most similarly to *C. melitensis*, although the overall species effect indicated that it

was generally much larger than either congener, regardless of environmental treatment (Fig. 2). The relationships among *Crepis* congeners and their responses to the environmental treatments were much more variable and idiosyncratic than among the *Centaurea* (Fig. 2).

The main effect of genus was significant for number of basal stems and suggestive for number of rosette leaves and branch order (Table 3); *Crepis* were more architecturally complex in all cases (Fig. 2a,d). As in the case of the phenological traits, the nested effect of species was significant for nearly all architectural and size traits examined (Table 3, Fig. 2).

FITNESS TRAITS

While the drought treatment had no significant effect on involucre diameter (Table 4, Fig. 3a), there was an appreciable, but species-dependent, effect on the number of inflorescences (Table 4, Fig. 3b). Although inflorescence production in *Centaurea* was not responsive, among the *Crepis*, *C. capillaris* produced more inflorescences when exposed to drought, while *C. setosa* produced more inflorescences in the non-drought treatment (Fig. 3b). With respect to survival, although all species had reduced survival in drought conditions, this effect was not statistically significant in any particular case, nor overall (Table 4, Fig. 3c).

The effect of the phosphorus additions on fitness traits included increased involucre diameter, number of

Table 3 ANOVAS for each architectural and size trait (columns) and factor (rows) in the experiment. For each factor we report the degrees of freedom (d.f.), sums of Squares (SS) and P-values. Statistically significant effects at the $P < 0.05$ value are highlighted in bold (see 'Materials and methods' for discussion of Bonferroni correction). R^2 refers to the amount of variance explained by the entire model for a particular trait

		Number of basal stems	Stem diameter	Stem length	Number of leaves	Rosette diameter	Branch order	Above-ground biomass	Below-ground biomass
R^2		0.63	0.52	0.55	0.63	0.65	0.53	0.68	0.67
Genus	SS	**178**	0.12	783	6109	26803	141	0.8884	< 0.0001
d.f. = 1, 4	P	**0.0294**	0.8854	0.3471	0.0677	0.3082	0.0744	0.3651	0.9614
	Power (medium, small)	**0.90, 0.25**	0.89, 0.25	0.90, 0.25	0.91, 0.26	0.84, 0.22	0.90, 0.25	0.90, 0.25	0.87, 0.23
Species (Genus)	SS	130	**36.98**	**5227**	**6261**	**102766**	**192**	**6.5692**	**0.1027**
d.f. = 4, 6	P	0.1402	**0.0356**	**0.0166**	**0.0301**	**0.0196**	**0.0484**	**0.0066**	**0.0176**
	Power (medium, small)	0.69, 0.13	**0.67, 0.13**	**0.69, 0.13**	**0.70, 0.14**	**0.60, 0.12**	**0.69, 0.13**	**0.69, 0.13**	**0.64, 0.13**
Drought	SS	2	0.05	41	18	538	1	0.0004	0.0003
d.f. = 1, 4	P	0.6618	0.8099	0.5841	0.7752	0.2010	0.7678	0.9521	0.6190
	Power (medium, small)	0.90, 0.25	0.89, 0.25	0.90, 0.25	0.91, 0.26	0.84, 0.22	0.90, 0.25	0.90, 0.25	0.87, 0.23
Phosphorus	SS	< 1	**3.48**	**654**	23	4450	**108**	**0.6686**	**0.0030**
d.f. = 2, 8	P	0.7905	**0.0036**	**0.0317**	0.8689	0.1228	**0.0013**	**0.0096**	**0.0074**
	Power (medium, small)	0.83, 0.19	**0.82, 0.19**	**0.83, 0.19**	0.84, 0.20	0.76, 0.17	**0.83, 0.19**	**0.83, 0.19**	**0.79, 0.18**
Drought × Species (Genus)	SS	**44**	3.64	502	**886**	832	27	**0.4557**	**0.0054**
	P	**0.0002**	0.0583	0.0955	**0.0099**	0.9035	0.2471	**0.0183**	**0.0331**
d.f. = 4, 118–149	Power (medium, small)	**0.52, 0.10**	0.50, 0.10	0.52, 0.10	**0.54, 0.10**	0.43, 0.09	0.52, 0.10	**0.52, 0.10**	**0.47, 0.10**
Phosphorus × Species (Genus)	SS	6	1.50	578	691	7397	37	0.3970	0.0014
	P	0.9079	0.8668	0.3286	0.2244	0.3339	0.4991	0.2288	0.9434
d.f. = 8, 118–149	Power (medium, small)	0.42, 0.09	0.40, 0.09	0.42, 0.09	0.43, 0.09	0.34, 0.08	0.42, 0.09	0.42, 0.09	0.38, 0.08
Block	SS	3	1.30	412	290	3117	**63**	0.2755	0.0020
d.f. = 4, 118–149	P	0.8353	0.5045	0.1640	0.3439	0.4259	**0.0160**	0.1209	0.4061
	Power (medium, small)	0.73, 0.15	0.71, 0.14	0.73, 0.15	0.74, 0.15	0.64, 0.13	**0.73, 0.15**	0.73, 0.15	0.68, 0.14
Residual, d.f. = 118–149	SS	271	53.73	8976	9551	94661	719	5.3746	0.0642

Table 4 ANOVAS for each fitness proxy trait (columns) and factor (rows) in the experiment. For each factor we report the degrees of freedom (d.f.), sums of squares (SS) and P-values. Statistically significant effects at the $P < 0.05$ value are highlighted in bold (see 'Materials and methods' for discussion of Bonferroni correction). R^2 refers to the amount of variance explained by the entire model for a particular trait

|		Involucre diameter	Number of inflorescences	Survival to flowering
R^2		0.82	0.69	0.72
Genus	SS	60.926	**4696**	**32089**
d.f. = 1, 4	P	0.2844	**0.04**	**< 0.0001**
	Power (medium, small)	0.90, 0.25	**0.90, 0.25**	**0.57, 0.14**
Species (Genus)	SS	**308.198**	3947	**22520**
d.f. = 4, 6	P	**0.0135**	**0.0452**	**< 0.0001**
	Power (medium, small)	**0.69, 0.13**	**0.69, 0.13**	**0.32, 0.08**
Drought	SS	0.008	1	1734
d.f. = 1, 4	P	0.9402	0.9362	0.0789
	Power (medium, small)	0.90, 0.25	0.90, 0.25	0.57, 0.14
Phosphorus	SS	**4.645**	751	**9108**
d.f. = 2, 8	P	**0.0010**	0.0532	**0.0007**
	Power (medium, small)	**0.83, 0.19**	0.83, 0.19	**0.27, 0.08**
Drought × Species (Genus)	SS	5.117	**720**	300
d.f. = 4, 75–145	P	0.1001	**0.0044**	0.9670
	Power (medium, small)	0.52, 0.10	**0.52, 0.10**	0.21, 0.07
Phosphorus × Species (Genus)	SS	1.342	**852**	4906
d.f. = 8, 75–145	P	0.9775	**0.0210**	0.3554
	Power (medium, small)	0.42, 0.09	**0.42, 0.09**	0.16, 0.06
Block	SS	3.275	335	NA
d.f. = 4, 145	P	0.2848	0.1232	NA
	Power (medium, small)	0.73, 0.15	0.73, 0.15	NA
Residual, d.f. = 75–145	SS	93.513	65701	27515
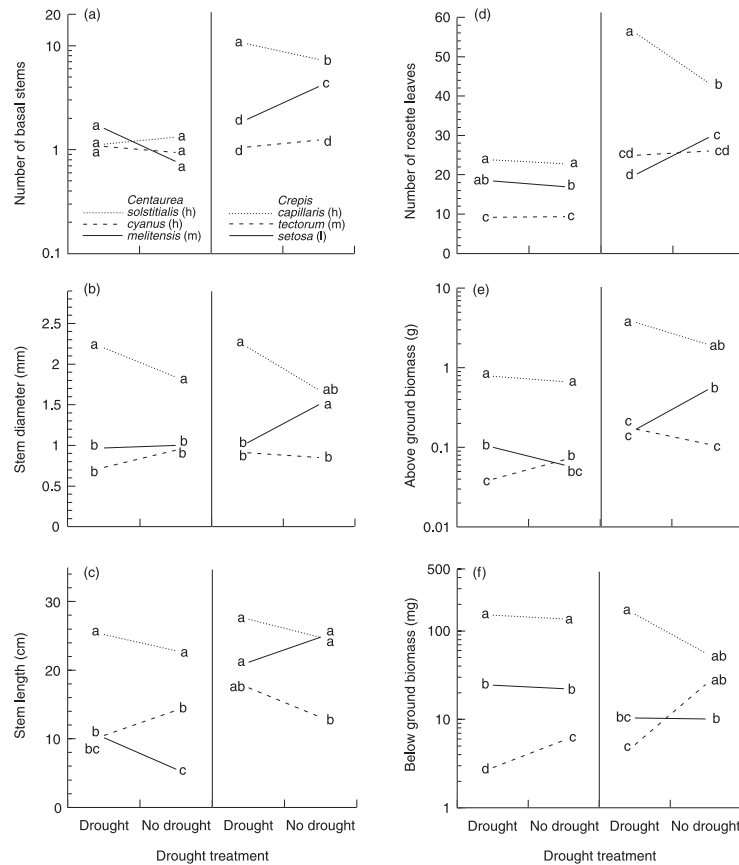
Fig. 2 Least squared means of morphological traits by drought treatment of (a) number of basal stems, (b) stem diameter, (c) stem length, (d) number of rosette leaves, (e) above-ground biomass and (f) below-ground biomass. Within each figure *Centaurea* is on the left, *Crepis* on the right. Invasive designation follows species name as (h)igh, (m)oderate or (l)ow. Among congeners, values sharing a letter do not differ significantly ($\alpha = 0.05$).

inflorescences and survivorship (Table 4, Fig. 3d,e,f). While species almost universally responded positively to the phosphorus additions, there was considerable variation in the degree of response for number of inflorescences and survivorship (Fig. 3e,f). In particular, with respect to the number of inflorescences, *C. melitensis* and *C. setosa* were the least responsive among their congeners (Fig. 3e). The effect on survivorship differed substantially as *C. melitensis* and *C. setosa* were both very plastic, *C. cyanus* and *C. solstitialis* both had flat or unresponsive portions of their fitness responses, and *C. tectorum* was entirely insensitive to phosphorus variation (Fig. 3f).

The main effect of genus was significant for number of inflorescences (Table 4) and survival (Table 4). *Crepis* species tended to have more inflorescences (Fig. 3b,e), while *Centaurea* species had higher survival rates (Fig. 3c,f). The

nested species effect was significant for involucre diameter, number of inflorescences (Table 4, Fig. 3) and survival (Table 4, Fig. 3c,f), indicating moderate across-environment variability among congeners in both genera.

With respect to multiple tests of significance, the probability of our finding one significant test (K = 1) for a specific effect (as was only the case for the block effect) out of 14 total tests ($n = 14$), by chance is $P = 0.3593$ (Moran 2003). This probability drops to $P = 0.1229$ and $P = 0.0259$ for K = 2 and K = 3, respectively. While power generally remained adequate (> 0.80) to detect effects of medium size for all main effects, the power to detect effects of medium size (or less) was considerably reduced for interaction effects (0.34–0.54), as may be predicted because of the fewer degrees of freedom. The loss of power for interaction effects makes it more likely for us to underestimate or
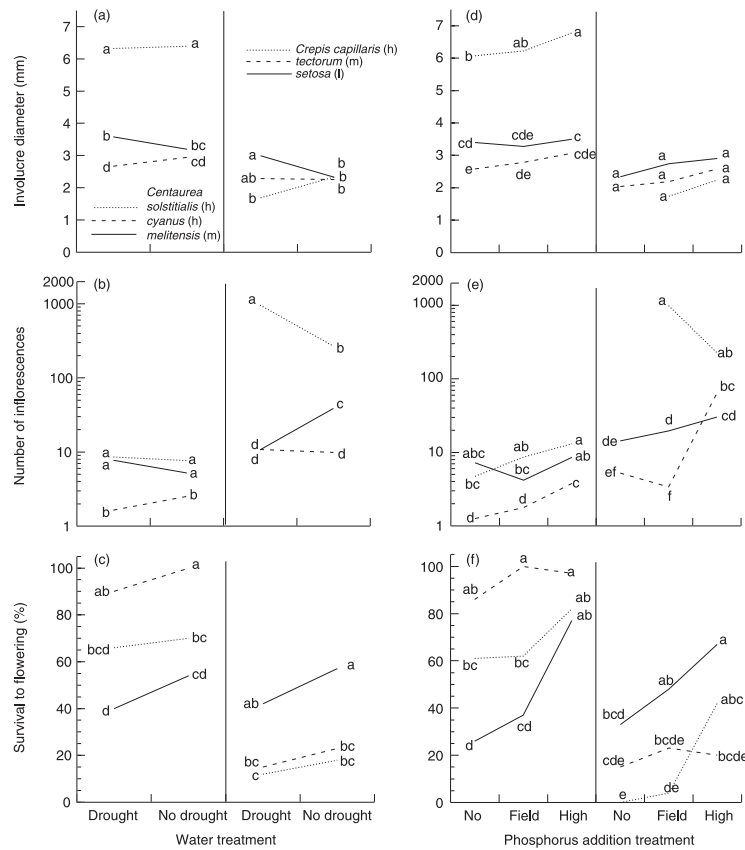
Fig. 3 Least squared means of fitness proxy traits by (a–c) drought treatment and (d–f) phosphorus addition treatment of (a and d) involucre diameter, (b and e) number of inflorescences and (c and f) survivorship. Within each figure *Centaurea* is on the left, *Crepis* on the right. Invasive designation follows species name as (h)igh, (m)oderate or (l)ow. Among congeners, values sharing a letter do not differ significantly ($\alpha = 0.05$).

miss real differences among species responses. With these caveats in mind, we interpret the results cautiously and infer biological significance as warranted based on the magnitude of the effect (SS) and the probabilities of committing type I and type II errors.

Discussion

PATTERNS OF FITNESS PLASTICITY

While stress tolerance and resource opportunism may both contribute to the invasiveness of certain introductions, our study suggests that differences in average fitness as opposed to plasticity may be a sufficient explanation of differential invasiveness. For instance, involucre diameter among *Centaurea* congeners was relatively non-plastic to both our environmental treatments (Fig. 3a,d). However, with respect to the

across-environment mean, *C. solstitialis* had seed heads about twice the size of the less invasive *C. cyanus* and *C. melitensis*. Differences in involucre size among *Crepis* congeners were slight and failed to shed much light on their differential invasiveness (Fig. 3a,d).

Differential plasticity of inflorescence production to varied P availability among *Crepis* congeners did suggest a possible role in invasiveness (Fig. 3e). While average inflorescence production of *C. capillaris* was an order of magnitude greater than that of the less invasive *C. tectorum* and *C. setosa*, differences were reduced or negligible in the high P treatment. We consider this lack of tolerance to reduced field P levels in *C. tectorum* and *C. setosa* relative to *C. capillaris* to have been an instance of the Jack-of-all-trades scenario, although the lack of survival (and thus no inflorescences to count) in *C. capillaris* under the lowest P conditions is of course an important limitation, and illustrates the

independence of plasticities of traits like inflorescence number and probability of survival. The positive response of *C. capillaris* inflorescence production to drought is puzzling. It is unlikely that the non-drought plants were over-wet as we observed no damage associated with over-watering and a large number of inflorescences were produced in both watering conditions.

Finally, while survival to reproduction seems to play no explanatory role relative to invasiveness among *Crepis* congeners, its role in *Centaurea* seems quite different (Fig. 3c,f). While *Centaurea* congeners all suffered greater premature mortality in the drought treatment, average premature mortality was highest for the least invasive *C. melitensis*. In the P treatment, we found that both *C. solstitialis* and *C. cyanus* exhibited appreciable tolerance to low P levels relative to *C. melitensis* (suggesting the Jack-of-all-trades scenario). Both these species also showed aspects of resource opportunism (increased survival in high P conditions). However, as the less invasive *C. melitensis* shared this responsiveness, its explanatory relevance is diminished (i.e. it is the resilience of the other species that separates them from *C. melitensis*, not their responsiveness).

PATTERNS OF PLASTICITY OF PHENOLOGY, ARCHITECTURE AND SIZE

The only cases where fitness tolerances suggested a role for plasticity in explaining invasiveness were restricted to the P treatments. Therefore, any mechanistic role of plasticity (or robustness) of underlying phenological, architectural or size traits must similarly be found across the P treatments (differential plasticity of non-fitness traits to the drought treatment apparently failed to significantly influence fitness components in any direct manner that would help explain differences in invasiveness). The only non-fitness character significantly sensitive to the P treatments, and therefore a candidate to explain fitness differences across the P treatments, was days from bolting to flowering (Table 2, Fig. 1c). As there were no significant differences in days from bolting to flowering among *Crepis* congeners, we do not have an explanation for the partial resilience of inflorescence production in *C. capillaris* to reduced P levels. However, among the *Centaurea* it seems plausible that the constancy and shortness of time to flowering in *C. solstitialis* and *C. cyanus*, compared with the long delay in *C. melitensis* under low P conditions (Fig. 1c), may partially account for the robust rates of survival to maturity in the former and the increased mortality of the latter under low P conditions.

RELATIONSHIPS TO PREVIOUS STUDIES AND BROADER RELEVANCE

Any single experimental study is of course too limited in scope to suggest that differential invasiveness is more likely to be explicable by fitness tolerance (Jack-of-all-trades) than fitness responsiveness (Master-of-some)

or some combination of both (Jack-and-Master). However, it is interesting to observe that Jack-of-all-trades is the only scenario for which we found evidence in our study. Richards *et al.* (2006) found that in the few other studies assessing the responses of fitness components in introduced species, the Master-of-some scenario was most commonly observed, followed by Jack-and-Master, and, least commonly, Jack-of-all-trades.

Specifically, assessing some of the same species examined here, Gerlach & Rice (2003) suggest that the invasiveness of *C. solstitialis* may be due to both its tolerance to competitors and its ability to respond to open disturbed patches, therefore fitting the Jack-and-Master scenario with respect to fecundity response to different gap sizes. Suding *et al.* (2004), in a study including the invasive *Centaurea diffusa*, actually found evidence of an inverse relationship between P availability and survival, the opposite to that observed for three congeners here. In the same study, however, *C. diffusa* was significantly more responsive (greater survival) to the facilitative effects of neighbours than were co-occurring native species (under ambient and reduced phosphorus conditions, but not under reduced nitrogen conditions), thereby fitting the Master-of-some scenario.

That both of these studies were carried out in the field whereas the present study was conducted in a glasshouse with potted individuals may account for some of the differences in findings. In the field some *Centaurea* species are known to have deep rooting architecture and mycorrhizal associations (Marler *et al.* 1999; Zabinski *et al.* 2002; Suding *et al.* 2004), either of which could affect the pattern of response that we observed. In our comparative approach, the use of multiple species and genera imposed certain experimental constraints; a common field garden would have risked spreading introduced species outside of their current ranges and glasshouse space further restricted our ability to include additional variables of interest (e.g. presence or absence of mycorrhizal associates).

While Richards *et al.* (2006) have noted that the Jack-of-all-trades and Master-of-some scenarios are not necessarily equivalent to specialists or generalists, it is worth considering that different scenarios of fitness response may correspond to different demographic properties. It seems reasonable that the Jack-of-all-trades scenario, indicating maintenance of fitness across an environmental gradient, is particularly relevant to explaining broader ecological success, relative to less invasive controls. The Master-of-some scenario, as it relates directly to the opportunistic fitness response of a species, may be particularly relevant in explaining differential densities of species. If this is the case it should be expected that introduced species most consistent with the Jack-of-all-trades scenario are ubiquitous, though not necessarily locally dense, while introductions more similar to the Master-of-some scenario might only be able to reach high local densities (or near monocultures) in a few environments. The

expectations for the Jack-and-Master scenario would include both wide ecological success as well as occasional environment-specific high local densities. All of these predictions are contingent upon the scale of relevant environmental variation.

LIMITATIONS

The framework for studying phenotypic plasticity in biological introductions employed here has certain limitations. Most notably, the determination of whether any particular scenario is relevant is necessarily relative to the particular comparative group(s) used (the responses of other species, populations or genotypes used as controls).

Another limitation is that there is necessarily a degree of subjectivity in deciding whether a flat response should be interpreted as evidence of tolerance, or alternatively, an absence of opportunism. While familiarity with the study systems and environmental conditions ought to give justification for appropriate distinctions (were the conditions relatively harsh or benign, compared with relevant natural conditions?), use of a wide range of environmental levels increases the likelihood that both stressful and relatively luxuriant conditions were examined. The number of levels or types of environments considered will have a crucial role in the ability to assess whether species exhibit both tolerance and responsiveness to any one gradient (Jack-and-master). At an absolute minimum, three levels are necessary to accomplish this (Richards *et al*. 2006).

Finally, as phenotypic plasticity is the responsive property of a particular genotype, the lowest level at which replication is present in a study will determine the precision with which a study is actually measuring genotypic norms of reaction (Richards *et al*. 2006). In our work, we present the average responses of species. As populations are expected to harbour genetic variants, these are necessarily averaged norms of reaction, which, as such, conceal the potentially varied underlying responses of individual genotypes. Our use of seed accessions from different sources (e.g. European or North American, wild collected or from botanical gardens) may not be representative of the species introductions, thereby obscuring the realized differences between introduced populations. However, a previous study suggests that the effect of these different sources may be minimal in our case (Muth & Pigliucci 2006). Logistical constraints are always likely to impinge on a study of this type, resulting either in a focus on greater precision and exploration of the variability of responses between genotypes of a few populations (of a few species), or greater sampling of species, and populations within species, at the cost of statistical power at the level of genetic families. While the substitution of species or populations for genotypes may be a practical compromise, it must be made knowing that the averaged norms of reactions that are generated necessarily result from an unknown combination of potentially variable individual genotypic responses.

Conclusions

It is widely considered and conceptually grounded that phenotypic plasticity is likely to be an important type of trait influencing the relative success of introduced species (Baker 1965). Here we employ an explicit framework, based on Baker's (1965) seminal work on colonizing plant species, as subsequently formalized by Richards *et al*. (2006). Specifically, differential success (spread, impact, etc.) of species may in part result from certain species being more tolerant of stressful conditions (Jack-of-all-trades), more responsive to increasingly luxuriant conditions (Master-of-some), or both (Jack-and-Master).

While we only found evidence for the Jack-of-all-trades scenario in the current study, as tolerance and responsiveness both confer potential advantages, we believe the prevalence of any particular model of fitness reaction among other invasive species is an open empirical question. Furthermore, as any one species may exhibit aspects of different scenarios (either for different fitness traits, or under different environmental conditions), we cannot even suggest that particular invasions necessarily have their success or failure pinned to a specific scenario. Rather, as introductions are historically contingent events and the culmination of numerous successive (and potentially iterative) stages, the importance of particular attributes of a species in conferring invasiveness is not likely to be consistent from one instance to another. It seems much more likely that success or failure will be the result of the plasticity, or lack thereof, of different traits to different environments at different stages along the way.

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IV. Phenotypic integration among variably invasive *Crepis* and *Centaurea* introductions

Abstract

That closely related species differ widely in their invasiveness upon introduction to novel regions is as much an interesting ecological and evolutionary question as it is an applied conservation problem. We employed path analysis in modeling the integrated phenotypes of introduced congeneric species differing in degree of invasion success in two genera of Asteraceae (*Crepis* and *Centaurea*). Path models for highly invasive species generally exhibited fewer non-zero path coefficients (suggesting less overall integration) and fewer negative path coefficients (suggesting fewer allocation trade-offs). Path models revealed potential trade-offs in allocation among architectural traits within less invasive species that were absent in more invasive congeners. These results differ from univariate analyses of these species that revealed few common associations of traits with invasiveness, within or across genera.

INTRODUCTION

Finding general predictors of which introduced species will become problematic invasives has proven difficult (Mack et al., 2000; Kolar and Lodge 2001). An often used criterion in assessments of weedy potential is close evolutionary relationship to a known invasive species (e.g. Scott and Panetta, 1993; Pheloung et al., 1999). Despite the prospective utility of "guilt by (taxonomic) association" (Mack et al., 2000), one of the most intriguing aspects of invasion biology has been the ubiquity of closely related species of vastly differing degrees of invasion success (Rejmanek and Richardson, 1996; Grotkopp et al., 2002; Gerlach and Rice, 2003; Mandak, 2003; Bellingham et al., 2004; Muth and Pigliucci, 2006; Ashton, 2006). Studies of this type, by taking phylogeny into account, stand to improve the accuracy of future predictive attempts within a restricted domain, as well as contribute to our fundamental understanding of biological diversity.

A common byproduct of studying closely related taxa is a reduction in the qualitative phenotypic variability that many studies of invasive

species document. While variation among these types of traits may account for a substantial portion of broad taxonomic patterns of invasiveness (e.g. Reichard and Hamilton, 1997), it is largely unable to explain differences among close relatives that share many character states. Greater examination of quantitative characters, and the relationships among these traits (i.e. measures of phenotypic integration), provides an alternative methodology to better understand differences among closely related variably invasive species.

In this study we used structural equation modeling (SEM) to investigate the integration of multivariate phenotypes in variably invasive congeners within two genera of Asteraceae (*Crepis* and *Centaurea*). Specifically, we sought to address the following questions: (1) Do more invasive introductions exhibit patterns of phenotypic integration consistent with fewer constraints? We predicted that the more invasive species would have fewer tradeoffs between the production of necessary developmental and architectural structures (i.e., invasive species should exhibit non-significant or weakly positive relationships between production of leaves, stem material, branches and fecundity). (2) Do the differences in phenotypic integration of variably invasive congeners more often occur as differences in the magnitude or sign of effect? We predicted that differences among these closely related congeners would more likely be differences in degree, not sign, of effect. (3) How similar are patterns of trait integration among variably invasive species across genera? We predicted that there would be substantial correspondence between invasive species of the phenotypically and ecologically similar *Crepis* and *Centaurea*.

MATERIALS AND METHODS

Plant material---All study species are short-lived, predominantly annual, herbaceous plants that commonly occur in a variety of disturbed habitats including roadsides, rangeland and agricultural fields. Although intraspecific phylogenetic relationships within *Crepis* and *Centaurea* are poorly understood, the clades themselves are well supported (Bremer, 1994; Bayer and Starr, 1998; Panero and Funk, 2002). Twenty-eight different seed accessions were obtained of the study species from wild collected populations, European botanical gardens, and North American collaborators (see Table 1 for details).

We chose to study *Centaurea* and *Crepis* (both Asteraceae) because they include many problematic invasives as well as more benign introductions (Table 1). We characterized invasive status as high, moderate, or low based upon recorded presence of the species in the

lower 48 U.S. as presented in the USDA PLANTS Database (2002). Muth and Pigliucci (2006) have previously shown that intra-state level (county by county) presence/absence records confirm our characterization of the invasive status at a smaller spatial scale (i.e., for the species used in this study invasives are largely widespread both across and within states, while non-invasives are largely restricted at both scales). Estimates of dates of introduction to North America from native Eurasian ranges are given as the earliest found herbarium record compiled from various North American herbaria and available floras (Table 1). Additional details on the merits and drawbacks of our characterization of invasiveness are discussed in Muth and Pigliucci (2006).

Plant handling and experimental set-up---For all 28 populations, two to four seeds (based on availability) were planted into each of ten 4 by 4 by 4.5cm starter pots with standard autoclaved pro-mix potting soil and placed in the University of Tennessee White Avenue greenhouse. Ambient lighting and photoperiod were augmented with greenhouse lights (400 watt metal halide at one meter intervals) set at 16h per day. Once established, individual seedlings were transplanted into 13 by 13 by 13.5cm pots and distributed in a randomized block design comprising ten complete blocks. Planting was carried out in October and November of 2001.

All traits were measured at the individual level on plants transplanted to larger pots. These traits included: 1) number of rosette leaves at bolting, an estimate of investment in the vegetative phase of growth; 2) stem length, a component of the size and architecture of the reproductive phase; 3) branch order (number of branch nodes encountered in tracing the longest stem backwards to the rosette), a second characterization of plant architecture during the reproductive phase; and 4) number of inflorescences, a component of reproductive fitness.

Data analysis--- The results of standard univariate ANOVAs for all traits appear in Muth and Pigliucci (2006). We checked data for violations of assumptions of normality and homoscedasticity and, following relevant transformations (number of rosette leaves and number of inflorescences were log transformed in both genera, in *Crepis* stem length was log transformed and branch order was square root transformed), standardized each variable to its arithmetic mean.

We used AMOS (version 7.0, James L. Arbuckle, 2006) to create path models and perform SEM. We created our phenotypic path model (Fig. 1) based on the following understanding of the architecture and

development of the study species and prior analyses of Muth and Pigliucci (2006). The growth of rosette leaves precedes and normally subsides prior to the development of all other measured traits. As most branching occurred prior to the achievement of greatest stem length, the relationship between these variables was assumed to be unidirectional with branching preceding and potentially affecting stem length. We allowed the fitness measure, number of inflorescences, to be influenced by all traits.

Within each genus we performed a nested multi-group model analysis. The paths of the fully constrained model (assuming equal regression weights among species models) were sequentially relaxed to assess improvements in model fit (measured as a statistically significant decrease in X^2). Paths were retained as unconstrained where this resulted in significantly improved model fit. The resulting model performance for groups was evaluated according to the following criteria:

1. Root mean square error of approximation (RMSEA), together with its confidence interval and p-value for the hypothesis of a RMSEA = 0. This statistic characterizes the fit of a given model to the observed covariance matrix while accounting for model complexity. RMSEA values range from zero to one with models having values approaching zero indicating better fit, or greater parsimony, compared to those with larger values.

2. Akaike's information criterion (AIC) is an alternative joint measure of fit and simplicity. The AIC values are given for the reduced model of interest, as well as for the saturated and independence models. The saturated model has no degrees of freedom and includes all possible paths, yielding an over-parameterized model with the maximum possible fit, to be used as a comparison standard. The independence model assumes independence of all variables and is essentially equivalent to the simple path diagram generated by a multiple regression analysis. The more a particular model accurately reflects the observed covariance structure of the data, the closer its AIC will be to that of the saturated model and more distant from that of the independence model.

3. We also examined the goodness-of-fit using the comparative fit index (CFI) in which the covariance of the observed data is compared to that expected assuming the path model is true. Values close to 1 indicate very good fit, and values > 0.9 generally indicate acceptable fit (Bollen 1989).

RESULTS

Models and model fit---As described above, we began our analyses with the analytical model consistent with our biological

understanding of the development of our study species (Fig. 1). Our subsequent relaxation of path constraints (common path coefficients across the species groups) yielded three free paths among *Centaurea* (leaves to branches, branches to stem length and stem length to inflorescences) and three as well among *Crepis* species (leaves to stem length, branches to inflorescences and stem length to inflorescences). Only one free path was shared among the different genera models; in both *Centaurea* and *Crepis* allowing the path from stem length to inflorescences to be unconstrained led to significantly improved model fit. Based on the summary statistics, both multi-group models fit the observed covariance structure well (Table 2). The RSMEA values for both models were very close to zero, model AIC statistics were less than those for the saturated models, and the CFI were very close to the rule of thumb criteria of 0.90 (Table 2).

   Comparison of path coefficients---We used the respective structural equations from the aforementioned models to construct direct, indirect and total path coefficients for all congeners. We present the direct effects of traits along with each species path model for both *Centaurea* (Fig. 2) and *Crepis* (Fig. 3). Total and indirect effects for all species are presented separately for both *Centaurea* (Fig. 4) and *Crepis* (Fig. 5).
   Within *Centaurea*, the free parameters of the invasive species differed from the less invasive species primarily in the following ways. With regard to direct effects, the highly invasive species (*C. cyanus* and *C. solstitialis*) were both characterized by weak path coefficients from leaves to branches and stem length to inflorescences (Fig. 2). In the less invasive species these relationships were more strongly negative, in the case from leaves to branches, and more strongly positive in the relationship between stem length and inflorescences. There was no obvious relationship with invasiveness in the variable coefficients between branches and stem length (although they were both strongly positive in *C. cyanus* and *C. solstitialis*, this was also the case in less invasive species). The fixed relationships across species were moderately positive, in the case between branches and inflorescences, or did not differ from zero (leaves to stem length and leaves to inflorescences).
   In examining the total and indirect relationships in *Centaurea* (Fig. 4), it was generally the case that the more invasive species had fewer strong effects compared to the less invasive species, which tended more towards strongly negative (in the case of *C. diluta*) or strongly positive (in the case of *C. calcitrapa*) trait relationships. The exception to this pattern was *C. sulphurea*, which instead differed from the highly invasive species in having a moderately negative relationship between branch production

and stem length, where all other species had moderately or strongly positive relationships (Figs 2 and 4).

Within *Crepis*, the free parameters of the invasive species differed from the less invasive species primarily in the following ways. With regard to direct effects, the more invasive species (*C. capillaris* and *C. tectorum*) were both characterized by moderately negative path coefficients from leaves to stem length and moderately positive effects between stem length and inflorescences (Fig. 3). In the less invasive species these relationships were either similar in sign but stronger (as between leaves and stem length in *C. setosa*), or of the opposite effect (as in the significant negative effect of stem length on inflorescences in *C. zacintha*).There was no obvious relationship with invasiveness in the variable coefficients between branches and inflorescences (although neither were strong in the more invasive *C. capillaris* or *C. tectorum*, this was also the case in less invasive *C. zacintha*). The fixed relationships across *Crepis* congeners did not differ significantly from zero (in the case between leaves and branches and leaves and inflorescences) or was moderately positive (branches to stem length). As in *Centaurea*, the total and indirect relationships in *Crepis* (Fig. 5) were generally stronger among the less invasive species.

DISCUSSION

Multivariate phenotypes and invasiveness---We observed appreciable differences in trait interactions between variably invasive species in both *Crepis* and *Centaurea* (Figs. 2-5). It was generally the case that the most integrated phenotypes (those having the most non-zero path coefficients) were found in the less invasive species (*Centaurea diluta*, *Centaurea calcitrapa*, and *Crepis setosa*). In *Centaurea*, the highly and moderately invasive species had only two non-zero path coefficients each, while two of the less invasive species, *C. calcitrapa* and *C. diluta*, had three and four significant path coefficients respectively. A similar pattern is seen in *Crepis* where the highly and moderately invasive species had two or fewer non-zero path coefficients while the least invasive *C. setosa* had four significant trait relationships.

With respect to the direction and magnitude of trait relationships, in both genera differences among variably invasive species were largely of degree and not sign, of the relationship. The two exceptions to this pattern were the less invasive *Centaurea sulphurea* and *Crepis zacintha*. *Centaurea sulphurea* had a strong negative relationship between branch order and stem length while this relationship was strongly positive in the most invasive *Centaurea* species. Similarly *C. zacintha* had a strong

trade-off between stem length and inflorescence production where this relationship was positive or non-significant in the more invasive *Crepis* species.

The good fit of our path models to the observed data in both genera confirms their overall architectural similarity. This analysis suggests that more invasive species within both genera exhibited fewer non-zero path coefficients and generally weaker relationships where they were significant. Furthermore some less invasive species had significant negative path coefficients where highly invasive congeners had positive or non-significant relationships.

Broader relevance of path analytic approaches to invasive species---Although path analytic approaches have been widely employed in studies of phenotypic variation (e.g. Scheiner et al., 2000; Valladares et al., 2002; Huber et al., 2004; Pigliucci and Kolodynska, 2006; Shipley, 2006) and biological invasions (Grotkopp et al., 2002; Taylor and Irwin, 2004; Vazquez and Simberloff, 2004; Garcia-Berthou et al., 2005; Keeley et al., 2005; Mullerova et al., 2005; Williams et al., 2005; Harrison et al., 2006; Seabloom et al., 2006), their use in studies of phenotypic variation of biological invasions is uncommon (but see Grotkopp et al., 2002). Within the field of invasion biology path analysis has been largely restricted in use to questions above the level of the individual phenotype, including population biology studies (Mullerova et al., 2005), the influence of environmental factors on invasions (Taylor and Irwin, 2004; Garcia-Berthou et al., 2005; Keeley et al., 2005; Mullerova et al., 2005; Seabloom et al., 2006), the effects of introduced species on ecosystem processes (Williams et al., 2005), and the effects of introduced species on native species demography (Vazquez and Simberloff, 2004; Harrison et al., 2006; Seabloom et al., 2006).

Other multivariate approaches more commonly used in studies of invasive phenotypes include principal component analysis (Grotkopp et al., 2002; DeWalt et al., 2004; Erfmeier and Bruelheide, 2004), discriminant analysis (Rejmanek and Richardson, 1996; McDowell, 2002; Richardson and Rejmanek, 2004; Muth and Pigliucci, 2006), and multiple regression (Scott and Panetta, 1993; Goodwin et al., 1999; Hamilton et al., 2005). Although these statistical methods all have the benefit of being able to allow for trait interactions, they do not provide insight into specific causal structures. Path analytical models differ from these previous methods in that interactions among traits can be directional (i.e. implying specific causal relationships) and can be restricted to those interactions conforming to our prior knowledge of the phenotypes in question. The result is that path models have the capacity to provide robust descriptions

of complex integrated phenotypes.

   Although the analysis of non-quantitative trait variation (e.g. perennation, reproductive syndromes, growth form) may be effective in providing predictive information about invasive and noninvasive species, this type of variation is less likely to be found among the many interesting cases where close relatives differ in their success. In studies of closely related introduced species, path analysis can be a useful tool in determining which particular phenotypic relationships differ between variably invasive introductions.

Conclusions---We found substantial differences in phenotypic integration between variably invasive species within two genera of short-lived forbs. The phenotypes of highly invasive species were generally less constrained than those of less invasive congeners. However, similarly flexible phenotypes were also found among certain less invasive species. While highly constrained phenotypes may preclude invasiveness, not all flexible phenotypes are likely to be highly invasive.

Table 1. Species list, invasive status (as characterized for this study, see text for details); life history characterization as annual (a), biennial (b), or perennial (p); earliest known record for North America; and population accession source data for the 28 populations used in this study.

species	invasive status	life history	date of introduction	population source
Centaurea calcitrapa L.	low	a,b,p	1827	wild collected by J. DiTomaso (CA, USA) Botanical Garden of the University of Gottingen, Germany Civico Orto Botanico, Trieste, Italy Kew Royal Botanical Gardens, United Kingdom
Centaurea cyanus L.	high	a	1880	wild collected by NZM (Blount county, TN, USA) wild collected by NZM (Knox county, TN, USA) Botanical Garden of the University of Gottingen, Germany Kew Royal Botanical Gardens, United Kingdom National Botanical Garden of Belgium
Centaurea diluta Aiton	low	a	1952	Kew Royal Botanical Gardens, United Kingdom
Centaurea melitensis L.	moderate	a,b	1881	wild collected by J. DiTomaso (CA, USA) wild collected by M. Pitcairn (Solano Co., CA, USA)
Centaurea solstitialis L.	high	a	1879	wild collected by J. DiTomaso (CA, USA) Civico Orto Botanico, Trieste, Italy
Centaurea sulphurea Willd.	low	a	1923	wild collected by J. DiTomaso (CA, USA)
Crepis capillaris (L.) Wallr.	high	a,b	1903	wild collected by NZM (Haywood county, NC, USA) wild collected by NZM (Johnson county, TN, USA) National Botanical Garden of Belgium University Botanical Garden, Marburg, Germany
Crepis setosa Haller f.	low	a	1904	wild collected by NZM (Polk County, OR, USA) wild collected by A. Liston (Benton Co., OR, USA) Botanical Garden of the University of Gottingen, Germany National Botanical Garden of Belgium
Crepis tectorum L.	moderate	a	1891	wild collected by NZM (Dane County, WI, USA) wild collected by NZM (Marquette County, WI, USA) Botanical Garden of the University of Gottingen, Germany Friedrich-Schiller-Universitat, Jena, Germany
Crepis zacintha (L.) Babcock	low	a	1993	Botanical Garden of the University of Gottingen, Germany

Table 2. Summary statistics for the structural equation model as applied to *Centaurea* and *Crepis* species. The Root Mean Square Error of Approximation together with its 90% confidence interval and P-value (low values indicate significant departure between observed covariances and those expected from the model structure) and the Akaike Information Criterion both take into account model fit and complexity (penalizing unnecessarily complex models). The Comparative Fit Index is a goodness-of-fit index where values close to 1 indicate very good fit.

Genus	RMSEA	Confidence Interval for RMSEA (*P*-value for RMSEA ≤ 0.05)	saturated AIC	Model AIC	Independence AIC	CFI
Centaurea	0.09	0.03 - 0.15 (0.11)	168.0	164.9	224.7	0.87
Crepis	0.08	0.00 - 0.16 (0.22)	112.0	108.4	114.1	0.87
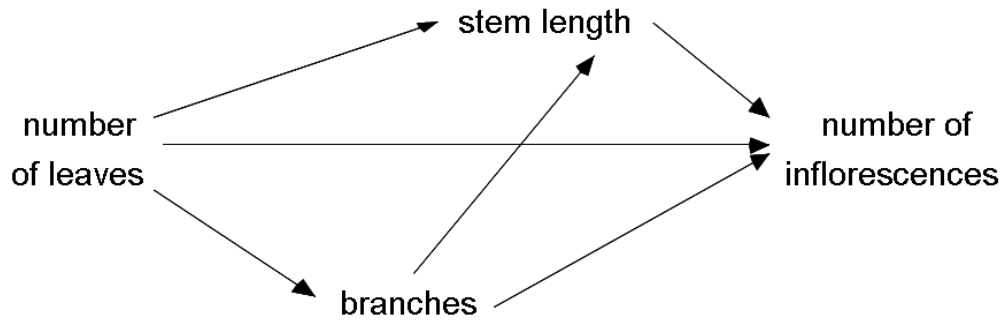
Figure1. The path analytical phenotypic model used to fit the data for all species. The model structure is based on prior knowledge and analysis of *Crepis* and *Centaurea* species (see text for details).
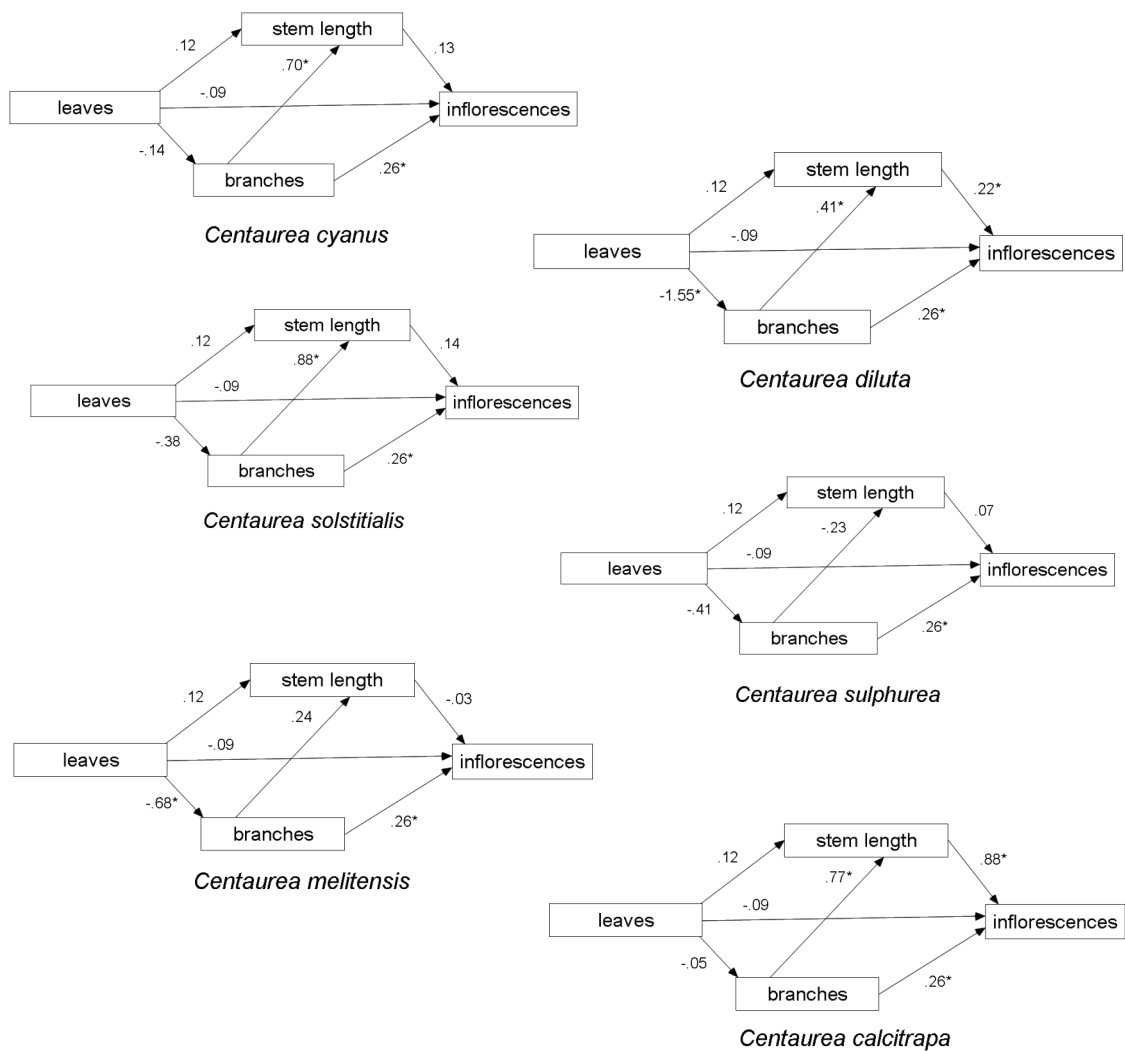
Figure 2. Path models and direct effects for all *Centaurea* species. Path coefficients indicated by an asterisk are statistically different from zero (p < 0.05).

stem length

-.11   .36*   .23*

leaves   -.04   inflorescences

-.12   branches   -.16

Crepis capillaris

stem length

-.51*   .36*   .44*

leaves   -.04   inflorescences

-.12   branches   .20*

Crepis setosa

stem length

-.27   .36*   .32

leaves   -.04   inflorescences

-.12   branches   .03

Crepis tectorum

stem length

-.15   .36*   -.26*

leaves   -.04   inflorescences

-.12   branches   -.17

Crepis zacintha

Figure 3. Path models and direct effects for all *Crepis* species. Path coefficients indicated by an asterisk are statistically different from zero (p < 0.05).

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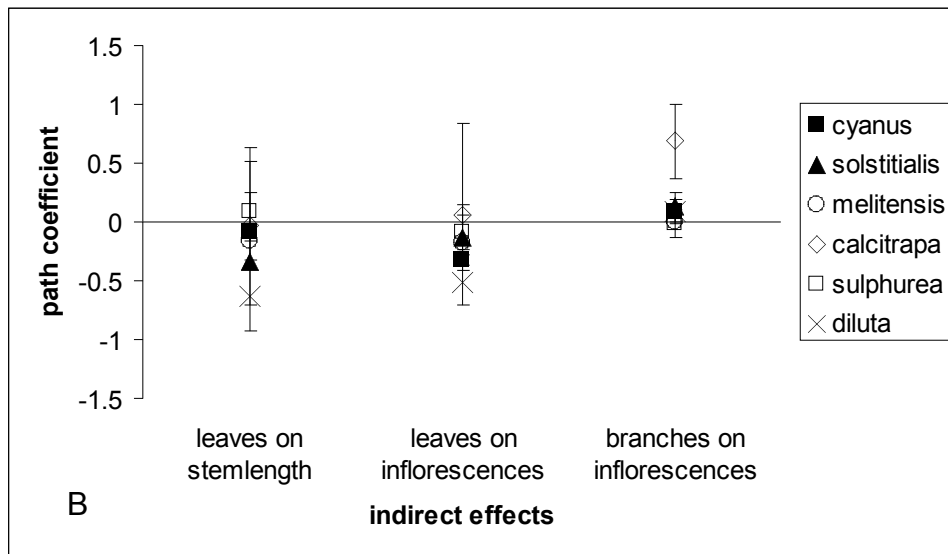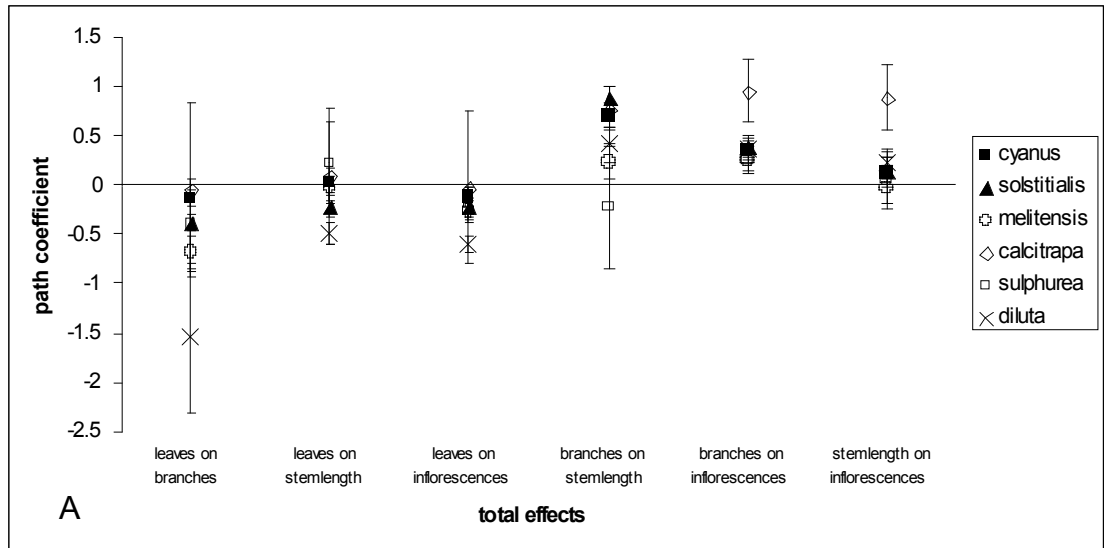
Figure 4. Total (a) and indirect effects (b) for all *Centaurea* species. Bars indicate standard errors.
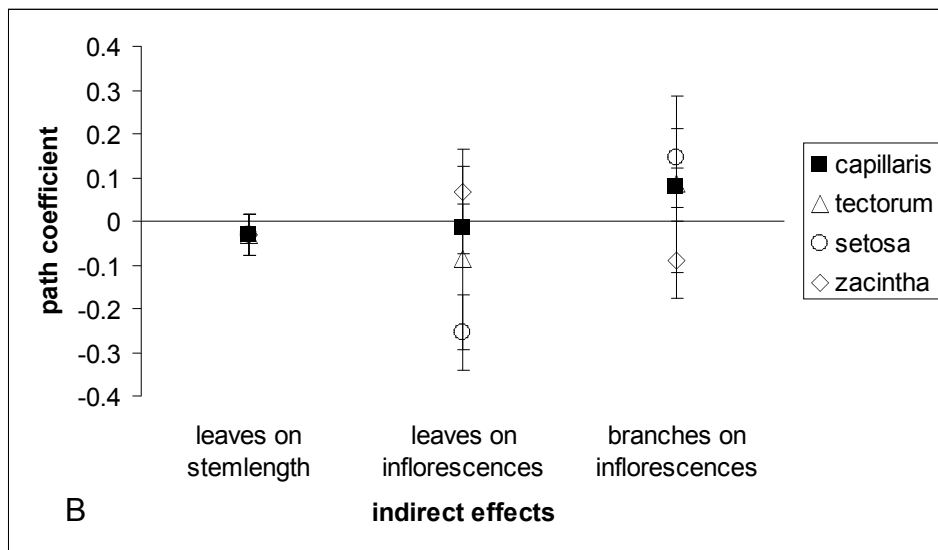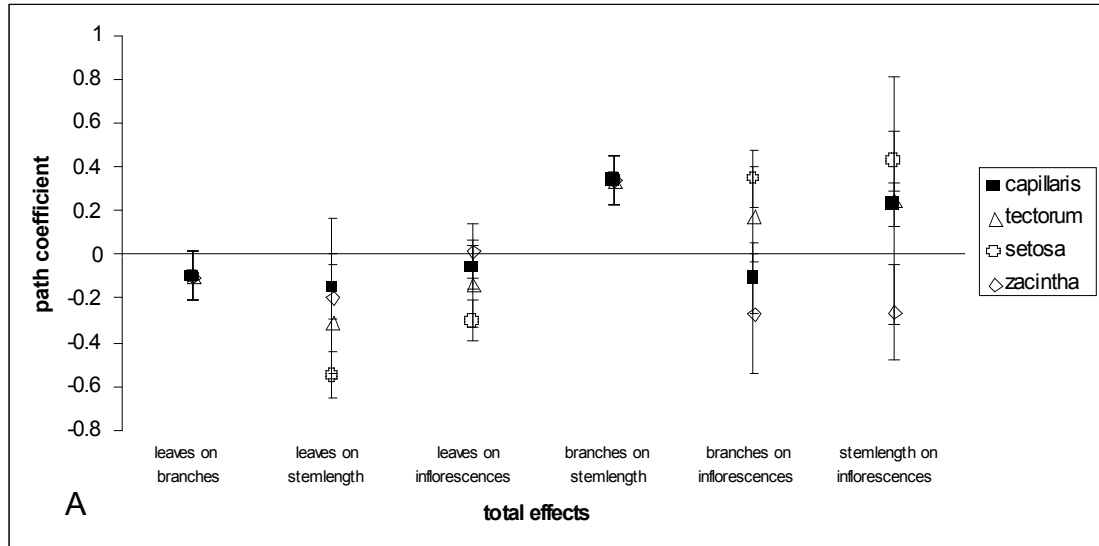
Figure 5. Total (a) and indirect effects (b) for all *Crepis* species. Bars indicate standard errors.

V. Plasticity of phenotypic integration among variably invasive *Centaurea* introductions in response to phosphorus and drought

Abstract

Why certain species become widespread following introduction while others remain restricted is a longstanding and practically important biological question. In an effort to better understand the complex phenotypes underlying variably invasive *Centaurea* species (Asteraceae), we employed path analysis to model their integrated architectures and subsequent fitness differences across two environmental gradients (phosphorus additions and drought conditions). While the significant direct effects suggested greater constraints in the more invasive species, the total and indirect effects pointed to a more complex and interconnected web-like architecture in the less invasive species. We observed no significant plasticity of trait interactions across our drought treatments. While the observed plasticity of architectures across the phosphorus gradient was modest, the variability th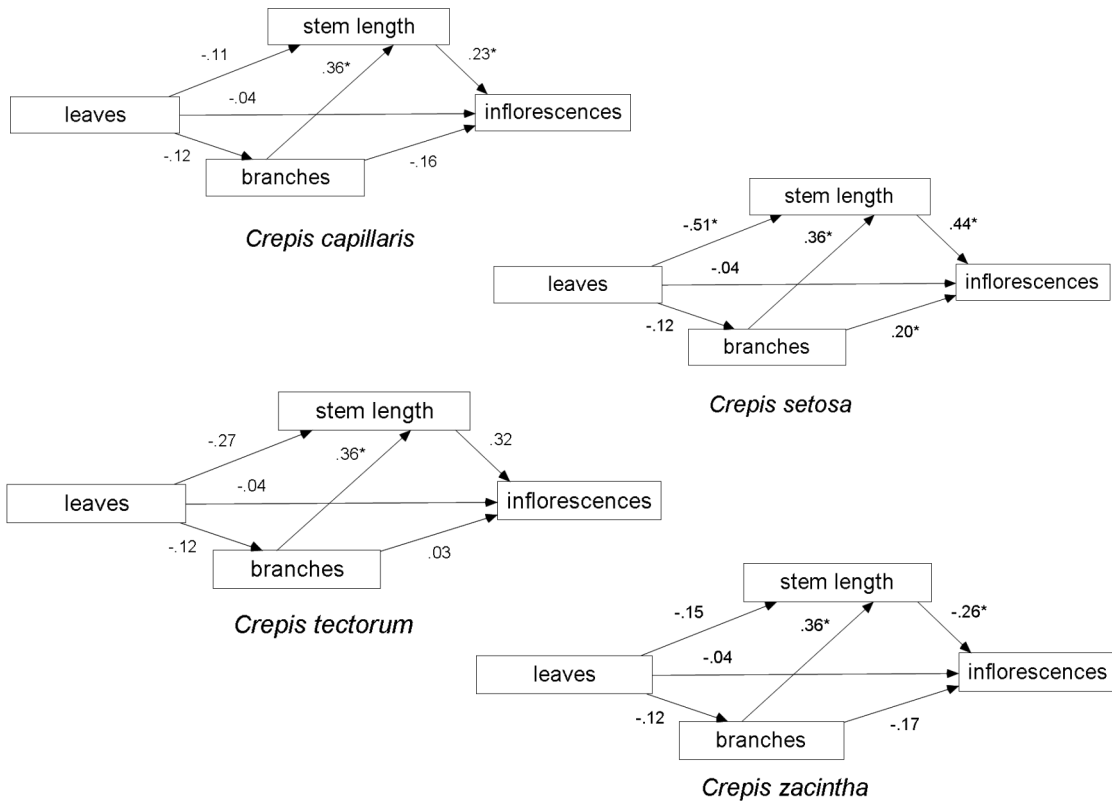at existed revealed a decrease in fitness-related constraints in a highly invasive species under favorable conditions. This specific shift in architecture may help explain the substantial fitness responsiveness of *C. solstitialis* to high phosphorus availability.

INTRODUCTION

In the face of vast amounts of research on invasive species, the continuing struggle to identify traits associated with invasiveness suggests both a potential problem with approaches as well as the general question itself of "what makes some species invasive?" Recent studies have detailed the nature of some of these problems (Rejmanek, 2000; Colautti and MacIsaac, 2004; Colautti et al., 2004; Hierro et al., 2005; Muth and Pigliucci, 2006; Richards et al., 2006) and have suggested alternative experimental methodologies and conceptual frameworks. Here we address several of these frameworks in using variably invasive species

introductions to investigate the plasticity of their integrated phenotypes under stressful and favorable conditions.

The use of variably invasive species introductions allows us to better address the question of why some introduced species are more invasive than others. This is an important question considering that most species introductions fail, many more are largely benign (Williamson, 1996), and many native species are often as weedy as problematic introductions (Muth and Pigliucci, 2006). Examining the responses of species to stressful and favorable conditions allows us to determine how phenotypic plasticity affects invasiveness and to what degree more invasive species are particularly robust in the face of harsh conditions (a Jack-of-All-Trades *sensu* Richards et al. 2006), able to significantly increase fitness in favorable conditions (a Master-of-Some), or both (a Jack-and-Master). Finally, the analysis of trait-trait interactions in a causally connected phenotypic framework may facilitate a better understanding of how certain species achieve their reproductive advantages and their robust or responsive architectures.

In this study we used structural equation modeling (SEM) to investigate the plasticity of integrated plant architectures of variably invasive *Centaurea* species (*C. cyanus*, *C. solstitialis*, and *C. melitensis*, all Asteraceae) across two environmental gradients, phosphorus addition and drought condition. Specifically, we sought to address the following questions: (1) Across environments, do more invasive introductions exhibit patterns of phenotypic integration consistent with fewer constraints? We predicted that the more invasive species would have fewer tradeoffs between the production of necessary developmental and architectural structures (i.e. more invasive species should exhibit non-significant or weakly positive relationships between traits). (2) Does trait integration vary in a predictable manner with increasing environmental stress? We predicted that exposure to the more stressful environments, P-limitation and drought, would result in more rigidly interconnected architectures. (3) Do more invasive species show evidence of having more robust or responsive phenotypes? We predicted that the highly invasive species would be more likely to maintain similar architectural and fitness relationships under stressful conditions and better able to respond to favorable conditions with increasingly flexible phenotypic relationships.

MATERIALS AND METHODS

Plant material---*Centaurea* are short-lived herbaceous plants commonly found in a variety of disturbed habitats including roadsides, rangeland and agricultural fields. Although there are *Centaurea* species

native to North America, many others are classified as problematic noxious weeds throughout North America. For the purposes of our study, we have previously characterized the invasive status of *C. cyanus, C. solstitialis* and *C. melitensis* (Table 1). The designations of highly and moderately invasive is based upon recorded presence of the species in the lower 48 U.S. as presented in the USDA PLANTS Database (2002). Muth and Pigliucci (2006) have previously shown that intra-state level (county by county) presence/absence records confirm our characterization of the invasive status at a smaller spatial scale (i.e., for the species used in this study invasives are largely widespread both across and within states, while non-invasives are largely restricted at both scales). It is important to note however, that these range characterizations may not capture the population structure of the species. Where any of these species occur, it is often at high densities and can cause serious environmentally deleterious impacts.

Estimates of dates of introduction to North America from native Eurasian ranges are given as the earliest found herbarium record compiled from various North American herbaria and available floras (Table 1). Additional details on the merits and drawbacks of our characterization of invasiveness are discussed in Muth and Pigliucci (2006).

Plant handling and experimental set-up---In April 2003, two to four seeds (based on availability) were planted into each of thirty 11.5 by 11.5 by 12.7cm pots of autoclaved sand and distributed in a randomized block design in the University of Tennessee White Avenue greenhouse. We used 400 watt metal halide lights at 16h per day (at one meter intervals) to augment ambient lighting and photoperiod.

Upon initial germination we hand thinned pots to one individual and maintained them as such for the remainder of the experiment. Following successful development to true leaf production (at approximately three to five weeks post germination), half of all pots within each block were exposed to a single week-long drought. Controls were watered twice weekly, as were drought-exposed plants following the drought treatment. Concurrent with the post-drought treatment, we established three phosphorus (P) treatments. All levels of P treatment received a background of modified Hoagland's solution of 5 mmol $Ca(NO_3)_2$ x $4H_2O$, 5 mmol KNO_3, 2 mmol $MgSO_4$ x $7H_2O$, 1ml/L 1% Fe-EDTA, and 1ml/L micronutrient solution (Boron 0.5 mg/L, Manganese 0.5 mg/L, Zinc 0.05 mg/L, Copper 0.02 mg/L, Molybdenum 0.01 mg/L). The P levels varied as follows: a No-P treatment contained only the background modified Hoagland's solution (although trace amounts of P were likely present in our potting material, pots and watering equipment); a Field-P treatment

included 0.025 mmol $Ca(H_2PO_4)_2 \times H_20$; and a High-P treatment contained 0.5 mmol $Ca(H_2PO_4)_2 \times H_20$. Phosphorus treatments were administered bi-weekly (at 250 ml. per administration) throughout the duration of the experiment.

With the exception of number of rosette leaves at bolting, all traits were measured at senescence. These traits included: 1) number of rosette leaves at bolting, an estimate of investment in the vegetative phase of growth; 2) stem length, a component of the size and architecture of the reproductive phase; 3) branch order (number of branch nodes encountered in tracing the longest stem backwards to the rosette), a second characterization of plant architecture during the reproductive phase; and 4) number of inflorescences, a component of reproductive fitness.

Data analysis--- The results of standard univariate ANOVAs for all traits appear in Muth and Pigliucci (in Press). We checked data for violations of assumptions of normality and homoscedasticity and standardized each variable to its arithmetic mean following relevant transformations (number of rosette leaves and number of inflorescences were log transformed).

We used AMOS (version 7.0, James L. Arbuckle, 2006) to create path models and perform SEM. We employed a phenotypic path model (Fig. 1) previously developed for a single common environment greenhouse study that included our current study species, among others. The structure of the path model was originally based on the following understanding of the architecture and development of the study species, as well as prior analyses by Muth and Pigliucci (2006; in press): rosette leaf development precedes and normally ceases prior to the development of the architectural and fitness traits. As most branching in these species occurs prior to the achievement of greatest stem length, the relationship between these variables was assumed to be unidirectional with branching antecedent to, and potentially influencing, stem length. Number of inflorescences is modeled to assess the direct and indirect influences of all other traits on this aspect of lifetime fitness.

For each species we separately modeled the responses to the environmental factors, drought condition and phosphorus addition. This analysis allowed us to determine the sensitivity of the modeled trait relationships ranging from stressful (drought and no P addition) to favorable conditions, within each species. In developing the best fit models from each species-environmental gradient combination, each path of the fully constrained model (one assuming equal regression weights across variable Phosphorus levels or across watering regimes) were

sequentially relaxed to assess improvements in model fit (measured as a statistically significant decrease in X^2). Paths were retained as unconstrained where this resulted in significantly improved model fit. The resulting model performance for each species-environmental gradient combination was evaluated according to the following criteria:

      1. Root mean square error of approximation (RMSEA), together with its confidence interval and p-value for the hypothesis of a RMSEA = 0. This statistic takes model complexity into account while characterizing the fit of a given model to the observed covariance matrix. RMSEA values range from zero to one with models having values approaching zero indicating better fit, or greater parsimony, compared to those with larger values.

      2. Akaike's information criterion (AIC), a second joint measure of fit and simplicity. The AIC values are given for the reduced model of interest, as well as for the saturated and independence models. The saturated model has no degrees of freedom and includes all possible paths, yielding an over-parameterized model with the maximum possible fit. The independence model assumes independence of all variables and is essentially equivalent to the simple path diagram generated by a multiple regression analysis. Smaller AIC values reflect a better balance of fit and parsimony.

      3. Finally, we report the goodness-of-fit using the comparative fit index (CFI) in which the covariance of the observed data is compared to that expected assuming the path model is true. Values close to one indicate very good fit, and values > 0.9 generally indicate acceptable fit (Bollen 1989).

RESULTS

Model fit across environments---As described above, we began with the analytical model based on the phenotypic development of our study species (Fig. 1). Relaxation of path constraints (common path coefficients for a species across an environmental gradient, phosphorus addition or drought treatment) yielded six path multigroup models: *Centaurea cyanus* across three phosphorus treatments, *C. solstitialis* – across three phosphorus treatments, *C. melitensis* – across two phosphorus treatments, *C. cyanus* across two drought treatments, *C. solstitialis* – across two drought treatments, and *C. melitensis* – across two drought treatments (Table 2). Due to low sample size in the No-P and Field-P treatments in *C. melitensis*, these groups were lumped together as a single Low-P treatment and compared to the High-P treatment. Summary statistics indicate relatively good model fit for all six

combinations of species and environmental gradients (Table 2).

In only one case, *C. solstitialis* modeled across P treatments, did relaxing path constraints result in a better fit model. In this model the path between stem length and number of inflorescences was allowed to vary across treatments (Fig. 2). In both the No-P and Field-P addition treatments there was a strong positive effect of stem length on the number of inflorescences. However, this effect became non-significant in the High-P treatment (Fig. 2). This was the only significant trait interaction observed in our species models across P environmental treatments. There was no statistically significant path variation observed within species in response to the drought treatment.

Comparison of path coefficients across species--- When compared to the characterization of variation within species across environmental treatments, there were substantially greater differences observed across species in similar environments. In all species branch order contributed positively to stem length (Fig. 2). This was the only similar path coefficient shared across all species. In *C. solstitialis* the effect of leaves on branch order and stem length were both significant and strongly positive. In *C. cyanus* the number of leaves had a positive effect on inflorescence production, but this was the only case where this effect was statistically significant. The effect of branch order on inflorescence production was significant and strongly positive in both *C. cyanus* and *C. solstitialis*. Lastly, in *C. solstitialis*, the effect of stem length on inflorescence production varied from strongly positive in No-P and Field-P conditions to non-significant in the High-P treatment. This effect was non-significant in both *C. cyanus* and *C. melitensis*.

In examining the total and indirect relationships among traits in these species it was generally the case that these effects were positive or not significantly different from zero (Fig. 3). Where these effects differed, the less invasive *C. melitensis* exhibited stronger effects than either of the more invasive species.

DISCUSSION

Multivariate phenotypes and invasiveness---In a previous study in which these species, and others, were grown in a single environment we observed appreciable differences in trait interactions between variably invasive species. In this previous study the most integrated phenotypes (those having the most non-zero path coefficients) were found in the less invasive species. In the current study we have, on the surface, an apparently contradictory finding where the more invasive *C. cyanus* and *C.*

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solstitialis appeared to have more integrated phenotypes than the comparatively less invasive *C. melitensis*.

There are, however, two lines of evidence against this interpretation. First, while *C. melitensis* had very few significant direct relationships among traits (Fig. 2), the sum of these effects and indirectly mediated effects suggests a very different picture of *C. melitensis* exhibiting a tightly connected (i.e. relatively constrained) architecture, mediated through the combined effects of interacting traits. Second, the change in the strength of the direct effect of stem length on inflorescence production in *C. solstitialis*, from strongly positive in No-P and Field-P conditions, to non-significant in High-P conditions (Fig. 2), hints at the broader relationship between this study and the previous single-environment study. The previous study was carried out in comparatively lush conditions, much more favorable even than the High-P treatment in this study (due largely to the use of a more nutrient rich potting medium and larger containers). Therefore, if we expect to see greater trait integration in stressful environments (e.g. Schlichting, 1986, 1989; Chapin, 1991; Pigliucci, 2004, Tonsor and Scheiner, 2007; but see Pigliucci and Kolodynska, 2006), as we do in *C. solstitialis* here, we should also expect to have seen greater overall trait integration in this study compared to the previous one, and this is also the case.

Finally, the plasticity of the relationship between stem length and inflorescence production observed in *C. solstitialis* contributes to our understanding of how this species is able to increase its fitness under high phosphorus conditions (as observed by Muth and Pigliucci, *in Press*). The decoupling of these otherwise tightly linked traits in favorable conditions may represent a significant shift in developmental architecture from one with a particular and relatively rigid stem growth to inflorescence ratio, to a different form where plant height reaches a plateau but continued inflorescence production is still feasible.

Conclusions---We found substantial differences in phenotypic integration between variably invasive species of *Centaurea*. The phenotypes of highly invasive species were generally more constrained with respect to the direct interactions of traits than that of a less invasive congener. However, the total and indirect effects of the less invasive species were generally greater, indicating a more complicated architecture than suggested by the direct effects alone. We also found evidence for some species having more constrained phenotypes (stronger trait interactions) under more stressful phosphorus conditions. Furthermore, the plasticity of this particular trait interaction suggests a potential mechanism by which an invasive species exploits favorable phosphorus conditions.

Table 1. Species list, invasive status (as characterized for this study, see text for details); life history characterization as annual (a), or biennial (b); earliest known record for North America; and population accession source data for the 6 populations used in this study.

species	invasive status	life history	date of introduction	population source
Centaurea cyanus L.	high	a	1880	wild collected by NZM (Blount Co., TN, USA)
				wild collected by NZM (Knox Co., TN, USA)
Centaurea melitensis L.	moderate	a, b	1881	wild collected by J. DiTomaso (CA, USA)
				wild collected by M. Pitcairn (Solano Co., CA, USA)
Centaurea solstitialis L.	high	a	1879	wild collected by J. DiTomaso (CA, USA)
				wild collected by E. Grotkopp (Solano Co., CA, USA)

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Table 2. Summary statistics for the structural equation model as applied to *Centaurea* species grown in two environmental gradients, (P)hosphorus and (D)rought conditions. The Root Mean Square Error of Approximation together with its 90% confidence interval and P-value (low values indicate significant departure between observed covariances and those expected from the model structure) and the Akaike Information Criterion both take into account model fit and complexity (penalizing unnecessarily complex models). The Comparative Fit Index is a goodness-of-fit index where values close to one indicate very good fit.

Species	RMSEA	Confidence Interval for RMSEA (*P*-value for RMSEA ≤ 0.05)	saturated AIC	Model AIC	Independence AIC	CFI
C. cyanus (P)	0.06	0.00 - 0.14 (0.36)	84.0	69.2	193.9	0.98
C. solstitialis (P)	0.00	0.00 - 0.16 (0.56)	84.0	69.7	150.0	1.00
C. melitensis (P)	0.15	0.00 - 0.31 (0.19)	56.0	50.3	82.8	0.88
C. cyanus (D)	0.13	0.00 - 0.25 (0.14)	56.0	52.1	130.6	0.93
C. solstitialis (D)	0.00	0.00 - 0.17 (0.58)	56.0	45.3	105.1	1.00
C. melitensis (D)	0.00	0.00 - 0.18 (0.76)	56.0	43.0	76.4	1.00
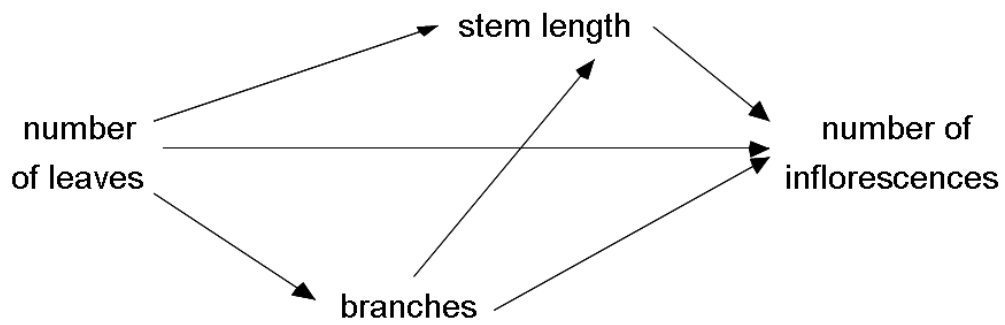
62

Figure1. The path analytical phenotypic model used to fit the data for all species. The model structure is based on prior knowledge and analysis of *Crepis* and *Centaurea* species (see text for details).

stem length

-.08

.33*

.11

leaves

.17*

inflorescences

.32

branches

.78*

Centaurea cyanus

stem length

.57*

.49*

.42*, .47*, -.06

leaves

-.03

inflorescences

.67*

branches

.53*

Centaurea solstitialis

stem length

.34

1.00*

.23

leaves

.60

inflorescences

.71

branches

.29

Centaurea melitensis
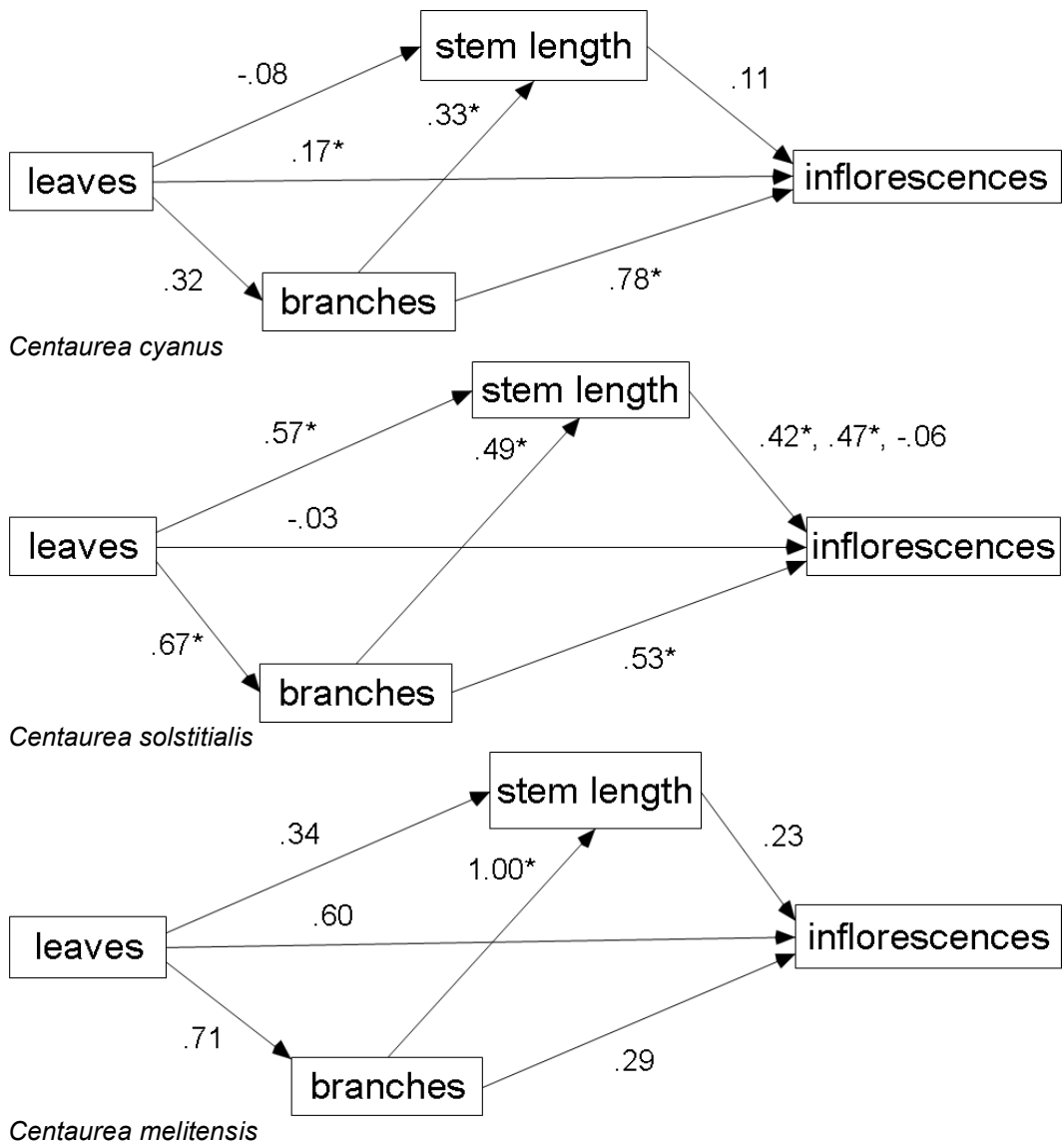
Figure 2. Path models and direct effects of plant architecture for all *Centaurea* species across a phosphorus gradient. Path coefficients indicated by an asterisk are statistically different from zero (p < 0.05). Multiple coefficients (in the case of *C. solstitialis*) indicate the change in the direct effect with increasing phosphorus (No-P, Field-P, and High-P additions).
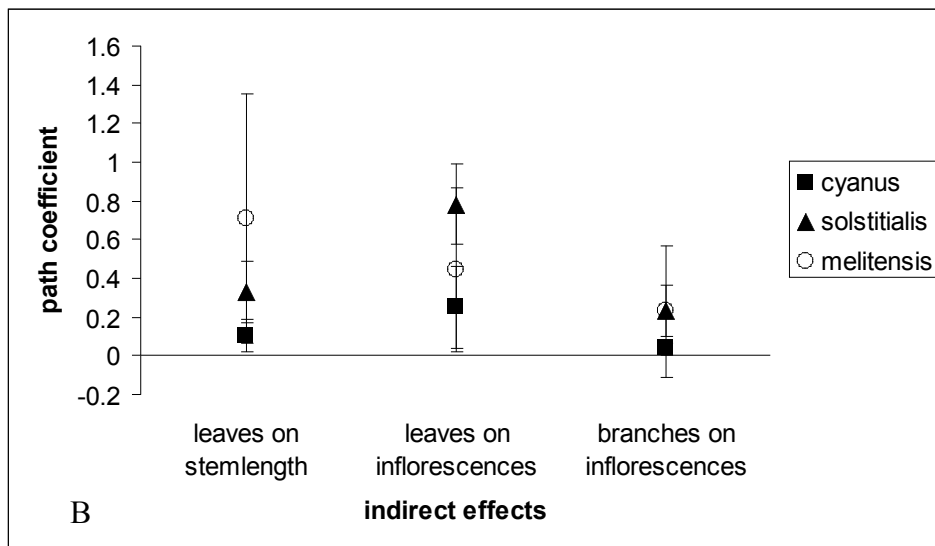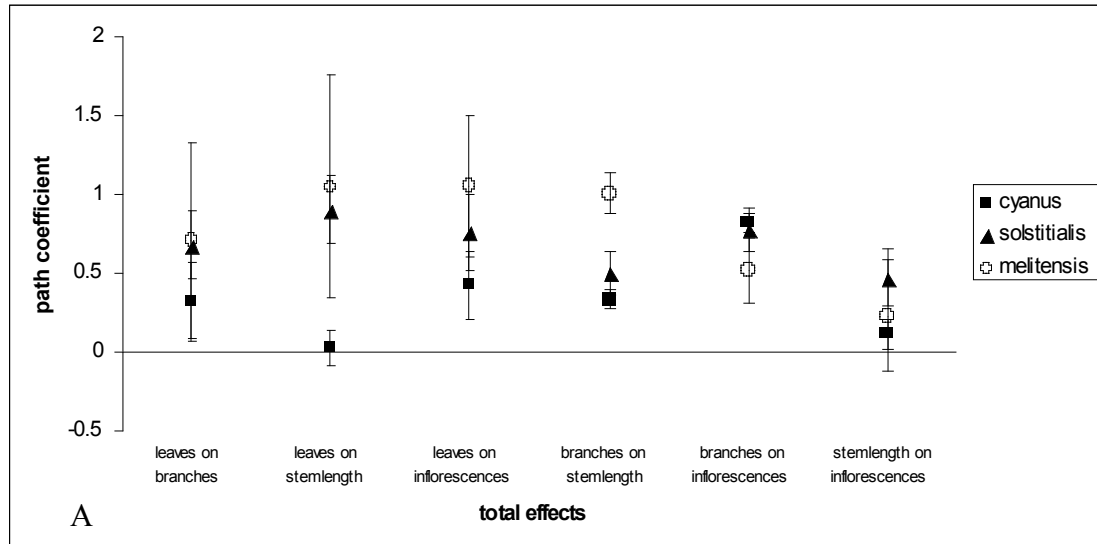
Figure 3. Total (a) and indirect effects (b) for all *Centaurea* species. Bars indicate standard errors.

VI. Summary

In chapter one we provided an outline of the patterns of interest central to biological invasion research and suggested that better articulated questions, and consequently more appropriate experimental designs, could significantly improve our understanding. Furthermore, we suggested that invasion biologists can benefit from accepting the discipline as more of a "soft" science in that: (1) generalizations are likely to be few and relatively straightforward; (2) compelling explanations are likely to be pluralistic in character; and (3) adequate studies may feel more like "puzzle solving" than "hard" science.

In chapter two we implemented a study design, suggested by the preceding chapter, that compared phenological, architectural, size, and fitness traits of several introduced invasive species to introduced noninvasive species within two genera of Asteraceae (*Crepis* and *Centaurea*). While there were many significant differences between the genera, there were few shared attributes among invasive or noninvasive congeners, even for traits as seemingly important as the number of inflorescences produced and the size of seed heads.

In chapter three we showed evidence that the more invasive species of *Crepis* and *Centaurea* had greater stress tolerance to low phosphorous conditions, but we observed no relationship between invasiveness and opportunism to favorable conditions. Overall, trait responses were highly variable and largely idiosyncratic with respect to invasive categorization.

In chapter four we employed path analysis in modeling the integrated phenotypes of introduced congeneric species differing in degree of invasion success in two genera of Asteraceae (*Crepis* and *Centaurea*). Path models for highly invasive species generally exhibited fewer non-zero path coefficients (suggesting less overall integration) and fewer negative path coefficients (suggesting fewer allocation trade-offs). Path models revealed potential trade-offs in allocation among architectural traits within less invasive species that were absent in more invasive congeners. These results differed from univariate analyses of these species that revealed few common associations of traits with invasiveness, within or across genera.

In chapter five path models of the integrated architectures and

fitnesses of variably invasive species across two environmental gradients (phosphorus additions and drought conditions) pointed to a more complex and interconnected web-like architecture in the least invasive species. While the observed plasticity of architectures across the phosphorus gradient was modest, the variability that existed revealed a decrease in fitness-related constraints in a highly invasive species under favorable conditions. This specific shift in architecture may help explain the substantial fitness responsiveness of this species to high phosphorus availability.

      While some aspects of our results conformed to our expectations regarding the phenotypes of variably invasive species, there were many results counter to these predictions. Despite that our empirical assessments of the plasticity and integration of invasive phenotypes provided us with a greater understanding of their biology, it seems likely that the breadth of relevant factors involved in these studies (which genotypes, which populations, which traits, which environments) reduces the likelihood of detecting robust general patterns. As suggested in chapter one and supported by the idiosyncrasies of our own results, a synthetic view of these studies suggest that while explanations of specific cases were forthcoming, broader predictions were largely unrealized.

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