SUPPORTING INFORMATION

Supplemental discussion of methodology

Factors to consider when deciding if a given study organism is amenable to invasibility

experiments

Invasibility experiments cannot be performed using all study organisms. For some organisms, it

might be logistically intractable to quantify all components of population growth (e.g., hatching

rates, survival, fecundity) or to perform competition experiments. The former difficulty could be

overcome by focusing on one or few components of population growth (allowing option 2 in

Figure S1), by experimenting on multiple life history stages and integrating them via

demographic modeling, or by pairing estimable components with literature estimates (allowing

option 1 in Figure S1). Care must be taken with small populations that could be subject to Allee

effects [3], which may emerge in some organisms and would underestimate the rate of increase

expected in a larger population. For other organisms, competition experiments might not be

possible or ethical (i.e., genes being moved among localities and the possibility of escape).

Figure S1 provides a flowchart to be used to decide if a given organism is amenable to a full

(option 1) or partial (option 2) test of invasibility. Though researchers should certainly consider

the ethics of moving relocating genetically-distinct populations, our proposed method is

analogous to reciprocal transplant tests of local adaptation (e.g., [4]), which are commonly

employed, as are species pool manipulations in ecological studies (e.g., [5]).

Performing invasibility experiments if single-species equilibria are not known

To make inferences about coexistence outcomes from invasibility experiments, it is

recommended to compete each species when rare and its competitor is at its single-species

equilibrium abundance [3]. In practice, however, equilibrium abundances are difficult to know

ahead of time, given that they vary through space and time and are affected by other community members. As such, simplifying the experiment to rare vs. abundant is often the best way forward. Here we discuss the benefits of making this simplification, how to decide what population sizes are most appropriate, and how doing so might affect competitive outcomes. The most obvious benefit of a 'rare vs. abundant' contrast rather than 'rare vs. at single-species equilibrium' is that the former approach overcomes the difficulty of quantifying the latter. A less obvious benefit is that, because equilibria can evolve in different environmental contexts, technically each population of origin would need to be maintained at a population-specific single-species equilibrium abundance, which would result in different replicate population pairs receiving different community densities. We recommend that population sizes of the "abundant" competitor against which each species competes when rare be based on a rough average of the population sizes of allopatric populations in their natural environment – or at least a population density that is ecologically plausible. Doing so is the most tractable way to achieve population sizes that approximate single-species equilibria while allowing all replicates to experience the same competitor density. Note that, unlike invasibility experiments, carrying capacities are estimated via model fitting and need not be known *a priori* when estimating stabilizing and fitness differences (Box 1).
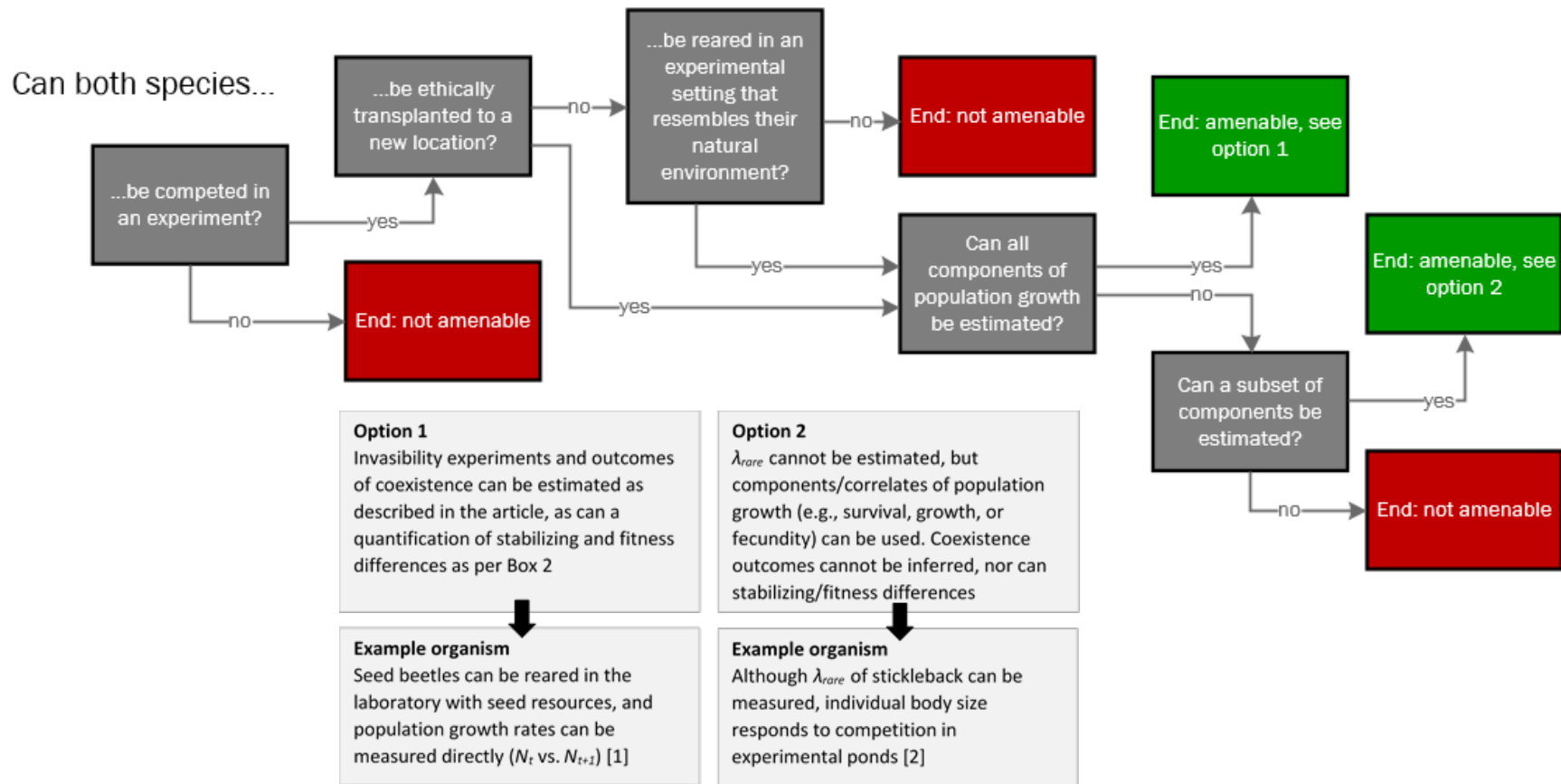
Can both species...

...be competed in an experiment?

...be ethically transplanted to a new location?

...be reared in an experimental setting that resembles their natural environment?

Can all components of population growth be estimated?

Can a subset of components be estimated?

End: not amenable

End: amenable, see option 1

End: amenable, see option 2

Option 1
Invasibility experiments and outcomes of coexistence can be estimated as described in the article, as can a quantification of stabilizing and fitness differences as per Box 2

Example organism
Seed beetles can be reared in the laboratory with seed resources, and population growth rates can be measured directly (N_t vs. N_{t+1}) [1]

Option 2
λ_{rare} cannot be estimated, but components/correlates of population growth (e.g., survival, growth, or fecundity) can be used. Coexistence outcomes cannot be inferred, nor can stabilizing/fitness differences

Example organism
Although λ_{rare} of stickleback can be measured, individual body size responds to competition in experimental ponds [2]

Figure S1. Decision tree to help researchers answer the question of "is my study system amenable to an invasibility experiment?"

Supplementary Boxes

Box S1. Weighing the invasibility framework against six criteria for character displacement

Schluter and McPhail [4] highlighted six criteria that should be addressed when examining putative cases of character displacement. The criteria were designed to help rule out alternative explanations for a pattern of character divergence in sympatry and allopatry. More than twenty years later, Stuart and Losos [5] reported that few studies have met those criteria. As a solution, they called for more rigorous assessments of character displacement, and studies of experimental evolution that better control factors that tend to confound observational studies. Our proposal to adopt community ecology's invasibility framework overcomes many shortcomings of prior observational approaches, while still describing past evolutionary outcomes of competitive interactions and looking ahead to its ecological consequences. Here we demonstrate how the invasibility framework addresses each criterion.

Criterion 1: Phenotypic differences result from evolved genetic differences, not environmentally induced plasticity. Competition trials that pit populations of each species against one another in a common arena address this criterion by avoiding phenotypic comparisons amongst individuals living in different environments. Ideally, populations would be reared in a common environment for one or more generations prior to competition experiments to control for maternal effects [6].

Criterion 2: Patterns suggestive of character displacement could not arise by chance. By sampling replicate sympatric-allopatric pairs of populations, researchers have met this criterion more often than any other – repeated divergence of the same character across

replicate population pairs has become the hallmark of character displacement. However, focusing on phenotypes potentially biases our understanding of the prevalence and mode of character displacement in nature. Divergence that is non-repeatable (characters displaced in different directions) among populations (Figure 1C,D) could fail to meet this criterion, as would convergence in characters that reduce differences in resource use (Box 2, Figure 3). The invasibility framework offers a robust way to identify non-random competitive divergence between species, even when the underlying causes are heterogeneous across population pairs.

Criterion 3: Character displacement results from evolution in sympatry, not species sorting after allopatric divergence. Meeting this criterion requires ruling out pre-existing differences that are also found in allopatry. By comparing the strength of competition between allopatric versus sympatric population pairs, the invasibility framework tests this criterion directly.

Criterion 4: Phenotypic differences among competitors affect resource use. This criterion ranked second in frequency met, suggesting that researchers have focused on study systems where the character-resource use link is obvious or well-documented. In contrast, the invasibility framework reveals the net effect of all ecologically-relevant characters, even when they are unknown or hard to measure. Knowing how specific characters map onto resource use is not a required criterion of an invasibility approach, as competitive differences are directly measured rather than inferred.

Criterion 5: No confounding differences in environment between sympatry and allopatry. This criterion is rarely met, given that real-world environmental gradients vary in many dimensions in addition to the presence or absence of a competitor, and characters that affect resource use can be under selection by the abiotic and biotic environment. It is possible for

character evolution to be driven by the abiotic environment but lead to patterns of invasibility that are consistent with character displacement, as is also the case with a character-centric approach [7]. For either approach, character displacement and environmental variation are most likely to be confounded when sympatry occurs at species' range boundaries [7], compared to when species' ranges overlap and co-occurrence patterns are a mosaic determined by colonization and extinction. Although one solution is to statistically partition variation due to the abiotic environment [8], we present the interplay between adaptation to the biotic and abiotic environment (i.e., is selection synergistic or antagonistic?) as an outstanding question that could be tested via their effects on λ_{rare}.

Criterion 6: Evidence that similar phenotypes actually compete for resources. The invasibility framework is a direct and informative way to measure the strength of competition and its consequences for population growth. To date, this criterion has been met less frequently than any other, suggesting that the invasibility framework fills a critical need. However, what remains is evaluating whether species interactions measured in an experimental setting indeed reflect resource competition, or if other forms of interactions are present (e.g., apparent competition, intraguild predation, mutualisms), how they contribute to competitive differences among species as measured via invasibility. Other forms of interactions are viewed as features of the environment unrelated to coevolutionary history with a competitor, and can influence the relative competitive abilities of competitors if each is differentially affected by the biotic environment (as is also the case with the abiotic environment).

<u>Box S2. Invasibility outcomes in the presence of divergence vs. convergence in resource use</u>

Estimating the degree to which characters have diverged or converged in sympatry, and what that means for the overall intensity of interspecific competition, is not trivial. Not only does doing so run into the same challenges presented in Figure 1, but additionally, a single character might simultaneously experience evolutionary pressure for divergence and convergence. The results of invasibility experiments described in the main text can be used to differentiate whether character displacement primarily drives divergence or convergence in resource use. We have already argued that classic character displacement (i.e., divergence in characters resulting in divergent resource use) is predicted to weaken interspecific competition among sympatric population pairs and thus lead to increased invasibility (both species $\lambda_{\text{rare[sympatric]}} > \lambda_{\text{rare[allopatric]}}$ in Figure S2A). By contrast, if one species is an inferior competitor for limited resources prior to evolution, then individuals of the inferior competitor that use resources more similarly to the superior competitor will have higher fitness [9], resulting in character convergence and higher invasibility in sympatry (Species A $\lambda_{\text{rare[sympatric]}} > \lambda_{\text{rare[allopatric]}}$ in Figure S2B). As a consequence of the inferior competitor evolving to become a stronger competitor in sympatry, the strength of interspecific competition experienced by the superior competitor should be higher in sympatry than in allopatry, resulting in reduced invasibility in sympatry (Species B $\lambda_{\text{rare[sympatric]}} < \lambda_{\text{rare[allopatric]}}$ in Figure S2B). Taken together, divergence in resource use in response to character displacement is characterized by a mutual increase in invasibility in sympatry for both species, whereas convergence in resource use is characterized by opposing directions of response.
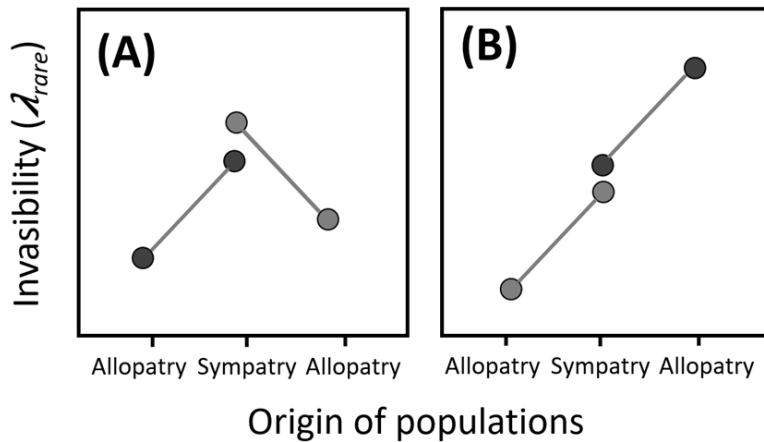
Figure S2. Predicted consequences of character displacement for invasibility outcomes in the presence of divergence vs. convergence in resource use. (A) Resource use divergence as a consequence of character displacement should give rise to higher invasibility in sympatry for both species (presented in main manuscript), whereas (B) convergence in resource use should give rise to increased and decreased invasibility in sympatry depending on whether the species was the inferior or superior competitor prior to evolution, respectively.

Image sources

Darwin's finches: open to the public domain, accessed via wikipedia.org

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Small milkweed bug: photograph by Tom Murray © bugguide.net

Common milkweed: image created by Karen A. Johnson © karensnatureart.files.wordpress.com

Supplementary references

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