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Commentary

The role of facilitative interactions in tree invasions

Many ecologists studying the invasion biology of exotic plants have adopted the Gleasonian view that plant communities are primarily structured on the basis of competitive individualistic interactions and are primarily not structured on the basis of interdependent interactions (i.e. direct and indirect facilitation) (e.g. Bruno *et al.*, 2005). However, some have speculated that facilitative interactions affect plant invasions (reviewed in Simberloff & Von Holle, 1999); for example, some invasive grasses increase the prevalence of fires, which negatively affects resident species and indirectly facilitates additional invasion (D'Antonio & Vitousek, 1992). A literature survey reveals that competitive interactions may be overemphasized relative to their actual occurrence in nature compared with facilitative interactions; however, this conclusion is based primarily on studies describing associational patterns (Bruno *et al.*, 2005). It is clear that many more studies have been conducted that have tested for competitive effects than have tested for facilitative effects (Bruno *et al.*, 2005). Furthermore, most studies relating to invasion biology are based on associational patterns and relatively few have included rigorous field experiments that characterize individual direct and indirect interactions (Simberloff, 2004; Bruno *et al.*, 2005). A clever and insightful study by Saccone and colleagues in this issue of *New Phytologist* (pp. 831–842) addresses this concern with manipulative experiments that help to elucidate the effects of direct and indirect interactions on the invasion process occurring in a European floodplain.

'*… A.* platanoides *was observed to actually reduce its internode lengths with reductions in R : Fr in an apparent attempt to 'sit-and-wait' rather than compete for light in an unfair duel with canopy trees.*'

Saccone and colleagues performed a field experiment along a successional gradient and manipulated the effects of different canopy types and the presence of the understory herbaceous layer on juvenile tree species. Disturbance by flooding drives the successional gradient at the study site. Existing theory, on the organization of natural plant communities, predicts that direct facilitative effects are most likely in environments with a high level of physical stress (Bertness & Callaway, 1994). Consistent with this prediction, they found that the resident *Salix alba* tree canopy, in the early-seral habitat of the floodplain (Fig. 1), had direct facilitative effects on the invasive *Acer negundo* and the late-seral *Fraxinus angustifolia*. This was counterbalanced by the competitive effects of the herbaceous layer, and results suggest a net facilitative effect of the two vegetative layers on juvenile *A. negundo* and *F. angustifolia*. Establishment of exotic invaders is affected by two important processes: propagule supply processes and post-dispersal recruitment processes. The reported facilitative effects are believed to buffer the *Acer* recruits from the impacts of flood disturbance. This and the species' ability to disperse via water appear to be essential for its invasion into the studied floodplain, while competitive effects exerted by the herbaceous layer help to limit their invasion into this zone of the floodplain (Fig. 1).

Saccone and colleagues report further that the invasive *Acer* populations facilitate their own continued presence by both direct facilitation and indirect facilitation effects of the *Acer* canopy on juveniles of this species. As the recruits grow, their population develops into reproductive trees that form a forest intermediate between the *Salix* and *Fraxinus* forests along the successional gradient (Fig. 1). The developing *Acer*-dominated forest accelerates structural changes along the successional gradient, leading to increased shading of the understory compared with the early-seral *Salix* forest (Fig. 1). A characteristic of other invaded systems is that they alter ecosystem processes and successional trajectories, which may negatively affect resident species while either directly or indirectly facilitating invasion (e.g. Reinhart *et al.*, 2006; Dehlin *et al.*, 2008). The increased shade caused by the *Acer* canopy appears to have a direct negative effect on the understory herbaceous layer and weakens the competitive effects of the herb layer on *Acer* juveniles. The *Acer* canopy, therefore, indirectly facilitates establishment of its recruits by ameliorating the negative effects of the herbaceous layer on its juveniles. Although not in an invasion context, *Acer pseudoplatanus* in its native European forests has also been observed to indirectly facilitate establishment of its seedlings by reducing the competitive effects of understory forbs on *A. pseudoplatanus* seedlings (Pagès *et al.*, 2003). Other invasive tree species are known to reproduce in intact forests, and then to 'sit-and-wait' and rapidly exploit any newly formed canopy gaps with great efficiency compared with the resident natives (Closset-Kopp *et al.*, 2007). Martin & Canham (2010) found variation among the survival characteristics of seedlings of native and invasive *Acer* species, indicating that variation among phylogenetically related species is important in determining which become invasive. Evidence is beginning to emerge suggesting a type of syndrome among some invasive plants that share the functional characteristic of shade tolerance and the ability of adults to alter canopy structure in systems where the resident species lack a similar combination of characters.

Parallels between the study by Saccone *et al.* and other studies suggest that invader-driven changes in the understory light environment, which differ from natural rates of succession and/or trajectories, may be a common mechanism by
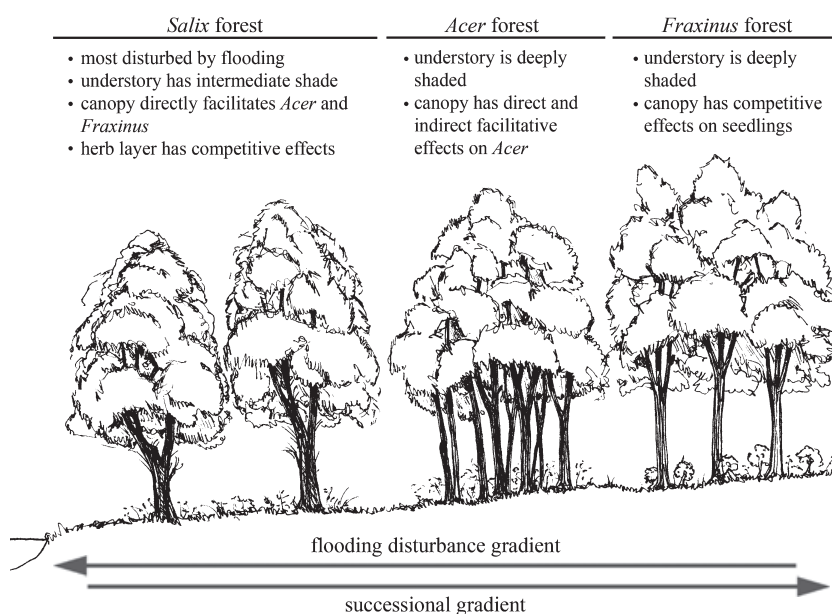
Salix forest	*Acer* forest	*Fraxinus* forest
• most disturbed by flooding • understory has intermediate shade • canopy directly facilitates *Acer* and *Fraxinus* • herb layer has competitive effects	• understory is deeply shaded • canopy has direct and indirect facilitative effects on *Acer*	• understory is deeply shaded • canopy has competitive effects on seedlings

flooding disturbance gradient

successional gradient

Fig. 1 A diagrammatic cross-section of the middle Rhône River, France, being invaded by *Acer negundo*. The diagram illustrates the effect of canopy and understory on the establishment and invasion of *A. negundo* along a successional gradient from early-seral *Salix* forest, through *Acer* invaded mid-seral forest, to late-seral *Fraxinus*-dominated forest (Saccone *et al.*, this issue, pp. 831–842). The figure is not to scale. The illustration is courtesy of D. A. Malcolm.

which invasive species disrupt natural communities and facilitate their own invasion (Reinhart *et al.*, 2006; Dehlin *et al.*, 2008; Valladares & Niinemets, 2008). *Acer platanoides*, which is native to Europe, has invaded North American forests, and in some invaded forests has caused canopy-driven changes that have quantitatively and qualitatively (red : far-red (R : Fr)) altered the understory light environment compared with uninvaded forests (Reinhart *et al.*, 2006). The study by Reinhart *et al.* (2006) also provides suggestive support for indirect facilitation, as the invader-driven alteration of the understory light environment reduced the survival of many resident species while its seedlings were uniquely capable of surviving and optimally allocating resources. This change in understory light quantity and quality with canopy closure is not surprising; however, the plants' responses to the change in R : Fr were relatively unusual. In response to reductions in R : Fr, most plants are thought to follow the shade avoidance syndrome and elongate their stems and increase their internode lengths in an attempt to aggressively compete for light with similar-sized neighbors (Smith, 2000). Much of this research, however, has focused on shade-intolerant and early-seral species in contexts where symmetric competition for light is likely. Seedlings that elongate in response to the light environment created by mature trees would probably experience increased mortality as a result of producing long slender stems. This may explain why *A. platanoides* was observed to actually reduce its internode lengths with reductions in R : Fr in an apparent attempt to 'sit-and-wait' rather than compete for light in an unfair duel with canopy trees (K. O. Reinhart, unpublished data). Different environmental cues are thought to elicit shade tolerance (photosynthetically active radiation (Smith, 1982) and blue light (Lin, 2000)) vs shade avoidance responses (R : Fr). Shade tolerance may depend on synergistic responses to the collinear changes in light quantity and R : Fr that help prolong survival until light conditions change and facilitate growth (Valladares & Niinemets, 2008).

Documenting associational patterns is likely to always be an important aspect of invasion biology. Advances in the field, however, are likely with studies that couple information on associational patterns with descriptions of species' interactions. Incorporating a more mechanistic understanding that can account for direct facilitative and indirect effects will require manipulative experiments to better understand the complex of interactions shaping natural communities and plant invasions. Combining knowledge of associational patterns with more reductionist and mechanistic experimental approaches will advance our understanding of invasion biology and improve our ability to predict and manage invaders and improve restoration (Simberloff, 2004). Increasing evidence is accumulating that shows exotic plants are invading mature undisturbed forests (reviewed in Martin *et al.*, 2008). However, the vast majority of invasion biology research has focused on early-seral habitats. Studies in these additional systems may be essential for building an understanding, especially if knowledge regarding early-seral environments is not transferable, as suggested in the above case of phenotypic responses to changes in R : Fr. Future studies are needed that continue to perform field experiments and advance our understanding of the interactions contributing to invader establishment, spread, and impact in multiple systems.

Kurt O. Reinhart

USDA-ARS Fort Keogh Livestock & Range Research Laboratory, Miles City, MT 59301-4016, USA
(tel +1 406 874 8211; email kurt.reinhart@ars.usda.gov)

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Understanding natural epigenetic variation

Recently, there has been increased interest in understanding the role of epigenetic processes in ecology and evolution (e.g. Richards, 2006; Bossdorf *et al.*, 2008; Johannes *et al.*, 2008; Richards *et al.*, 2010). We now know that some epigenetic marks are not reset each generation, but are faithfully transmitted across generations (Jablonka & Raz, 2009), that natural variation can exist not only in the DNA sequence but also at the epigenetic level (e.g. Vaughn *et al.*, 2007) and that epigenetic variation can cause significant heritable variation in phenotypic traits (e.g. Johannes *et al.*, 2009). Moreover, heritable epigenetic modifications can be triggered by exposure to different environmental conditions (e.g. Verhoeven *et al.*, 2010). If we put these different pieces of evidence together, then this clearly suggests that epigenetic mechanisms could add an additional layer of complexity to heritable phenotypic variation, and thus to the diversity and evolutionary potential of natural populations. However, in spite of abundant speculation about the potential ecological and evolutionary implications of epigenetic processes, most previous work has been carried out on only a few types of agricultural crops and on model species such as *Arabidopsis thaliana*, frequently under artificial conditions, and we therefore still have no idea of the true importance of epigenetic processes in natural populations. Because of this, several authors have argued for expanding research efforts into ecologically relevant circumstances across model and nonmodel organisms and have outlined experimental and statistical approaches that would facilitate the merging of molecular-based insight with sound evolutionary ecology (Bossdorf *et al.*, 2008; Johannes *et al.*, 2008; Richards, 2008). In this issue of *New Phytologist* (pp. 867–876), Herrera & Bazaga provide an intriguing example of how researchers are now beginning to respond to this call.

> '*… epigenetic mechanisms could add an additional layer of complexity to heritable phenotypic variation, and thus to the diversity and evolutionary potential of natural populations.*'

Herrera & Bazaga measured natural epigenetic variation in *Viola cazorlensis*, a long-lived violet endemic to southeastern Spain. They surveyed 14 natural populations across a 1000-m elevation gradient in the limestone mountains of the Sierra de Cazorla, sampled leaves from multiple individuals in each population and analysed the variation in DNA methylation among these plants using methylation-sensitive amplified fragment-length polymorphism (MS-AFLP) markers. One of the strengths of this study was that the same individuals had previously been analysed for DNA-sequence variation using standard AFLP markers (Herrera & Bazaga, 2008), which allows genetic and epigenetic data to be linked. It was found that epigenetic differences between populations and individuals are strongly correlated with the presence of specific AFLP loci that were previously implicated in divergent selection and adaptive differentiation in floral traits between the populations. They conclude that the observed epigenetic variation may thus be involved in population differentiation in ecologically important traits.

To our knowledge, the study of Herrera & Bazaga is the first attempt of a true population epigenetics study, and the authors are to be commended for their pioneering effort. They show how epigenetic variation is partitioned within and between *Viola* populations and search for indications that epigenetic variation may be involved in population adaptation. Studies like the one by Herrera & Bazaga are essential first steps for assessing the ecological and evolutionary relevance of epigenetics. However, observational epigenetic studies in natural populations do have some limitations, and these reflect some of the main current challenges in ecological and evolutionary epigenetics. Here, we discuss some of these challenges and the possible next steps towards understanding natural epigenetic variation.

One of the fundamental differences between genetic and epigenetic variation is that the latter is, to some extent, environmentally labile and reversible. Many of the developmental processes that underly the phenotypic plasticity of plants in response to different environments involve epigenetic changes (e.g. the plasticity of flowering time, Sung & Amasino, 2004). Therefore, patterns of epigenetic differentiation among field populations that are measured in different environments – like the ones observed in the study of Herrera & Bazaga – will probably include a reversible component as a result of phenotypic plasticity and a nonreversible or relatively stable component as a result of heritable epigenetic differentiation. Clearly, epigenetic contributions to phenotypic plasticity and to heritable variation are interesting from both an ecological and an evolutionary perspective. But in this respect, analyses of epigenetic variation are similar to analyses of phenotypic variation, and common garden experiments are necessary to firmly establish inheritance and to differentiate between plastic and heritable components of variation. For this reason, an informative