

## ORIGINAL ARTICLE

### Bridges and barriers to host shifts resulting from host plant genotypic variation

Timothy P. Craig<sup>a\*</sup>, Joanne K. Itami<sup>a</sup>, Takayuki Ohgushi<sup>b</sup>, Yoshino Ando<sup>b</sup> and Shunsuke Utsumi<sup>c</sup>

<sup>a</sup>Department of Biology, University of Minnesota Duluth, 1035 Kirby Drive, Duluth, MN 55812, USA; <sup>b</sup>Center for Ecological Studies, Kyoto University, 2-Hirano, Otsu 520-2113, Japan; <sup>c</sup>Department of General Systems Studies, University of Tokyo, 3-8-1 Komaba, Meguro, Tokyo 153-8902, Japan

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Host-associated differentiation is hypothesized to be one of the primary means by which new species of herbivorous insects evolve. Divergent selection for host plant use following a host shift is a critical assumption of host-associated differentiation. Host shifts are more likely to occur between closely related host species, and the probability of a host shift and of the evolution of reproductive isolation depends on the genetic variation within both the host plant population and the herbivorous insect. We have been studying the evolution of reproductive isolation in populations of *Eurosta solidaginis* that form galls on *Solidago altissima altissima* and *Solidago altissima gilvocanescens*. Each of the *Eurosta* populations is a host race that is partially but incompletely isolated from the other host races by its adaptation to its host plants. The *Solidago sp.* populations show high intraspecific variation within and among populations in their resistance to attack by the host races which are adapted to them. There is also evidence that they vary in their susceptibility to host races adapted to other *Solidago* species. We examine the hypothesis that the intraspecific *Solidago* variation determines the degree of gene flow among *Eurosta* populations and that this can create barriers or bridges to gene flow and therefore determine the course of speciation in these insects. The intraspecific host plant variation can influence the interactions with herbivores when it is an invasive species. The restricted range of genetic variation introduced into a new region may be a good or poor match with populations of herbivores that are also introduced into the new area.

**Keywords:** intraspecific variation; host race; invasive species; herbivory; host plant adaptation; gene flow

#### Introduction

A plant species is not a uniform resource for insect herbivores. There can be wide intraspecific phenotypic variation in species' attractiveness to herbivores, and in their suitability as food, and for entomophagous insects as an environment for the development of their offspring (Thompson 1988; Mayhew 2001; Craig and Itami 2008). There is a genetic basis to much of this variation that can have widespread effects on both the suitability for specific species and on the development of the entire herbivore community (Maddox and Root 1987). In this paper we examine the impact of the range of intraspecific host plant variation for interactions with specialist insects. We examine how the intraspecific variation can influence the colonization of new hosts, gene flow among populations adapted to different host plants, and the success of colonization by insects when it is an invasive species.

Differences between host plant populations or species can produce divergent selection for host-associated differentiation (HAD) (Funk and Nosil 2008; Nosil et al. 2009). Both the potential for a host shift and whether or not a population that shifts to a new host will become a genetically differentiated population depends on the distribution of genetic variation in the host plants. Many herbivorous insects

are highly specialized, either being monophagous or narrowly oligophagous (Weis and Berenbaum 1989; Novotny and Basset 2005). This is particularly true of endophagous insects such as gallers (Hardy and Cook 2010). We have been studying the host-associated populations of the gall-inducing tephritid fly *Eurosta solidaginis* on tall goldenrod *Solidago altissima* and early goldenrod *Solidago gigantea*, and two subspecies of tall goldenrod, *S. altissima altissima* and *S. a. gilvocanescens*. Each host race has a preference for oviposition on its natal host plant, and higher larval survival on its host plant (Craig et al. 1993, 1997, 2001; Craig and Itami 2010). Each host race also has a preference for mating on its host plant, and this is crucial for maintaining reproductive isolation (Craig et al. 2001; Craig and Itami 2010).

A phytophagous insect is most likely to expand to using a new plant species if that species is closely related to the usual host species and shares characteristics important to the herbivore with the original host plant (Berlocher and Feder 2002; Drés and Mallet 2002). The closer another plant's characteristics are to the normal host, the higher the probability of an oviposition mistake and the higher the probability that the larvae will survive on the novel host. *Eurosta* will completely reject species from another plant families or genus (Craig and Itami

\*Corresponding author. Email: tcraig@d.umn.edu

unpublished data), but it will occasionally oviposit on closely related *Solidago* species (Abrahamson et al. 1989; Craig et al. 1997, 2001), and *Eurosta* will survive at low levels on the most closely related *Solidago*. If novel hosts are utilized, the outcome of this accidental colonization will depend on the genotypic composition of the new host species. If there are strong similarities between plants and little divergent selection between the old and new hosts, then a host expansion will occur and the insect will become polyphagous. If there are strong differences between the host plants with no offspring resulting from oviposition “mistakes,” then there will be selection to select for greater host discrimination and no host shift. If there are differences between host plants creating divergent selection, but where there is some probability of survival on the “wrong” host plant, then a host shift and the evolution of reproductive isolation and speciation may follow.

Which of these three outcomes will occur depends not only on the mean differences between the characteristics of the plant species but on the distribution of plant genotypes on these characters. In a hypothetical model (Figure 1) we have two plant species with different mean characteristics along a continuum of plant characteristics critical for plant preference (such as a chemical that induces oviposition) or larval performance (such as a compound that influences larval survival). For simplicity we hypothesize a normal distribution of characters, but any distribution is possible, and it would be interesting to examine the consequences of other distributions. Within each plant species only a limited number of plant genotypes are

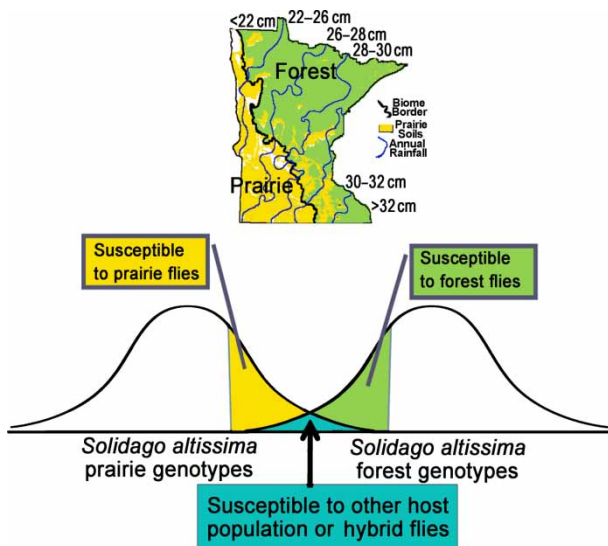


Figure 1. The genotypic bridge hypothesis. Host plants species have a wide range of genetic variation, and even adapted herbivore populations can utilize only a small proportion of those genotypes. A closely related plant species also has a range of genotypes, and some of those may overlap the genetic range of herbivores that are adapted to the closely related plant, allowing the colonization of the plant, and subsequently gene flow between populations that are adapted to other host plants.

suitable resources for survival even for the host race adapted to that population. The characters of the other host plant may lay completely outside the range where the plant can be colonized for a fly adapted to another *Solidago* sp. (Figure 2A). Alternately, if there are two very closely related plants, the fly could expand to utilize the genetic variation in the new host plant without evolving reproductive isolation (Figure 2B). Each of the host races studied so far fits the third possibility: that there is a difference in the means of the two host plants, but that there are extreme genotypes on which the alternate host race can survive (Figure 2C).

Several of the assumptions of this model are supported. First, there is variation among genotypes for the *Eurosta* host race that specializes in that species, in both *S. a. altissima* and *S. gigantea* for both oviposition preference and offspring performance (Andersen et al. 1989; Craig et al. 1999, 2000; Cronin et al. 2001; Wise et al. 2008). Oviposition preference varied in choice experiments from 0.2 ovipositions to 1.2 ovipositions per stem. Larval survival rates in experiments varied from 0 to 1 larvae per stem oviposited. The combination of oviposition preference and offspring performance meant that some genotypes lacked galls while others had nearly one gall per stem (Craig et al. 1999, 2000).

The second assumption of this model is that a *Eurosta* host race’s preference and survival on the alternate host plant will show wide variation with most of the alternate host genotypes being unsuitable, but with a small proportion of the plant genotypes permitting survival of the alternate *Eurosta* host race. Each

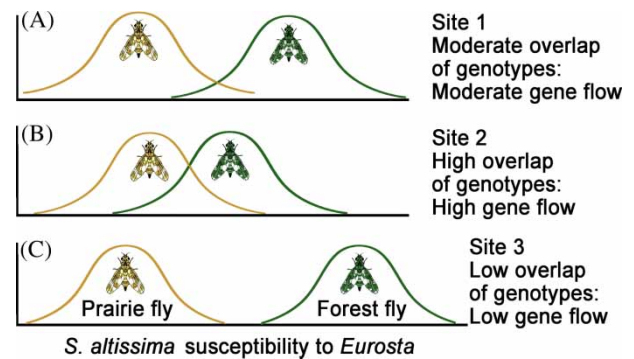


Figure 2. Geographic variation in the in the distribution of plant genotypes that vary in susceptibility to locally adapted populations of flies. (A) In area number one the plant populations are moderately differentiated in characteristics, causing divergent selection in the flies and resulting in the formation of host races with some gene flow facilitated by intermediate genotypes on which both host races can survive. (B) In area number two the populations are only slightly differentiated, leading to only slight differentiation of the fly populations and a large amount of gene flow because many plant genotypes of each species are susceptible to attack by both fly populations. (C) In area three the plant populations are so differentiated that there is no possibility for survival of each fly population and the other host plant forming a barrier to gene flow and selecting for the formation of two species.

*Eurosta* host race has a preference for its own host plant, but some individuals will oviposit on the alternate host plant (Craig et al. 1993, 1997, 2001; Horner et al. 2008). Each *Eurosta* host race has a much higher survival rate on its own host plant than on alternate hosts, but each did survive on the alternate host plant at a very low rate (Craig et al. 1997, 2001, 2010). Thus these rare phenotypes provide a “bridge” whereby some gene flow can pass from one population to another if two populations have already been established. These phenotypes may have also facilitated the colonization of the new host plant. If a few genotypes of one host plant bore strong similarity to those of the original host, a few individuals with a mutation for host preference could have become established on that host, and subsequent mutations would have perfected their adaptation to that host plant, permitting a wider colonization of genotypes.

The critical questions are, first, whether there is a genotypic basis to intraspecific host variation, and, second, whether each fly host race responds to variation within the alternate host plant in the same manner as it does to variation within the host plant to which it is adapted. Craig et al. (2007b) provided a partial answer to this question. They examined the response of hybrid larvae between the gigantea fly host race and the forest altissima fly host race to intraspecific genotypic variation. The hybrids showed significant differences in survival among plant genotypes, with survival ranging from zero on most genotypes to a rate equal to that of the pure fly host races on their host species. This supports the bridge hypothesis in that it demonstrates that there are plant genotypes that can provide gene flow between the two host races (Craig et al. 2007b). It also indicates that because each hybrid larva has half its genes derived from the alternative host race, the interaction of the herbivore’s genes with the host plant’s genes influence fly survival.

We artificially formed hybrids by mating male and female flies in a no-choice environment where host plants were not available. In a natural situation assortative mating occurs due to host plant preference. So another second crucial part of the bridge hypothesis is that there are genotypes of the host plant that are attractive to the alternate host race. We are currently testing this hypothesis.

Another hypothesis to be tested is that there is intraspecific genotypic variation in the pure host race’s ability to survive on the alternative host plant. This would not directly influence gene flow, as there is no evidence that larval conditioning influences host preference (Craig et al. 2001). If the pure fly host races did have variable survival among genotypes on the alternate host plant, then that would indicate that there was the potential for host expansion rather than divergence selection resulting in speciation, and that the probability for host expansion was mediated by the intraspecific host variation.

The distribution of intraspecific host plant genetic variation in susceptibility to the alternate host plants among sites may determine the variation in gene flow between the host races among sites. Host plants may evolve geographic differences for a variety of reasons that may influence susceptibility to the gall fly. For example, *S. altissima* plants show geographic variation in a number of morphological traits (Craig and Itami 2010), and as stated previously there is genotypic variation in susceptibility to *Eurosta*. We are currently testing the hypothesis that there is geographic variation in the frequency of genotypes that are susceptible to the alternative host race. The differences in character between two host plants may vary among locations from the situations depicted in Figure 2A–2C. Differences in the degree of overlap of susceptibility will mean that there will be differences in the degree of gene flow among geographic areas. As a result, the relationships between populations of the herbivore may range from relatively undifferentiated populations to host races to good species in different parts of the range.

The distribution of intraspecific variation in the host plant will influence the distribution of genotypic variation in the phenotypic variation in the phytophagous insect. Genetic variation in the herbivore should be proportionate to variation in the host plant: if the host plants are genetically uniform, then their herbivores will also be uniform; if the host plants are highly polymorphic, their phytophagous parasites will be too. Increased genetic variation in the host plants could result from a wide geographic distribution where the plants are exposed to a wide variation in environmental selection, or it could result from exposure to a wide range of herbivorous insects. Intraspecific host plant genetic variation that resulted in a genetically diverse herbivore population would increase the potential for utilizing or colonizing other host plants.

### Intraspecific variation and invasive species

Intraspecific variation will have an impact on the outcome of interactions when species are introduced to new environments. Introduced species typically experience a strong founder effect where a very limited range of genetic variation is introduced to the new location (Sakai et al. 2001). This limited amount of genetic variation will influence the evolution of interactions with other species in their new environment. It may also have a strong effect on the interaction of the plant and herbivorous species that attack it in its native range if both are invasive species.

Both the reduction of genetic variation in the host plant and the specific genetic variation in both species will influence the outcome of interactions in the invaded environment. As a hypothetical example we examine the results of introducing a limited range of genetic variation in both *S. altissima* and *E. solidaginis* to a new environment (Figure 3). If a limited number of susceptible *S. altissima* genotypes were introduced, it

could lead to population outbreaks of the *E. solidaginis* in the new environment (Figure 3A). Alternately, if only highly resistant genotypes were introduced, followed by the introduction of *E. solidaginis*, then the flies might fail to survive or their densities would be held to very low levels (Figure 3B). Low herbivore population densities could also result from introducing *E. solidaginis* populations that were a mismatch with the *S. altissima* genotypes (Figure 3C). For example, prairie populations of *E. solidaginis* have very low survival rates on forest *S. altissima*. Recent analysis indicates that the *S. altissima* in Japan are closely related to forest plants in North America (Ando

unpublished data). Even if *S. altissima* genotypes were introduced that were highly susceptible to forest altissima flies, if prairie altissima flies were introduced to Japan they would not survive or their survival rate would be very low. *Eurosta solidaginis* has not been introduced to Japan, but we are currently testing the genotypic variation in Japanese plants' susceptibility to *E. solidaginis* attack in common gardens in the state of Minnesota in the Midwestern US.

Two species of herbivores, an aphid *U. nigrotuberculatum* and a lacebug *Corythuca marmorata* that feed on *S. altissima* in North America, were recently introduced into Japan, and we are exploring the impact

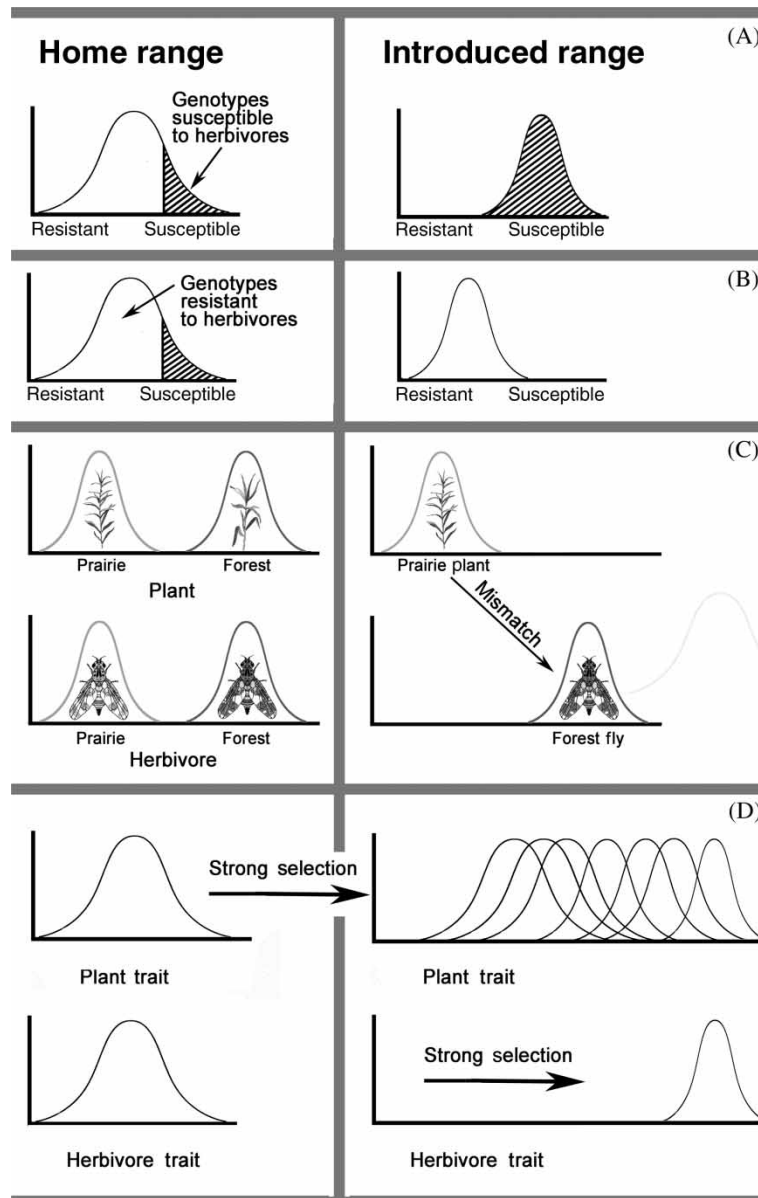


Figure 3. Four possible outcomes of species interactions based on a plant's introduced range depending on host plant genotypic variation. (A) A highly susceptible range of genotypes of the host plant is introduced, and when an herbivore from the original region is introduced it undergoes a population outbreak. (B) A highly resistant range of genotypes from the host plant native range is introduced, and when an herbivore from the original region is introduced it cannot become established, or is held at low population densities. (C) There are locally adapted populations in the original range of the interaction, and mismatch of populations is introduced, preventing the establishment of the herbivore population, or creating one that has very low densities. (D) The host plant is introduced and undergoes rapid evolution to adapt to its new environment. When the herbivore is introduced, it either cannot survive or initially has low densities until it evolves to adapt to the host plant.

of *S. altissima* genotypic variation on the population dynamics of these herbivores. Both of these species have much higher densities in Japan than in the USA. *Solidago altissima* host plant genetic variation could influence this in multiple ways. First, there could be limited genetic variation in the *S. altissima* in Japan, facilitating rapid adaptation in the herbivore. Second, the lack of genetic diversity within patches may increase host plant vulnerability by making it easier to locate the susceptible genotypes. An experimental study showed that the population density of aphids in a patch was influenced by the genotypic composition of *S. altissima* in the patch. Third, the plant may have undergone rapid evolution to adapt to its new environment in the absence of these herbivores (Figure 3D). The adaptation of the host plant to its new abiotic environment, combined with the relaxation of selection for defenses against these herbivores, may have resulted in a highly vulnerable plant population.

We are currently testing the role of genotypic variation in the host plant in reciprocal transplant experiments between Japan and North America. Preliminary evidence indicates that a limited range of genetic variation of *S. altissima* was introduced into Japan, and that this may help explain the high densities of aphids and lacebugs. We are measuring the variation in the susceptibility of *S. altissima* plant genotypes from a range of sites in North America and Japan to a range of herbivores. We are also analyzing genetic variation among these host plant genotypes. These herbivores show various degrees of host-specific or geographic-specific adaptation. Through these studies we will gain insight into the range of genetic variation in the host plants and their herbivores in determining the evolution of plant-herbivore interactions.

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