



Adaptive divergence in host plant use and historical demography in the grasshopper *Hesperotettix viridis*

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Insect herbivores, host use, & divergence

Broadly distributed species offer a spatial and temporal context for unraveling the evolutionary forces that drive lineage diversification and speciation. As a broadly distributed oligophagous species that feeds on different hosts in different areas, the grasshopper *Hesperotettix viridis* provides unique opportunities for investigating the process of divergence in a typically generalist group of herbivores. In our model of host race formation we envision four dominant evolutionary processes that are involved with diversification: local adaptation, genetic drift, gene flow, and reinforcement of sexual isolation. The importance



Figure 1. *Hesperotettix viridis* feeding on *Gutierrezia sarothrae*.

of each evolutionary process is dependent on the time since isolation of each host race and their geographic proximity. Scenarios include: (a) allopatric differentiation in host plant use followed by secondary contact, where prior host use affinity is retained after contact; (b) a two-stage model of differentiation in which allopatric differentiation of host races is incomplete. Following dispersal and secondary contact, selection and limited gene flow reinforce differentiation on new host plants under local sympatric conditions; and, (c) sympatric host race formation on alternate host plants, where new host associations evolve locally in response to divergent selection and are reinforced by premating isolation. Ecological data from populations in the Great Plains indicate that sympatric populations are specializing on different hosts; local adaptation with fitness consequences (Traxler and Joern 1999) and assortative mating (preliminary data) suggest that divergence of host races is occurring. Preliminary genetic data for Great Plains populations indicate that ecological divergence is recent; gene flow is ongoing and genetic drift is minimal and it appears that each host-race population descended from the same late Pleistocene refugium. Nonetheless, parallel patterns in AFLP loci under selection among host races are evident, providing further evidence for the role of local adaptation in a common refugium followed by a Holocene radiation.

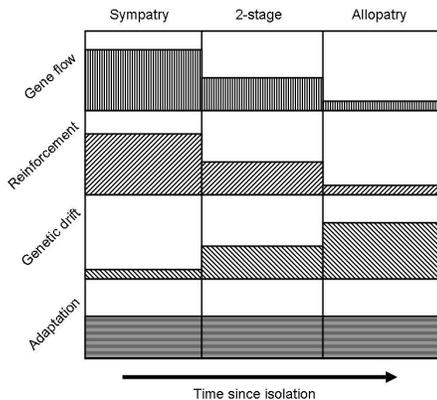


Figure 1. Model of host race formation indicating the relative importance of different evolutionary processes (indicated by height of bars) in three biogeographic scenarios.

Detecting a selection signature related to host plant use

We conducted AFLP analysis of 676 *Hesperotettix viridis* individuals from 23 populations associated with either *Solidago*, *Gutierrezia*, or *Chrysothamnus* host plants. We compared the distribution of F_{ST} estimates of pairs of populations for many individual AFLP loci to a null of neutrality to identify outlier loci, which exhibit greater differentiation than expected and are thus potentially experiencing divergent selection in the two populations compared (Beaumont & Nichols 1996). In 8 pairwise comparisons of *H. viridis* feeding on different host plants, we identified significantly more outlier loci than in 8 pairwise comparisons of populations that fed on the same host plants (paired $t = -2.96$, $df = 7$, $p = 0.021$). This pattern was primarily driven by comparisons involving *Solidago* and *Gutierrezia*; the number of outlier loci in comparisons of *Chrysothamnus*- and *Gutierrezia*- associated populations did not differ substantially from same-host comparisons (Table 1).

In 11 different-host and 8 same-host population comparisons, we identified 57 outlier loci. By examining patterns in the distribution of these loci across different comparisons, we can identify candidates that may be potentially involved in adaptation to alternative host plants. Of the 38 outliers that appeared in appeared in *Gutierrezia/Solidago* comparisons, 24 were involved in multiple independent comparisons, and 11 of these appeared in 6 or more of the 8 *Gutierrezia/Solidago* comparisons performed. We observe a very different pattern in the 24 outliers that appeared in *Chrysothamnus/Gutierrezia* comparisons, however; none of these occurred in more than one such pair. Same-host population comparisons produced 34 outliers; some of these same loci appeared in different-host comparisons, but half of such cases were associated with particular populations and thus may be attributed to local effects (Bonin et al. 2006). The high degree of identity in outlier loci identified in different-host comparisons suggests that similar genetic mechanisms may be driving divergent selection, at least with respect to particular host plants, across a large geographic area.

Table 1. We detected a greater number of AFLP outlier loci (loci with F_{ST} values higher than the 99 quantile of simulated values generated by assuming neutrality) in pairs of populations that used different host plants. Note that geographic distance between populations (in miles) was held constant among pairwise comparisons of same hosts and different hosts. C=*Chrysothamnus*, G=*Gutierrezia*, S=*Solidago*. Numbers of outlier loci and F_{ST} values were determined using Dfdist (<http://www.rubic.rdg.ac.uk/cgi-bin/MarkBeaumont/dlist1.cgi>).

Same-host comparisons					Different-host comparisons				
Population	Hosts	Distance	Outlier loci	F_{ST}	Population	Hosts	Distance	Outlier loci	F_{ST}
Comparisons involving <i>Solidago</i>									
NE-RW/KS-Sher	G/G	100	1	0.006	NE-RW/NE-Thom	G/S	110	18	0.162
KS-Mar/NE-Cust	S/S	201	8	0.010	KS-Mar/NE-RW	S/G	213	16	0.166
NE-Log/NE-Cust	S/S	44	0	0.003	NE-Log/NE-Keith	S/G	67	15	0.168
NE-Keith/NE-RW	G/G	92	2	0.009	NE-Keith/NE-Thom	G/S	90	19	0.138
KS-Sher/OK2	G/G	180	1	0.010	KS-Sher/NE-Cust	G/S	196	15	0.159
Comparisons involving <i>Chrysothamnus</i>									
KS-Ham/CO-Com	G/G	110	7	0.027	KS-Ham/CO-Chey	G/C	88	13	0.046
CO-Gar/CO-Chey	C/C	249	7	0.233	CO-Gar/CO-Com	C/G	233	7	0.205
WA-Gr/OR-Cel	C/C	101	10	0.048	WA-Gr/OR-Mor	C/G	78	4	0.039

Parallel patterns of divergence

As a means of testing for parallel selection on traits related to host use, we identified loci that exhibit parallel trends of divergence (Campbell & Bernatchez 2004); these are loci in which the frequency of AFLP band presence is greatest for the same host plant association across all 6 independent different-host pairwise comparisons. Outlier loci were significantly more likely to exhibit parallel trends of divergence than neutral loci ($\chi^2 = 80.5$, $p < 0.001$; Table 2). Parallel patterns of divergence in loci associated with differentiation in host plant use suggest a role for selection in divergence of different host-associated populations.

	Outlier loci	Neutral loci
Parallel trend	25	82
No parallel trend	8	488

Table 2. Number of neutral and outlier loci exhibiting parallel trends of divergence across 8 independent different-host (*Solidago* vs. *Gutierrezia*) population comparisons.

The biogeographic context of host-race formation

We sequenced 92 individuals from 25 populations at a 372-bp segment of the cytochrome oxidase I mtDNA gene. The dominant haplotype was observed in 56 individuals and included representatives of all three host-plant associations and 5 geographic regions. The lack of structure with respect to host affiliation and the star phylogeny suggest divergence is relatively recent. Molecular clock analysis of this network supports our inference of a single Pleistocene refugium that expanded into the Central Great Plains 71,142 ± 15,918 (SD) years ago.

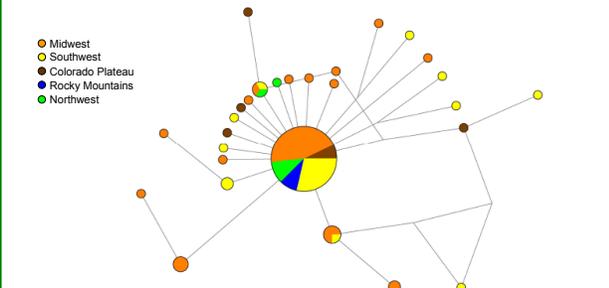


Figure 3. mtDNA haplotype network for a 372-bp region of the cytochrome oxidase I mtDNA gene. Nodes are coded according to geographic region and are sized proportional to frequency.

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