

Host plant-associated genetic differentiation in the snakeweed grasshopper, *Hesperotettix viridis* (Orthoptera: Acrididae)

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Abstract

Studies of herbivorous insects have played a major role in understanding how ecological divergence can facilitate genetic differentiation. In contrast to the majority of herbivorous insects, grasshoppers as a group are largely polyphagous. Due to this relative lack of intimate grasshopper–plant associations, grasshopper–plant systems have not played a large part in the study of host-associated genetic differentiation. The oligophagous grasshopper, *Hesperotettix viridis* (Thomas), is endemic to North America and feeds on composites (Asteraceae) within the tribe Astereae. Previous work has shown both preference and performance differences between *H. viridis* individuals feeding on either *Solidago mollis* or *Gutierrezia sarothrae*. Using 222 AFLP markers, we examined the genetic relationships among 38 *H. viridis* individuals feeding on these plants both in sympatry and allopatry. Neighbour-joining analysis resulted in two distinct host-associated clades with 71% bootstrap support for host-associated monophyly. Analyses of molecular variation (AMOVA) revealed significant genetic structuring with host plant accounting for 20% of the total genetic variance while locality accounted for 0%. Significant genetic differentiation was detected between *S. mollis*-feeders and *G. sarothrae*-feeders even when the two were present at the same locality. These results are consistent with observed differences in preference and performance between *H. viridis* grasshoppers feeding on either *G. sarothrae* or *S. mollis* and indicate that *H. viridis* is comprised of at least two genetically distinct host plant-associated lineages.

Keywords: AFLP, ecological divergence, *Gutierrezia sarothrae*, performance, preference, *Solidago mollis*

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Introduction

The role of natural selection in promoting reproductive isolation is a cornerstone in models of speciation (Mayr 1942, 1963) and has been the subject of renewed interest among evolutionary ecologists during the past decade (e.g. Schluter 1996a, b, 2001; Mopper & Strauss 1998; Orr & Smith 1998; Funk *et al.* 2002; Coyne & Orr 2004). Divergent selection pressures experienced by members of different populations under alternate environmental conditions can facilitate the evolution of reproductive isolation. Gene exchange between populations undergoing divergent selec-

tion can be reduced if the resulting adaptations lead to pre- or postzygotic isolation mechanisms such as assortative mating or inferior hybrid offspring (Bush 1994; Feder 1998; Nagel & Schluter 1998; Nosil *et al.* 2002). This process, referred to as ecological speciation, initially forms genetically structured local demes and may eventually give rise to new species (Feder *et al.* 1998; Mopper & Strauss 1998; Via 1999, 2001; Schluter 2001).

Studies of herbivorous insects have played a central role in our understanding of the evolution of ecological specialization (Jaenike 1990; Bernays & Chapman 1994; Thompson 1994; Mopper & Strauss 1998) and how such specialization may influence speciation (reviewed in Funk *et al.* 2002). A vast majority of herbivorous insects restrict their diets to a closely related group of plant species and

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are sometimes monophagous on single species (Bernays & Chapman 1994; Bernays 1998). The intimate relationships between specialist herbivores and their host plants in feeding, oviposition, mate finding and predator avoidance make their populations particularly susceptible to disruptive selection following shifts to novel host plants (Mopper & Strauss 1998; Berlocher & Feder 2002; Funk *et al.* 2002). Host plant-associated ecological divergence among populations that utilize different plants is often assessed in terms of traits such as preference to feed, mate, or oviposit in association with the different hosts as well as measures of performance in utilization of the different hosts such as growth, survivorship and fecundity (Funk *et al.* 2002).

As a group, grasshoppers (Orthoptera: family Acrididae) are somewhat unique among herbivorous insects in that they are largely polyphagous, feeding selectively on host plant species from a number of unrelated plant families (Otte & Joern 1977; Joern 1979; Chapman 1990; Chapman & Sword 1997). Because of their polyphagy, most grasshopper populations are not expected to experience disruptive selection associated with host shifts. There are, however, some grasshopper species with restricted host ranges and a small minority that are truly host specific (Otte & Joern 1977; Chapman 1990; Sword & Chapman 1994; Chapman & Sword 1997; Sword & Dopman 1999). Given that host plant specialization in grasshoppers appears to be the exception rather than the rule, it is of interest to examine in grasshoppers the generality of the mechanisms that in other herbivorous insects are thought to promote ecological diversification and reproductive isolation.

The snakeweed grasshopper, *Hesperotettix viridis* (Thomas) (Orthoptera: Acrididae: Melanoplinae), is native to North America. It is an oligophagous feeder that consumes only composites (Asteraceae) in the tribe Astereae (Mongkolkei & Hosford 1971; Joern 1979, 1985; Parker & Root 1981; Parker 1982, 1984). Traxler & Joern (1999) compared populations of *H. viridis* from western Nebraska, USA, (Keith and Arthur counties) that fed primarily on one of two composites, either snakeweed, *Gutierrezia sarothrae*, or goldenrod, *Solidago mollis*. Using reciprocal transplant experiments, they found substantial divergence in both performance and preference traits between the different host plant-associated grasshoppers. Individuals collected from *G. sarothrae* developed faster, survived longer and grew larger when fed *G. sarothrae* relative to those fed *S. mollis*. Similarly, individuals collected from *S. mollis* developed faster and gained more weight on *S. mollis* than on *G. sarothrae*. In this case, survivorship did not differ between the two hosts. Paired choice experiments also revealed strong preferences for feeding on the host plant from which they were collected. Importantly, these differences in performance and preference were found between individuals using different hosts at the same site and could not

be explained by either previous experience with the plant from which they were collected or by environmental parameters unrelated to host use (Traxler & Joern 1999).

The observed trade-offs in preference and performance on different hosts in *H. viridis* (Traxler & Joern 1999) indicate the possibility of divergent selection for host plant specificity on the alternative hosts, *S. mollis* or *G. sarothrae* (Futuyma & Moreno 1988; Via 1990; Joshi & Thompson 1995). This negative correlation across host plants in preference and performance suggests that gene flow may be restricted between different host plant-associated forms. Using multilocus AFLP markers, we tested the null hypothesis of no host plant-associated genetic structuring of *H. viridis* grasshoppers using specimens retained from the original Traxler & Joern (1999) study along with individuals from an additional allopatric, *G. sarothrae*-feeding population from New Mexico, USA. We observed significant host plant-associated genetic structuring of *H. viridis* populations that is maintained even when the different host-associated forms co-occur at the same site.

Methods

Insect sampling

Thirty second instar *Hesperotettix viridis* were collected off multiple plants, one individual at a time, from populations of each plant species dispersed within a radius of about 1000 m at two sites separated by 30 km in Nebraska, USA, (Keith and Arthur counties) as reported in Traxler & Joern (1999). The primary Keith County site has recently been destroyed by movement of earth because of new construction. For consistency across studies, we retain the terminology employed in Traxler & Joern (1999) to describe the location and host plant affiliation of the insects examined in this study. At the Keith County site, both *Solidago mollis* and *Gutierrezia sarothrae* were utilized by *H. viridis*. The 10 grasshoppers collected at this site from *S. mollis* are referred to as K-S (#1–10) and the 10 grasshoppers collected from *G. sarothrae* are referred to as K-G (#1–10). At the Arthur County site, only *S. mollis* was present and the insects from this site are referred to as A-S (#1–10). An additional 10 *H. viridis* specimens associated with *G. sarothrae* were collected approximately 900 km from the Nebraska sites in Sevilleta, New Mexico, USA, and are referred to as S-G (#1–10). The same basic sampling scheme described above was used in New Mexico, where grasshoppers were collected individually from multiple plants. Insects were individually stored in ethanol.

AFLP protocol

Hind femur muscle tissue from each of the 30 Nebraska specimens was ground with sterile white quartz sand in a

Primer pair	Preselective		Selective		No. of markers	PIC \pm SD
	<i>EcoRI</i> + 2	<i>MseI</i> + 2	<i>EcoRI</i> + 4	<i>MseI</i> + 4		
1	CT	CA	CTCC	CAAT	74	0.38 \pm 0.10
2	CT	CG	CTCC	CGAG	44	0.35 \pm 0.12
3	CT	CC	CTCC	CCTG	55	0.36 \pm 0.13
4	CT	CA	CTCC	CAAG	49	0.37 \pm 0.10

Table 1 The number of polymorphic AFLP makers and average polymorphic information content (PIC) obtained using four different preselective and selective amplification primer combinations

1.5 mL microfuge tube using disposable plastic pestles. For the New Mexico specimens, a combination of hind femur and thorax muscle was ground under liquid nitrogen in a 1.5 mL microfuge tube using disposable plastic pestles. DNA extractions were conducted in accordance with the manufacturer's protocol for animal tissue using the DNeasy Tissue Kit (QIAGEN). DNA concentration was estimated using a fluorometer-based PicoGreen assay (Molecular Probes). Extractions were stored at -20°C .

Amplified fragment length polymorphism (AFLP) (Vos *et al.* 1995) can be used to assess phylogenetic relationships and genetic structuring of populations in a wide variety of organisms (Mueller & Wolfenbarger 1999). While the use of AFLP is widespread, occasional modifications to the original Vos *et al.* (1995) protocol are required in some taxa, particularly those with large genomes, to reduce the number of amplified products so that homologous markers can be scored reliably (Han *et al.* 1999; Tatsuta & Butlin 2001). Acridid grasshoppers have unusually large genomes relative to other insects (Bensasson *et al.* 2001a). This, along with problems associated with the prevalence of nuclear-mitochondrial pseudogenes in grasshopper genomes (Zhang & Hewitt 1996; Bensasson *et al.* 2000, 2001b) can complicate molecular phylogenetic analyses that are otherwise relatively straightforward in other insect groups. Tatsuta & Butlin (2001) found that long primer sets (*EcoRI* + 4 and *MseI* + 4) could be used during the selective amplifications stage of AFLP to reduce the number of markers in acridid grasshoppers to a manageable size. Our initial application of their modified protocol using *EcoRI* + 4 and *MseI* + 4 primers in *H. viridis* grasshoppers still resulted in a large number of fragments, many of which were indistinguishable from similarly sized fragments. To further reduce the number of fragments, we adopted the AFLP protocol developed by Han *et al.* (1999) for organisms with large genomes in which an additional nucleotide is added during the preselective amplification stage (*EcoRI* + 2 and *MseI* + 2) in addition to the use of *EcoRI* + 4 and *MseI* + 4 primer pairs during selective amplification. Our preliminary screens using this approach on a subset of individuals yielded a reduced number of fragments for each primer pair that repeatedly appeared in independent amplifications and could be reliably scored. Our preselective

and selective amplification primer sets are listed in Table 1.

EcoRI and *MseI* restriction digestion of approximately 500 ng of DNA and the subsequent ligation of adapters was performed in accordance with the protocol provided with the Invitrogen Core Reagent Kit (Invitrogen), with the only modification being an extension of the digestion time. The samples were allowed to digest overnight at 37°C , and the adapters were ligated the following morning. The ligated DNA was diluted 1:10 with $1 \times$ TE buffer (10 mM Tris-HCl, 0.1 mM EDTA) prior to preselective amplification. Preselective amplifications were conducted with three different primer pairs each containing two selective nucleotides (Table 1). Polymerase chain reaction (PCR) was carried out using 30 ng of each primer in a total final volume of 20 μL with thermal cycling parameters of 20 cycles of 30 s at 94°C , 1 min at 56°C , and 1 min at 72°C , followed by a 4°C hold. The resultant products were diluted 1:10 with $1 \times$ TE buffer. Four selective nucleotides were used in the selective amplification in conjunction with the appropriate preamplification products (Table 1). The *EcoRI* + 4 primer was labelled with a phosphoramidite dye (D4-PA, WellRED dye, Prologo LLC). PCRs were carried out using the labelled *EcoRI* + 4 primer at $0.05 \mu\text{M}$ and *MseI* + 4 primer at $0.1 \mu\text{M}$ for 12 cycles of 10 s at 95°C , 40 s at 65°C less 0.7°C per cycle, and 1.5 min at 72°C ; followed by 35 cycles of 11 s at 95°C , 40 s at 56°C , and 1.5 min at 72°C , and a final hold at 4°C .

Samples were electrophoresed on a Beckman Coulter CEQ2000XL DNA analysis system using the fragment analysis application. One microlitre of each selective PCR amplification reaction was mixed with 29 μL of sample loading solution and 0.5 μL of an internal lane standard (600 bp size standard, Beckman Coulter), permitting the detection of fragments in the range of 60–640 bp. Fragment data were exported for analysis using GENOGRAPHER (version 1.6, Montana State University 1998, <http://hordeum.msu.montana.edu/genographer/>). Fragments from 60 to 500 bp were scored as presence/absence with peaks of low intensity included in the data set when unambiguous scoring was possible. Two individuals, A-S#9 and K-S#8, failed to yield quality fragment profiles and were omitted from the analysis.

Data analysis

The polymorphic information content (PIC) of markers generated from each primer combination was calculated as $PIC_i = 2f_i(1 - f_i)$; where PIC_i is the polymorphic information content of marker i , f_i is the frequency of the amplified band, and $1 - f_i$ is the frequency of the null allele (band absent).

We used a combination of distance-based and molecular variance methods to examine phylogenetic structure and genetic relationships among different host plant-associated *H. viridis* grasshoppers found in both sympatry and allopatry. Using the presence/absence character matrix, we conducted a neighbour-joining (NJ) analysis in PAUP* 4.0b10 (Swofford 2002) using the mean character difference as a distance measure. Bootstrap support was assessed with 1000 replicates. AMOVA was conducted in ARLEQUIN version 2.000 (Schneider *et al.* 2000) with significance tests estimated using 1023 permutations. We performed three separate analyses to test hypotheses of genetic structure attributable to variation: (i) among individuals across the different host plant-associated groups (i.e. variation among all host plant \times locality combinations), (ii) among individuals nested within host plant nested within locality, and (iii) among individuals nested within locality nested within host plant. A fully balanced experimental design was not possible because grasshoppers feeding on both plants were not present at each locality. Pairwise short-term genetic divergence estimates (F_{ST} values) were calculated and tested for significance in ARLEQUIN to assess the relative degree of divergence between individuals in all host plant \times locality combinations.

Results

The four primer sets employed yielded a total of 222 unambiguously scorable polymorphic fragments (Table 1). Average PIC values for markers obtained with each AFLP primer set were relatively high (Table 1) and exclusion of rare bands occurring in five or fewer individuals had no significant effect on our analyses.

NJ analysis revealed substantial genetic structure associated with host plant use. Individuals associated with *Solidago mollis* and *Gutierrezia sarothrae* formed distinct host plant-associated clades with 71% bootstrap support for host form monophyly (Fig. 1). Insects feeding on either *S. mollis* or *G. sarothrae* at the Keith County site were more closely related to individuals feeding on the same plant in allopatric populations than they were to each other. There was no evidence of significant structure within the different host-associated clades either among the *S. mollis*-feeders from two sites separated by 30 km or among the *G. sarothrae*-feeders from sites separated by over 900 km.

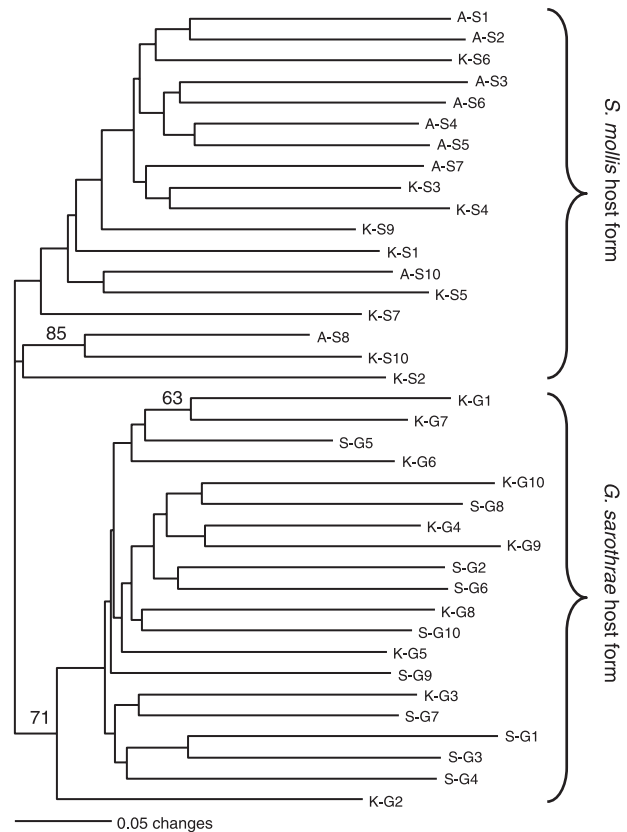


Fig. 1 Neighbour-joining analysis of *Hesperotettix viridis* grasshoppers associated with either *Gutierrezia sarothrae* or *Solidago mollis*. Phylogram depicts the relationships among individuals feeding on *S. mollis* from Arthur County, NE (A-S) and Keith County, NE (K-S) along with individuals feeding on *G. sarothrae* from Keith County (K-G) and Sevilleta, NM (S-G). Numbers indicate clades with > 50% bootstrap support. Scale bar represents 5% character divergence.

Analyses of molecular variance (AMOVA) confirmed the genetic structuring detected in the NJ analysis. The overall nonhierarchical AMOVA revealed significant variation among the different host plant \times locality combinations of insects with 15% of the total variation attributed to variation among groups (Table 2a). A hierarchical AMOVA supported the hypothesis of no effect of locality on genetic structure, but rather indicated significant substructuring within localities (Table 2b). Substructuring within locality was clearly attributable to genetic differentiation between the insects utilizing *G. sarothrae* and *S. mollis* together at the Keith County site as evidenced by the hierarchical AMOVA designed to test for the effect of host plant affiliation. There was significant genetic structuring along host plant lines with 20% of the total genetic variance explained by host plant use (Table 2c). Similar to the NJ analysis results, there was no evidence of significant substructuring between insects feeding on the same plant at different sites (Table 2c).

Table 2 Analysis of molecular variance (AMOVA) results for *Hesperotettix viridis* grasshoppers feeding on either *Solidago mollis* or *Gutierrezia sarothrae*. Variation was partitioned (a) among individuals within host plant \times locality groups, (b) among individuals nested within host plant nested within locality, and (c) among individuals nested within locality nested within host plant. Negative values can arise due to a negative intraclass correlation resulting from alleles being more related between than within populations (Weir 1996)

Source	d.f.	SS	Variance	Variation (%)	P
(a) Among plant \times locality groups	3	299.20	6.63	15.26	< 0.0001
Individuals within groups	34	1251.36	36.81	84.74	
(b) Among localities	2	193.81	-0.73	-1.69	0.78
Among host plant within locality	1	105.39	7.24	16.72	< 0.0001
Individuals within groups	34	1251.36	36.81	84.97	< 0.0001
(c) Among host plants	1	219.36	9.49	20.36	< 0.0001
Among locality within plant	2	79.60	0.32	0.68	0.15
Individuals within groups	34	1251.36	36.81	78.96	< 0.0001

Table 3 Pairwise comparisons of short-term genetic divergence estimates (F_{ST}) between all locality and host plant combinations

	1	2	3	4
1. Arthur County, NE (<i>S. mollis</i>)	0			
2. Keith County, NE (<i>S. mollis</i>)	-0.01	0		
3. Keith County, NE (<i>G. sarothrae</i>)	0.23***	0.16***	0	
4. Sevilleta, NM (<i>G. sarothrae</i>)	0.26***	0.19***	0.03†	0

*** $P < 0.0001$; †NS after controlling for multiple comparisons.

Pairwise comparisons of F_{ST} values between all possible host plant \times locality combinations further supported the pattern of host plant-associated genetic structure reported above, but also suggested additional genetic divergence. F_{ST} values were greatest in comparisons between insects associated with different host plants (Table 3). In contrast to the NJ and AMOVA results, the pairwise comparison between the *G. sarothrae* feeders from Nebraska and New Mexico revealed a slight degree of genetic differentiation (Table 3). This difference, however, fails to be significant after controlling for multiple comparisons ($\alpha = 0.05/6 = 0.008$).

Discussion

The snakeweed grasshopper, *Hesperotettix viridis*, is more than just a snakeweed grasshopper. Although *H. viridis* has been known to utilize at least 34 host plant species across its range in North America (Pfadt 2002), local host adaptation and divergence was only suspected following the detailed preference and performance assays conducted by Traxler & Joern (1999). This study suggests that ecological divergence by *H. viridis* grasshoppers in their utilization of *Solidago mollis* and *Gutierrezia sarothrae* as host plants has been accompanied by substantial genetic differentiation. Similar patterns in which insects utilize a

number of host plants across their range, but are found after detailed study to consist of locally adapted host-specific populations have been found in a number of phytophagous insects (Fox & Morrow 1981; Futuyma & Peterson 1985; Thompson 1994; Mopper & Strauss 1998). Such demonstrations, however, have been quite rare in grasshoppers (Sword & Dopman 1999).

Genetically distinct lineages of *H. viridis* grasshoppers have evolved in association with at least two of their host plants, *S. mollis* and *G. sarothrae*. Importantly, genetic differentiation between these two host-associated forms appears to be maintained even when the two co-occur at the same site. Although the different genetically distinct forms and their respective host plants can be found in syntopy, our data suggest that these lineages arose via a single ancestral host shift as opposed to local adaptation and subsequent genetic differentiation. This is indicated by the fact that *G. sarothrae*-feeding insects from Keith County, Nebraska, were more closely related to other grasshoppers feeding on the same plant over 900 km away at Sevilleta, New Mexico, than they were to conspecifics at the same site feeding on *S. mollis* (Fig. 1, Table 3). Additional sampling across the range of *H. viridis* will be required to determine if additional lineages occur in association with other plants in its host range. An intriguing possibility could be the presence of multiple genetically distinct lineages, each of which is oligophagous and associated with a particular suite of resources as opposed to monophagous on individual plant species.

Due in part to the limited sample sizes used in this study, the extent of reproductive isolation between the different host-associated *H. viridis* lineages cannot yet be determined. For example, the possibility of undetected shared lineages or the presence of hybrid individuals cannot be ruled out. Our data do, however, suggest a relatively recent origin of the different lineages. We found no fixed diagnostic differences in AFLP markers between the different host-associated forms. In addition, a preliminary analysis

of 850 bp of 12S-valine-16S mtDNA revealed no significant genetic structuring of insects in accordance with either locality or host plant affiliation (G.A. Sword *et al.*, unpublished). Similar discordance between mtDNA and AFLP loci has been found in other systems thought to have undergone recent divergence (Parsons & Shaw 2001; Shaw 2002).

The timing and polarity of host shifting within *H. viridis* is of interest due to the relatively recent influence of human activity on *G. sarothrae* abundance patterns in western North America. *Gutierrezia sarothrae* is an endemic perennial that is noxious to livestock and rarely fed upon by vertebrate herbivores (Smith *et al.* 1991; McDaniel & Ross 2002). Its populations are cyclic and influenced by climatic factors (Ralphs & Sanders 2002), but it is also suspected to have increased in local abundance across its range due to overgrazing by livestock (McDaniel & Torell 1987). Plant communities that come to be dominated by *G. sarothrae* are much lower in forage quality available to vertebrate herbivores due to the competitive exclusion of grasses relative to communities in which it is a minor component (Ueckert 1979; McDaniel & Torell 1987; McDaniel & Ross 2002). As such, *H. viridis* has been identified as a potential biocontrol agent that may augment the effectiveness of existing weed management practices (Gardner & Thompson 2001).

The recent history of *G. sarothrae* gives rise to two possible scenarios for the historical phylogeography of host plant use in *H. viridis*. One possibility is that an *H. viridis* lineage adapted to utilize *G. sarothrae* existed in North America prior to human-mediated changes in the abundance of its host plant. An alternative possibility is that a host shift to *G. sarothrae* occurred as a result of its increased abundance and availability as a potential resource following human-mediated plant community changes (Futuyma & Moreno 1988; Jaenike 1990; Bernays & Chapman 1994). If the latter scenario is the case, our data suggest that one or a limited number of local host shifts followed by a rapid range expansion of *G. sarothrae*-feeding individuals was more likely than multiple independent local host shifts (Nason *et al.* 2002). Though a rapid range expansion following human-mediated changes is possible, it does seem unlikely given the extent of genetic divergence observed between the different host-associated *H. viridis* grasshoppers and the brief amount of evolutionary time since human intervention. The recent finding of Feder *et al.* (2003) in which genetic divergence between the apple and hawthorne host races of *Rhagoletis pomonella* was found to predate the introduction of the apple in North America supports this notion. A broader sampling effort in the future will enable explicit tests of these phylogeographical hypotheses. In any event, our results suggest that the genotype and host affiliation of local *H. viridis* individuals will be an important concern if they are to be considered as an augmentative component of a *G. sarothrae* management

scenario (Thompson *et al.* 1996; Gardner & Thompson 2001).

In identifying the role of ecology in speciation, it is important to differentiate between the evolution of reproductive isolation as a by-product of divergent selection on other traits or as a result of selection against hybrids (Schluter 2001). The precise roles of pre- and postreproductive isolation mechanisms in host plant-associated genetic divergence have not yet been examined in *H. viridis*. However, this grasshopper shares with other phytophagous insects a number of characteristics that can act as mechanisms to restrict gene flow between different ecologically specialized forms (reviewed in Funk *et al.* 2002) and that warrant further study. In terms of prereproductive mechanisms, habitat selection almost certainly plays a role in reproductive isolation by promoting assortative mating in *H. viridis* through host plant affinity. Adults have wings, but are weak fliers whose dispersal between host plants becomes reduced at distances from several to tens of metres between plants (Parker 1983, 1984). Movement among nearby plants, however, does appear to be quite frequent (Parker 1984). Although different potential host plant species may coexist at this scale (Traxler & Joern 1999), the different forms studied here tend to feed on their respective host species relative to available alternatives (Traxler & Joern 1999). Given that *H. viridis* individuals spend most of their lives roosting, feeding and mating on the host plant (Parker 1983; Pfadt 2002), encounters among members of the same host form should be much more likely than mating between host forms. In the event that matings between the different host-associated forms do occur, the observed negative trade-offs in preference and performance across hosts (Traxler & Joern 1999) could feasibly result in reduced viability of hybrids and further restrict gene flow as a postreproductive isolating mechanism.

The advantages of polyphagous feeding to grasshoppers appear to be the benefits accrued from diet mixing, though the mechanisms underlying host plant selection in polyphagous feeders may be quite diverse (Simpson & Simpson 1990; Bernays & Bright 1993; Bernays & Minkenberg 1997; Simpson & Raubenheimer 2000). Mechanisms that have favoured the evolution of restricted host range in *H. viridis* grasshoppers have yet to be identified (see Jaenike 1990; Singer 1994; Futuyma & Moreno 1988). To date, there has been only one other demonstration of host plant-associated ecological divergence and genetic differentiation in grasshoppers. *Schistocerca lineata* (Cyrtacanthacridinae) grasshoppers (taxonomy according to Song 2004) were found to exist as distinct mtDNA lineages in populations associated with either *Ptelea trifoliata* (Rutaceae) or *Rubus trivialis* (Rosaceae) in central Texas, USA (Sword & Dopman 1999; Dopman *et al.* 2002). In this case, performance differences were detected across hosts and host plant-mediated deterrence to predators was identified a primary factor promoting

host range restriction as well as ecological and genetic divergence (Sword 1999, 2001, 2002; Sword & Dopman 1999; Dopman *et al.* 2002).

Despite the prevalence of polyphagy among grasshoppers (Otte & Joern 1977; Joern 1979; Chapman 1990; Chapman & Sword 1997), studies of host plant use ecology in *H. viridis* and *S. lineata* clearly demonstrate that host plant-associated ecological and genetic divergence can and does occur in this group. Furthermore, when it does occur in grasshoppers, these studies indicate that the evolutionary consequences of host plant-associated divergence are consistent across phytophagous insect groups. A common conclusion from studies of both *S. lineata* and *H. viridis* host-use ecology is that detailed examinations of local host plant use patterns, which are surprisingly rare in grasshoppers, are critical to elucidate instances of local adaptation and resource associated divergence (Sword & Dopman 1999; Traxler & Joern 1999). Additional examples are likely to be found in grasshoppers as properly designed investigations of host use patterns are conducted in the future. Indeed, if an estimated 64% of all grasshopper species are polyphagous (Chapman & Sword 1997), then the remaining 36% exhibit some degree of resource specificity and are candidates for ecological divergence and subsequent diversification. Because it is thought that estimates of grasshopper polyphagy are likely to be inflated by a temperate study bias (Rowell 1978; Marquis & Braker 1994), the number of grasshopper species in which host-associated ecological and genetic divergence may have occurred is possibly even greater.

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This project represents the mutual interests of Greg Sword and Tony Joern in understanding the ecology and evolution of grasshopper host plant use. Greg Sword is a Research Ecologist with the USDA-ARS conducting research on grasshopper–plant interactions, locust and Mormon cricket ecology, and the evolution of warning colouration. Tony Joern is a Professor of Biology at Kansas State University investigating grasshopper–plant interactions, population dynamics and community structure. Laura Senior is a Biological Science Technician with the USDA-ARS working with Greg Sword.
