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Association of genetic lineages with ecological features in a polyphagous montane grasshopper species

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Abstract

Understanding the relationships between intraspecific diversity and habitat has been greatly enhanced with the advent of molecular markers. Such understanding may help to elucidate complex microevolutionary processes. *Melanoplus alpinus*, a montane/alpine grasshopper species found in the central and northern Rocky Mountains, has a disjunct distribution with highly divergent mtDNA lineages, structured among meadows and drainages within mountain ranges. Previous analyses showed that genetic differentiation is not distance driven, so habitat factors may account for genetic structuring and diversity within the species. In this study, 3 mtDNA lineages were analyzed for their association with ecological variables, and PCR-RFLP haplotype genetic diversities were analyzed for relationships with elevation, elevation-latitude index, and meadow size. Chi-square analysis revealed mtDNA lineage predominance in particular soil textures, indicating the potential of soil to function as a limiting environmental factor in the distribution of lineages within this species. Genotype-habitat associations did not extend beyond soil, however. No significant relationships existed between genetic diversity within meadows and elevation, elevation-latitude index, or meadow size.

Key words

Orthoptera, Acrididae, genetic structuring, habitat type, soil

Introduction

The advent of molecular markers, both protein and DNA, has given ecologists quantitative tools for understanding the relationships between intraspecific diversity and habitat parameters, further helping to elucidate complex microevolutionary processes. Molecular markers are preferable to morphological characters for such correlations because significant genomic diversity may exist without obvious morphological evidence (Hewitt 1988). Polymorphism within or among populations is enhanced by genotype-specific habitat preferences, as seen in the selection of oviposition sites by the cactophilic fly, *Drosophila buzzatii* Patterson & Wheeler (Barker 1992, 1994). In species exhibiting host specificity with regard to food source and oviposition sites (for review see Jaenike & Holt 1991), genotype-specific habitat choice leads to assortative mating, potentially culminating in reproductive isolation between genotypes. In such cases, gene flow does not equate with dispersal capabilities, making for a more complex system than a one-variable solution such as migration potential would suggest (Slatkin 1987, Via 1999).

Regarding grasshopper species, across an altitudinal gradient in South America, allelic frequencies of *Trimerotropis pallidipennis* (Burmeister) were significantly correlated with both altitude and humidity (Matrajt *et al.* 1996). Sword and Dopman (1999) found genetic variability in host plant use among populations of a polyphagous grasshopper, *Schistocerca emarginata* (Scudder), in the nymphal stage. Though not at the level of genotype, grasshopper species presence and relative abundance were influenced by vegetative classifications based on presence and percent cover of plant species (Kemp *et al.* 1990). Egg pod densities for 8 grasshopper species in Pakistan were preferentially distributed, based on plant density and species composition, as well as soil texture (Mahmood & Qazi 1989). Soil texture was also found to be the primary habitat factor limiting grasshopper species distribution in Texas (Isely 1937). Schell and Lockwood (1997) found soils to be the primary factor accounting for spatial structuring of grasshopper outbreaks in Wyoming.

Habitat discrimination by species and genotypes can lead to greater biological diversity, but environmental pressures also greatly influence genetic diversity, a precursor to speciation. Polymorphism within populations has been shown to differ along environmental clines. A negative correlation between genetic diversity and latitude was found in lodgepole pine, *Pinus contorta,* in northwestern North America (Cwynar & MacDonald 1987). Decreased genetic diversity exists in populations of the European grasshopper, *Chorthippus parallelus* (Zetterstedt), at higher latitudes (Cooper *et al.* 1995, Hewitt 1993). And in a tropical tree species, *Alnus acuminata*, the frequency of an allele as well as overall genetic diversity, were negatively correlated with altitude (Murillo & Rocha 1999). Habitat size is also important to maintaining genetic polymorphism in species with disjunct distributions. Frankham (1996) found 16 of 19 studies involving diverse animal taxa to have a significant positive correlation between genetic variation and logarithm of habitat area.

The present study was conducted using disjunct populations of the alpine grasshopper, *Melanoplus alpinus* (Scudder), a common species found at relatively low densities in montane and alpine meadows in the central and northern Rocky Mountains. Though it is a polyphagous species possessing fully developed wings (Bergstrom 1955, Pfadt 1996), its range of distribution with regard to soil texture and dominant plant coverage were unknown. A previous study found that despite its apparent ability for dispersal, *M. alpinus* has highly divergent mtDNA lineages structured among meadows and drainages within mountain ranges (VanDyke 2002, 2004). The geographic scale for *M. alpinus* genetic diversity is at the level of the meadow, based on this earlier examination of hierarchical analysis of molecular variance. Furthermore, genetic differentiation was not found to be distance driven, suggesting that habitat may influence the genetic structuring and diversity of the species.

Based on these findings with *M. alpinus* and previous studies

of genetic structuring by ecological factors, we hypothesized that genetic lineages are associated with particular habitat features and lineages are preferentially distributed with regard to these factors (percentages of sand, silt, clay, and coarse material; percentages of bare ground, grass cover, and nongrass cover; elevation-latitude index; and meadow size). Furthermore, we hypothesized that genetic diversity has a negative relationship with elevation and elevation-latitude index and a positive relationship with meadow size.

Materials and Methods

Genetic Analysis.—In July and August of 1999, *M. alpinus* was collected in 3 mountain ranges in Wyoming, USA: Medicine Bow, Big Horn, and Wyoming Range. Three drainages and 1 to 5 meadows per drainage were sampled in each range, resulting in a total of 40 meadows. In August 2000, 3 meadows from 1 drainage were sampled in the Flathead Mountains, Montana, USA (VanDyke 2002, 2004). Five males were collected from each meadow, for a total of 215 individuals. Polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) analyses were done on 2 mitochondrial DNA (mtDNA) regions: a 555bp region of cytochrome oxidase I (COI), and a 310bp region of cytochrome oxidase II (COII). Sequences of a 496bp region of COI were determined for 23 individuals representing each PCR-RFLP haplotype and mountain range. MtDNA lineages were ascertained through phylogenetic analysis of sequence data using UPGMA and maximum parsimony algorithms (VanDyke 2002, 2004). Nei's (1987) genetic diversity was computed from the RFLP haplotypes for each of the 43 meadows using the software package Arlequin (Schneider *et al.* 2000).

Habitat Parameters. — In order to determine if the mtDNA lineages segregated among habitat types, 9 ecological variables were collected in July-August of 2000 for each of the 43 meadows surveyed: 1) elevation-latitude index; 2) meadow size (ha); 3 to 5) percentages of sand, silt, and clay in soil; 6) percent of coarse material (>2 mm in diameter) in soil sample; 7-9) percentages of bare ground, grass cover, and nongrass cover. The least dynamic variable (elevation-latitude index) was obtained by 2 steps. Global positioning system (GPS) readings were taken at each meadow, giving the latitude and longitude of each site (VanDyke 2002). Elevations for the coordinates were obtained from DeLorme Topo USA version 2.0 (DeLorme 1999); elevation (in meters) was multiplied by latitude (in degrees) to give the elevation-latitude index variable.

GPS readings were taken for each cardinal point of the meadow, providing 4 points of reference with which to estimate meadow size (rectangular estimation) using distances obtained by Topo USA.

Soil samples were taken at a random location in each of the 43 meadows by removing the top 8 cm of a 10 cm^2 area. The samples were later analyzed for percentages of sand, silt and clay at the University of Wyoming, based on the methods of Gee and Bauder (1986). Soil texture classifications based on USDA (1950) standards were obtained from the percentage composition. Nonsoil coarse material was weighed for its percentage within the soil sample.

The most dynamic habitat variables measured were percentages of bare ground, grass and nongrass cover. A qualitative classification of meadow vegetation was based on species dominance types (Whittaker 1962, Grossman *et al.* 1998). Percentages of bare ground and canopy coverage of the 5 most prominent plant species were visually estimated while walking a transect using a meadow habitat assessment protocol of the Wyoming Natural Diversity Database (Jones, pers. com.). Six canopy coverage classifications existed: 1 = 0 to 10% coverage, 2 = 10 to 25%, 3 = 25 to 40%, 4 = 40 to 60%, 5 = 60 to 80%, and 6 = 80 to 100%. The percentage of bare ground or plant species was based on the median of the appropriate coverage classification. The plant specimens were pressed and taken to the Rocky Mountain Herbarium (Laramie, WY) for identification to species. Two general classifications, grass and nongrass cover, were used for analyzing habitat parameters. Grass cover included the families Poaceae, Cyperacea, and Juncaceae. Nongrass cover included all shrub and forb families.

Data Analyses.—Both univariate analysis of variance (ANOVA) and multivariate principal component analysis (PCA) were used to discern differential habitat use by the mtDNA lineages. Due to problems with multicollinearity, unequal lineage sizes, and ill-conditioned data, PCA (rather than discriminant analysis) was conducted to determine spatial separation between the mtDNA lineages based on the 9 habitat variables. Principal components (PC) were chosen by the latent root criterion (Hair *et al.* 1998). Chi-square analysis (SPSS, 2002) was used to test for mtDNA lineage associations with soil texture. Cluster analysis was performed using Ward linkage and Minkowski distance to determine the amount of plant species similarity among meadows (SPSS, 2002). Three simple linear regressions were used to determine relationships between genetic diversity and meadow size, elevation, and the elevation-latitude index.

Results

Genetic Analysis.— As previously reported by VanDyke (2002, 2004), PCR-RFLP analysis of portions of the COI and COII mitochondrial genes revealed 9 haplotypes among the 215 individuals. Genetic diversity of *M. alpinus* ranged from 0.00 to 0.70 per meadow on a scale of 0.00 to 1.00. Phylogenetic analysis of mtDNA sequence data for 23 individuals representing the various RFLP haplotypes and ranges showed 3 major lineages within *M. alpinus*. The average sequence divergence between the 3 lineages ranged from 3.14% to 3.63%. Based on a molecular clock of 2.3% sequence divergence per million years, these lineages appear to have diverged early in the Pleistocene, approximately 1.4 to 1.6 MYA. Their distribution, however, is geographically paraphyletic.

Ecological Associations. — Seven soil textures were found among the 43 meadows surveyed: sandy loam, loam, silt loam, clay loam, sandy clay loam, clay, and silty clay loam (Table 1). Although this range of soil textures indicates broad habitat diversity on the part of *M. alpinus*, associations of mtDNA lineages with these soil classifications were not random. Chi-square tests on each of the 3 lineages revealed significant deviations from expected values. Lineage 1 ($p < 0.0001$) was primarily found in loam (55.7%) and sandy loam (23.0%) soils. Lineage 2 ($p < 0.0001$) was found only in loam soils. Lineage 3 ($p = 0.009$) was primarily found in clay loam (47.6%) and sandy loam (23.8%) soils.

Collectively, 60 species in 37 plant genera from 12 families were represented in the 43 meadows surveyed. Cluster analysis indicated high vegetation diversity within and among mountain ranges, resulting in 16 groups ranging from a minimum of 1 meadow per group to a maximum of 5 meadows. This degree of diversity was too great to test for plant habitat associations with the 3 lineages using chi-square analysis, since only 215 *M. alpinus* individuals were sampled.

No significant relationships were found between genetic diversity

Table 1. Percentages of individuals (n = 215) from the 3 mtDNA lineages found in the 7 soil types.

Soil Type	Lineage(%)		
	1	2	3
Sandy Loam	23	0	23.8
Loam	55.7	100	14.3
Silt Loam	9.8	0	14.3
Clay Loam	2.9	0	47.6
Sandy Clay Loam	2.9	0	0
Clay	2.9	0	0
Silty Clay Loam	2.9	0	0

and elevation [$F(1,41) = 4.06$, $p = 0.051$] nor the elevation-latitude index [$F(1,41) = 3.99$, $p = 0.052$] (Fig. 3). No relationship was found between meadow size and the genetic diversity therein [$F(1,41) = 1.31$, $p = 0.259$].

Habitat Discrimination.—With 3 highly divergent mtDNA lineages of *M. alpinus* occurring in a geographically paraphyletic distribution, the question of unique lineage discrimination of habitat was tested. Univariate ANOVAs for each habitat variable showed no significant differences among lineages. PCA on the 9 ecological variables resulted in 3 PC's with Eigenvalues greater than 1, accounting for 71.5 % of the total variance (Table 2). PC1 represented soil texture and coarse material, with percentages of sand and coarse material having a positive relationship with the principal component and percentages of silt and clay having a negative relationship. PC2 had a negative relationship with grass cover and elevation by latitude but a positive relationship with nongrass cover and meadow size. PC3 had a positive relationship with elevation-by-latitude and percent of bare ground (Table 3). Viewed in three-dimensional space, the mtDNA lineages failed to separate based on the 9 variables surveyed (Fig. 1).

Discussion

The 215 individuals from 43 meadows analyzed for this study represent a relatively large sample size for a molecular study. Genotypes are seldom represented in equal numbers in natural populations. Though lineage sizes differed in this study, an unfortunate reality that constrains our interpretations, some informative associations were found with regard to mtDNA lineages of *M. alpinus* and soil texture.

The 43 meadows surveyed between 1999 and 2000 represent suitable, but diverse, habitats for *M. alpinus*. Whether *M. alpinus'* phylogenetic structuring represents 3 cryptic species or 3 highly divergent lineages of a single species, these taxonomic units appear to predominate in particular soil textures. Lineage 3 was the only one to predominate in clay loam soils. Lineages 1 and 3 were both found with equal frequency (23%) in sandy loam soils. Lineage 2 was isolated to loam soils, but this distribution may be a limitation of sampling, since lineage 2 was found in only one drainage of the Wyoming Range. It was, however, fixed in 4 of the 5 meadows sampled in that drainage. Additional sampling in ranges west of the Wyoming Range may produce more individuals of lineage-2 descent, and a larger sample size to compare with soil texture. Lineage 1 was also found with a high frequency (56%) in loam soils.

Of the 7 soil textures represented, only 3 were highly associated with *M. alpinus* when considered collectively. Thus, soil texture could be a limiting factor for *M. alpinus'* distribution in the Rocky Mountain region of the United States. Differential associations between mtDNA lineages and soil texture suggest that genetic divergence may be reinforced by environmental association, possibly representing female philopatry. Soil might be a more important ecological indicator than biota for acridids, because soil is a function of numerous abiotic and biotic factors (Singer & Munns 1987) influencing plant community composition and grasshopper oviposition preferences (Isely 1937). Furthermore, Pfadt (1996) found 92% of *M. alpinus*

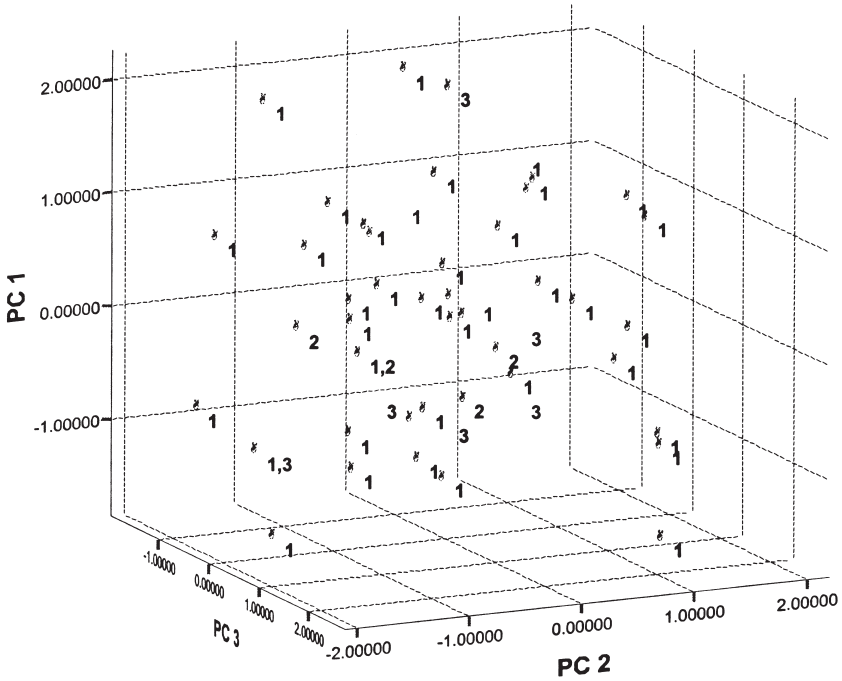

Fig. 1. Three mtDNA lineages of *M. alpinus* represented in three–dimensional space based on principal component analysis of ecological variables. The lineages do not occupy distinct habitats.

Table 2. Principal component analysis of meadow habitats of *M. alpinus*. Nine habitat variables reduced into 3 principal components (PC) that account for 71.5% of the total variance in the original 9 variables.

PC	Eigenvalues	% Variance	Total Variance
1	2.551	28.350	28.350
2	2.314	25.713	54.062
3	1.570	17.446	71.508

eggs to require 3 y of development before hatching, with an average nymphal period of 32.5 d. Assuming a generous month-long adult period, 94% of *M. alpinus*' lifecycle is spent in the soil. It appears suitable soils for embryonic/nymphal development are associated with grasshopper outbreaks in Wyoming (Schell & Lockwood 1997). Preferential selection of such soils at the genotype level for oviposition and subsequent embryonic development is a possible catalyst to grasshopper sympatric speciation.

Collective consideration of ecological variables did not result in differential habitat associations for the 3 lineages. However, even if habitat discrimination did exist among mtDNA lineages, it would be impossible to distinguish passive habitat sorting from active habitat selection through mere correlation (Jaenike & Holt 1991). At best, genotype eith habitat-type correlation suggests the likelihood of philopatry when seen in maternal markers or assortative mating when seen in recombining markers. Furthermore, lack of habitat discrimination among lineages may be a result of scale discrepancy between genetic and habitat types. There is an inherent difficulty in explaining genetic lineage divergence occurring on a scale of hundreds of thousands of years by habitat divergence occurring at a much more rapid rate. It is not surprising, therefore, that a dynamic variable such as vegetation would prove inconsequential. Soil texture is a moderately stable variable, while that of elevation-latitude is the most stable ecological variable accounted for in this study. For an ecological variable to influence genetic diversity or to reinforce established genetic lineages, that variable must change on a scale greater than or equal to those of the genetic indices. It seems that ecological variables which are too labile (vegetation) or too stable (elevation-latitude) may have less potential than moderately scaled (soil) features for association with genetic measures.

Genetic diversity (unlike lineage divergence) is a variable that may change (within centuries) due to dynamic forces of genetic migration, population size, and natural selection. Because present suitable habitat for *M. alpinus* was glaciated in the last glacial maxima, the meadows surveyed represent a relatively recent (10000 to 15000 y) habitat expansion (Denton & Hughes 1981). This was accompanied by a habitat contraction from the lower elevational basins. The lack of significance in the negative trends between genetic diversity and both elevation and elevation-latitude could be a result of scale discrepancy between a variable that changes within centuries (genetic diversity) and variables that represent change across millennia (elevation, elevation-latitude). Though genetic diversity generally decreases with increases in latitude (Cooper *et al.* 1995, Hewitt 1993, Cwynar & MacDonald 1987), the altitudinal expansion is believed to have been slower, thereby retaining greater genetic diversity in those species affected (Hewitt 1999). Altitudinal shifts are also characterized by shorter dispersal distances, further contributing to higher genetic diversity within populations (Hewitt 1996). Mitochondrial DNA did not, however, reveal a significant effect of habitat size in maintaining genetic diversity and minimizing the effects of drift for populations of *M. alpinus*. This might be expected if small meadows provide important migration corridors between larger ones.

Table 3. Component matrix of the principal component analysis of meadow habitats of *M. alpinus*. The 3 principal components are shown with loadings of the 9 habitat variables. Loadings of habitat variables, positive or negative, reflect importance of each variable to a principal component.

Variable	PC 1	PC 2	PC 3
% Sand	.935	.062	-.278
% Silt	-.695	-.344	.454
% Clay	-.775	.515	.064
% Coarse	.500	.091	.174
Elevation-Latitude	.359	-.587	.525
Non-grass Cover	.391	.605	.171
% Bare Ground	.081	.331	.818
Meadow Size	.096	.631	-.364
Grass Cover	-.216	-.836	-.382

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