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A HIERARCHICAL ANALYSIS OF GENETIC DIFFERENTIATION IN A MONTANE LEAF BEETLE *CHRYSOMELA AENEICOLLIS* (COLEOPTERA: CHRYSOMELIDAE)

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Abstract.—Herbivorous insects that use the same host plants as larvae and adults can have a subdivided population structure that corresponds to the distribution of their hosts. Having a subdivided population structure favors local adaptation of subpopulations to small-scale environmental differences and it may promote their genetic divergence. In this paper, I present the results of a hierarchical study of population structure in a montane willow leaf beetle, *Chrysomela aeneicollis* (Coleoptera: Chrysomelidae). This species spends its entire life associated with the larval host (*Salix* spp.), which occurs in patches along high-elevation streams and in montane bogs. I analyzed the genetic differentiation of *C. aeneicollis* populations along three drainages in the Sierra Nevada mountains of California at five enzyme loci: *ak-1, idh-2, mpi-1, pgi-1,* and *pgm-1,* using recent modifications of Wright's *F*-statistics. My results demonstrated significant differentiation (F_{ST} = 0.043) among drainages that are less than 40 kilometers apart. One locus, *pgi-1,* showed much greater differentiation than the other four (F_{ST} = 0.412), suggesting that it is under natural selection. *C. aeneicollis* populations were also subdivided within drainages, with significant differentiation 1) among patches of willows (spanning less than three kilometers) and 2) in some cases, among trees within a willow patch. My results demonstrate that this species has the capacity to adapt to local environmental variation at small spatial scales.

Key words.—Allozymes, *Chrysomela aeneicollis,* *F*-statistics, gene flow, genetic differentiation, insect population structure, natural selection, *phosphoglucose isomerase.*

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All species consist of populations that vary in size and degree of isolation from other populations. Local population sizes, migration rates, and the patterns of matings within a population all affect a species' population structure (Hartl and Clark, 1989; McCauley and Eanes, 1987; Slatkin, 1987). In many cases, local environmental variation causes natural selection to operate differently among local populations, and populations may differ genetically in response to this natural selection. Even in the absence of selection, genetic drift among small-sized populations increases their likelihood of becoming genetically differentiated. On the other hand, gene flow tends to make populations more genetically homogeneous (Slatkin, 1987). To measure the genetic structure of populations, Wright (1978) proposed that studies should use a hierarchical sampling design that corresponds to spatial subdivisions of natural populations. For example, if a herbivorous insect lives on trees that grow in distinct patches separated by geographical barriers, a study of that insect's population structure should include samples from individual trees from several host-tree patches. The advantage of a hierarchical study is that one can distinguish evolutionary processes such as isolation due to geographic barriers, isolation by distance, and isolation resulting from the organism's behavior itself. Until recently however, few studies have attempted to measure population structure in this way.

Willow leaf beetles (Family Chrysomelidae) occur on willow shrubs or trees that commonly grow in separated patches of boggy or moist habitat. These beetles feed on the same host species as adults and as larvae, and they usually overwinter below their host tree or under the bark of dead branches (Brown, 1956; Raupp and Denno, 1983; Rowell-Rahier, 1984; Pasteels et al., 1988; Smiley and Rank, 1986). Although they can fly, most willow leaf beetles do so rarely (Brown, 1956).

Because of their low vagility and because

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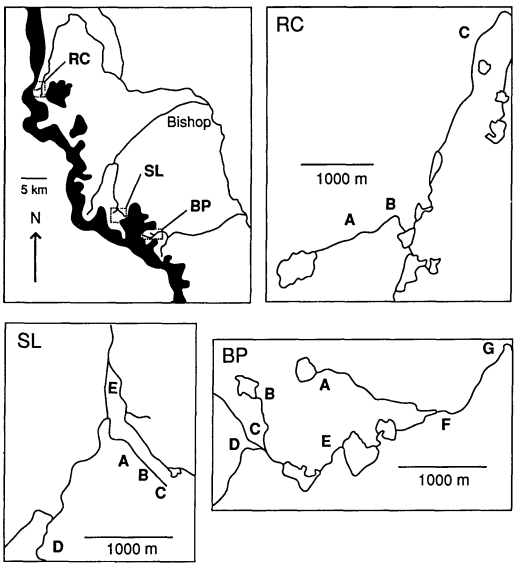

FIG. 1. Maps of the localities. *Top left*—locations of the three drainages in the eastern Sierra Nevada. The areas shaded in black indicate regions higher than 3500 meters. *Top right*—locations of the three localities in the Rock Creek drainage. *Bottom left*—locations of the five localities near South Lake in the Bishop Creek drainage. *Bottom right*—locations of the seven localities in the Big Pine Creek drainage.

they occur in distinct patches of host plants, one may predict that willow leaf beetles have a subdivided population structure. This subdivision may enhance their potential for local adaptation to small scale environmental variation. Indeed, an electrophoretic study of a willow leaf beetle, *Plagiodera versicolor,* which was introduced to North America at the turn of the century, found significant differentiation among patches of willows separated by several kilometers (McCauley et al., 1988). This study also demonstrated significant variation among patches in average relatedness of individuals within larval feeding groups, which suggests that genetic differentiation in average relatedness has occurred.

In this study, I used gel electrophoresis to quantify the population structure of a willow leaf beetle, *Chrysomela aeneicollis,* which occurs at high elevations in the eastern Sierra Nevada of central California (Smiley and Rank, 1986). *Chrysomela aeneicollis* is native to western North America from the Yukon to California (Brown, 1956). At this southern extreme of its range, *C. aeneicollis* populations are potentially subdivided at several levels. First, populations are localized in isolated drainages that are separated by high-elevation ridges. Second, *C. aeneicollis* populations occur on separated patches of willows in bogs, on talus slopes, or along streams. Finally, within these patches, the willows grow as distinct shrubs supporting populations of *C. aeneicollis* larvae and adults. Thus, for this species, which has a limited vagility, physical barriers and the patchiness of its host plants may favor genetic subdivision at each spatial scale. I tested this hypothesis by sampling *C. aeneicollis* populations hierarchically 1) among isolated drainages, 2) among patches of willows within a drainage (localities), and 3) among individual willows within a locality.

MATERIALS AND METHODS

Collection.—*Chrysomela aeneicollis* adults from the previous year can be found in May and June, but adults from the present generation do not emerge from their pupae until late August or early September. For this study, I collected newly emerged adult beetles on September 3–7, 1988. The beetles were collected along three drainages: Big Pine Creek (37°7′N/118°29′W), Bishop Creek (37°11′N/118°32′W, populations sampled near South Lake), and Rock Creek (37°25′N/118°44′W). These drainages are isolated by barren ridges that constitute part of the Sierra crest. Big Pine Creek is separated from Bishop Creek by about 10 kilometers and one ridge, but populations in both of these drainages are separated from the Rock Creek populations by several ridges and about 40 kilometers. I sampled beetles in a stepping stone fashion within each drainage at localities that were 0.3 to 5 kilometers apart (Fig. 1). In each locality, I sampled beetles from two to eight willows separated by 2 to 150 meters (Table 1). Because these willows are shrubs, all of the branches could be sampled. All of the beetles were collected from a single, salicin-rich willow species, *S. orestera,* except for those collected from six *S. boothi* individuals at locality BPf. The two willow species grew together at this locality.

Electrophoresis.—After collection, I brought the beetles to the University of Cal-

TABLE 1. Description of localities. *N* refers to the average sample size per willow, and the distance is the average distance between any two willows at the site.

Locality	Trees	*N*	Elevation m	Distance m	Site description
BPCa	6	27.2	3182	94	Large clones on talus slope
BPCb	3	15.3	3268	13	Bog by 4th lake
BPCc	3	27.3	3219	29	Open bog
BPCd	7	31.9	3127	32	Talus and bog
BPCe	3	31.7	3106	13	At edge of shaded pond
BPCf	8	28.8	2974	31	Bog and pine woodland
BPCg	4	13.0	2877	19	Shaded bog
RCa	3	12.3	3365	68	Stream-side clones
RCb	7	20.9	3264	90	Stream-side in steep canyon
RCc	3	16.3	3064	34	Bog in campground
SLa	3	51.0	3170	6	Shaded bog
SLb	3	45.0	3194	8	Shaded bog
SLc	2	38.5	3219	10	Shaded bog
SLd	2	21.0	3005	2	At edge of open pond
SLe	4	23.8	2883	74	Open boggy area near road

ifornia, Davis for long-term storage at −80°C. I prepared the samples for electrophoresis by homogenizing them with a glass rod in 0.1 ml of distilled water in a microcentrifuge tube. Electrophoresis was conducted according to the methods outlined in Harris and Hopkinson (1976), using a 12.5% potato starch solution (Sigma Chemical Co., St. Louis, Mo.). To determine which loci were polymorphic, I first screened *C. aeneicollis* (10 individuals per drainage) for polymorphism among 22 enzyme loci: *6-phosphogluconate dehydrogenase* (*6pgd*; E.C. 1.1.1.49), *aconitase* (*acon-1*; E.C. 4.2.1.3), *adenylate kinase* (*ak-1*; E.C. 3.5.4.4), *alcohol dehydrogenase* (*adh-1, adh-2, adh-3*; E.C. 1.1.1.1.), *alpha-gpd* (*alpha-glycerophosphate dehydrogenase* E.C. 1.1.1.8.), *aspartate aminotransferase* (*aat-1, aat-2*; E.C. 2.6.1.1), *creatine kinase* (*ck-1*; E.C. 2.7.3.2), *esterase* (*est-2*; nonspecific), *isocitrate dehydrogenase* (*idh-1, idh-2*; E.C. 1.1.1.42), *malate dehydrogenase* (*mdh-1, mdh-2*; E.C. 1.1.1.37), *malic enzyme* (*me-1*; E.C. 1.1.1.40), *mannose-6-phosphate isomerase* (*mpi-1*; 5.3.1.8), *peptidase* (*pep-b], pep-c, pep-d*; uncertain E.C. assignment, *peptidase* nomenclature based on Murphy et al., 1990), *phosphoglucomutase* (*pgm-1*; E.C. 5.4.2.2), *phosphoglucose isomerase* (*pgi-1*; E.C. 5.3.1.9), *superoxide dismutase* (*sod-1*; E.C. 1.15.1.1). Of these 22 loci, *acon-1, ak-1, idh-2, me-1, mp-1, pgi-1,* and *pgm-1* showed polymorphism. I subsequently dropped *acon-1* and *me-1* because the bands were not clearly readable for scoring of putative genotypes. Thus, five loci were used in the complete survey.

For these five loci, I used two buffer systems. For *ak-1, idh-2,* and *mpi-1,* I used a continuous tris-citrate buffer (pH 8.0), run at 100 mA for four hours. I added about 5 mg of NADP to the gel buffer just before pouring the gel. For *pgi-1* and *pgm-1,* I used a discontinuous tris-citrate buffer (pH 7.1) run at 75 mA for six hours (buffer recipe in Ayala et al., 1972). Agar overlays were used in staining all of the enzymes except for *idh-2.* For all five loci, I reran individuals to homologize alleles and to resolve scores that had been questionable on the first run.

Analyses.—I used the BIOSYS (Swofford and Selander, 1981; version 1.6 for personal computers) program on single-individual genotypic data to obtain allele frequencies and to test for significant deviations from Hardy-Weinberg equilibrium for each willow. Before testing for HWE, I pooled the genotypes into homozygotes for the most common allele, heterozygotes between the most common allele and other alleles, and homozygotes and heterozygotes for the other alleles. I used the exact test option in BIOSYS to obtain exact probabilities of the deviations, and I report the direct count average heterozygosities and their standard errors given in BIOSYS's step VARIAB.

To analyze differentiation among subpopulations, I used Weir and Cockerham's (1984) modification of Wright's *F*-statistics

(Wright, 1978). The F-statistics are calculated from the genotypic frequencies at each locus. F_{IS} measures the degree of nonrandom mating within a subpopulation. It takes a positive value when the observed heterozygosity is less than the expected heterozygosity, or a negative value when there is a heterozygote excess. F_{ST} measures the relative fixation of alternate alleles in different subpopulations by comparing the average of the subpopulation heterzygosities to the total heterozygosity expected under random mating. The magnitude of F_{ST} therefore depends on the amount of among-subpopulation divergence in allele frequencies (Wright, 1978; Hartl and Clark, 1989). The F-statistics are related by the equation $(1 - F_{IS}) = (1 - F_{IT})(1 - F_{ST})$.

In a multiallele system, the estimates from each allele are weighted by their frequencies to obtain the estimate for each locus. A jackknife procedure is used to combine the information across alleles within a locus and across loci and generate a mean and a standard error for the F-statistics. Because Weir and Cockerham's (1984) modification of Wright's F-statistics uses estimators that are corrected for sample size differences, F_{ST} may assume slightly negative values in their formulation, which indicates a lack of any differentiation among subpopulations (Slatkin and Barton, 1989).

In this paper, I calculated F_{ST} separately at each level in the spatial hierarchy. At the lowest level, F_{ST} was calculated using the genotype frequencies on each willow within a locality, and this calculation was repeated 15 times reflecting the total number of localities sampled (Table 1). I refer to this level as F_{WL} denoting differentiation among willows in a locality. Then I calculated an F_{ST} among localities within each drainage (F_{LD}), using the genotype frequencies for each locality. Three values were calculated at this level, one for each drainage. Finally, I calculated a single F_{ST} from the overall genotypic frequencies in each drainage, which I refer to as F_{DT}. This type of notation was used by Wright (1978) and McCauley et al. (1988). I have calculated the F statistics for each level in the spatial hierarchy separately because the individual measurements show more clearly how the patterns of differentiation vary at different locations.

To determine the statistical significance of the genetic variation, I have taken two approaches: G tests for heterogeneity of allele frequencies at each locus and t-tests of the jackknifed F_{ST} estimates. For the G tests, I pooled the rarer alleles into the next most common allelic classes when the expected values in any cell were less than 5. To test whether the jackknifed estimates were significantly different from zero, I used simple t-tests (cf. McCauley et al., 1988). Because F_{IS} and F_{IT} can take both negative and positive values, I evaluated their t-values with two-tailed probabilities, but I used one-tailed tests to determine the significance of F_{ST}. Additionally, I used the sequential Bonferroni procedure to control for Type I error resulting from multiple significance tests (Rice, 1989). Finally, I reported negative F_{ST} values as zero. The lowest value I obtained at an individual locus was -0.072, and for a jackknife estimate the lowest value was -0.012.

Another way of describing the geographical pattern of allele frequencies is to use principal components analysis. One can plot the principal components against other geographic variables (cf. Langercrantz and Ryman, 1990). In this analysis, I used the angular transformed allele frequencies on each willow. I included all alleles that had an overall frequency greater than 0.09, resulting in a total of 11 variables: *ak-1* allele 1, *idh-2* alleles 1, 3, 4, *mpi-1* alleles 1, 2, *pgi-1* alleles 1, 4, and *pgm-1* alleles 1, 4, 6. I then plotted the first versus the second principal component and elevation versus the first principal component separately for each drainage.

Results

In most cases, the loci that were polymorphic in the original screening were polymorphic on each willow (Appendix 1). However, at the level of drainages, populations in Rock Creek and South lake possessed alleles at *idh-2* (allele 2), *mpi-1* (allele 3), and *pgi-1* (alleles 2, 3) that were absent from Big Pine Creek, and the Big Pine Creek and South Lake populations possessed four alleles at *pgm-1* (alleles 2, 3, 5, 7) that were absent from Rock Creek. Additionally, at *pgi-1,* the most common allele at Big Pine Creek was quite rare at Rock Creek and

TABLE 2. F_{ST} values among drainages (F_{DT}) and among localities within each drainage (F_{LD}). Table-wide significance levels obtained from G tests for allele frequency heterogeneity as described in the text.

	Locus				
F-statistic	*ak-1*	*idh-2*	*mpi-1*	*pgi-1*	*pgm-1*
F_{LD} (BPC)	0.008	0.014***	0.011*	0.012**	0.011**
F_{LD} (RC)	0.022	0.007	0.018	0.016	0.000
F_{LD} (SL)	0.000	0.047***	0.016**	0.058**	0.026***
F_{DT}	0.016***	0.030***	0.040***	0.412***	0.067***

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.005$.

conversely, the most common allele at Rock Creek was rare at Big Pine Creek. The South Lake populations had intermediate frequencies of the two alleles at *pgi-1* (Appendix 1).

There were no pervasive departures from HWE in the sample. After the sequential Bonferroni adjustment of the P values for the number of tests made at each locus, only two of the deviations from HWE were statistically significant. Both of these deviations occurred on willow RV2 at locality SLe. At *pgm-1,* there was a heterozygote excess ($Fi = -0.82$, $P < 0.001$), while at *idh-2,* there was a deficiency of heterozygotes ($Fi = 0.32$, $P < 0.001$).

If a population has experienced a prolonged bottleneck, its average heterozygosity should be lower than others (Hartl and Clark, 1989). Average heterozygosities in this study were very similar for Big Pine Creek (0.430 ± 0.084) and for South Lake (0.430 ± 0.097), but they were somewhat smaller for Rock Creek (0.322 ± 0.099). Much of this difference results from the greatly reduced heterozygosity at *pgi-1* ($H = 0.078$) in Rock Creek relative to Big Pine Creek ($H = 0.322$) and South Lake ($H = 0.431$).

Among-Drainage Differentiation.—The frequencies at each locus were significantly heterogeneous among drainages (Table 2). The F_{DT} values for four of the five loci agree reasonably well, and the degree of polymorphism at the locus probably accounts for some of the variation in F_{DT} (Slatkin and Barton, 1989). However, the value of F_{DT} for *pgi-1* is nearly 10 times greater than any of the other four loci. This discrepancy suggests that natural selection is acting on one or several of the alleles at *pgi-1* at the level of drainages (Slatkin, 1987). Because differentiation at *pgi-1* appears to be influenced by natural selection, I made two jackknife estimates for F_{DT}, one that included *pgi-1* and one without *pgi-1*. In both cases, the overall among-drainage differentiation was significant (Table 3). However, when *pgi-1* is included in the jackknife estimate the standard error is proportionately greater, and the significance level is reduced to some degree.

Among-Locality Differentiation.—Localities within a drainage were genetically differentiated as well (Tables 2 and 3). The loci within each drainage are in basic agreement, except for the zero estimates of F_{LD} at *ak-1* in South Lake and at *pgm-1* at Rock Creek. It is worth noting that *ak-1* shows the least polymorphism at South Lake and *pgm-1* shows much less polymorphism at Rock Creek than at the other two drainages (Appendix 1). In all three drainages, the jackknife estimates of F_{LD} were lower than F_{DT}, indicating that the ridges indeed act as barriers to gene flow. Nevertheless, the jackknife estimate of F_{LD} was substantially greater in South Lake than in the other two drainages.

Among-Tree Differentiation.—The estimates for F_{WL} vary considerably among localities (Tables 4 and 5). At this level, there is greater variability in the estimates across

TABLE 3. Jackknife estimates of *C. aeneicollis* genetic differentiation among localities within each drainage and among drainages. Significance levels were determined by *t*-tests.

F-statistic	*F*	SE	*P*
F_{DT} with *pgi-1*	0.135	0.041	*
F_{DT} without *pgi-1*	0.043	0.005	**
F_{LD} (BPC)	0.012	0.001	***
F_{LD} (RC)	0.010	0.002	**
F_{LD} (SL)	0.037	0.004	**

Table-wide significance levels, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

TABLE 4. F_{WL} values among trees in a locality for each locus. Column-wide significances were determined separately for each locus after conducting G tests of allele frequency heterogeneity as described in the text. Dashes indicate that a locus was not polymorphic at that locality.

Locality	Locus				
	ak-1 F_{WL}	*idh-2* F_{WL}	*mpi-1* F_{WL}	*pgi-1* F_{WL}	*pgm-1* F_{WL}
BPCa	0.000	0.001	0.000	0.000	0.000
BPCb	0.066	0.253**	0.000	0.068	0.107
BPCc	0.033	0.005	0.000	0.036	0.133***
BPCd	0.018	0.038**	0.051**	0.049**	0.015
BPCe	0.000	0.001	0.000	0.100***	0.014
BPCf	0.009	0.033	0.018	0.128***	0.027
BPCg	0.042	0.000	0.000	0.000	0.004
RCa	0.000	0.045	0.001	0.063	0.000
RCb	0.167***	0.049*	0.025	0.051	0.161***
RCc	0.102	0.036	0.249**	—	0.268***
SLa	0.015	0.030*	0.000	0.097***	0.010
SLb	0.067**	0.104***	0.016	0.018	0.061***
SLc	0.005	0.008	0.000	0.004	0.022
SLd	0.023	0.000	0.000	0.000	0.000
SLe	0.980	0.386*	0.181	0.367***	0.397

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

loci, probably resulting from relatively greater sampling error. Despite this variability, the F_{WL} values for individual loci were generally consistently high or low within a population and usually if there was significant differentiation at any one locus, other loci indicated significant differentiation as well (Table 4). F_{WL} was greatest at locality SLe, but the high value for *ak-1* at SLe resulted from its fixation at several willows.

TABLE 5. Jackknife estimates of F_{IW}, the inbreeding coefficient, and F_{WL} among willows within a locality. All estimates are based on five loci except for RCc, which was based on four loci because *pgi-1* was fixed for one allele. Statistical significance was determined by t-tests.

Locality	F_{IW} SE	F_{WL} SE
BPCa	0.000 ± 0.024	0.000 ± 0.001
BPCb	0.002 ± 0.034	0.113 ± 0.022*
BPCc	−0.047 ± 0.034	0.043 ± 0.016
BPCd	−0.012 ± 0.015	0.036 ± 0.003**
BPCe	0.015 ± 0.009	0.015 ± 0.008
BPCf	−0.030 ± 0.007	0.039 ± 0.007*
BPCg	−0.005 ± 0.018	0.000 ± 0.004
RCa	−0.108 ± 0.028	0.023 ± 0.008
RCb	0.033 ± 0.025	0.079 ± 0.015*
RCc	0.015 ± 0.064	0.165 ± 0.041[1]
SLa	−0.064 ± 0.018	0.034 ± 0.009[1]
SLb	−0.097 ± 0.013*	0.016 ± 0.005
SLc	−0.053 ± 0.026	0.009 ± 0.002[1]
SLd	−0.153 ± 0.058	0.000 ± 0.002
SLe	0.024 ± 0.080	0.427 ± 0.048**

[1] $P < 0.1$, * $P < 0.05$, ** $P < 0.01$.

The jackknife estimates of F_{WL} indicated significant substructuring at the 95% column-wide significance level for 4 of the 15 localities sampled, with significant values ranging from 0.039 to 0.427. Additionally, another three localities were significantly differentiated at the 90% significance level (Table 5). In four cases, (BPb, BPf, RCb, RCc), the among-willow differentiation occurred in localities where the sample size on at least one tree was small ($N < 10$), and the highest value of F_{WL} occurred at locality SLe, where the subpopulations were clearly out of HWE. On the other hand, at locality BPd, where F_{WL} was also significant, sample sizes were consistently large. Finally, F_{WL} was not related to the average distance between willows within a locality ($m = 0.001$, $r = 0.35$, $P = 0.20$, $N = 15$ localities).

Neither the jackknifed estimates of F_{IS} at the drainage level (F_{ID}) nor the estimates for localities within a drainage (F_{IL}) were significantly different from zero. Additionally, at the level of individual willows (F_{IW}), most of the values were not significant (Table 5). This result suggests that there was no substantial genetic structuring within populations, and it corresponds with the finding that most of the individual populations were in HWE. For the one locality where F_{IW} was significant (locality SLb), it was negative (Table 5). It seems clear that *C. aeneicollis* populations are not generally inbred and

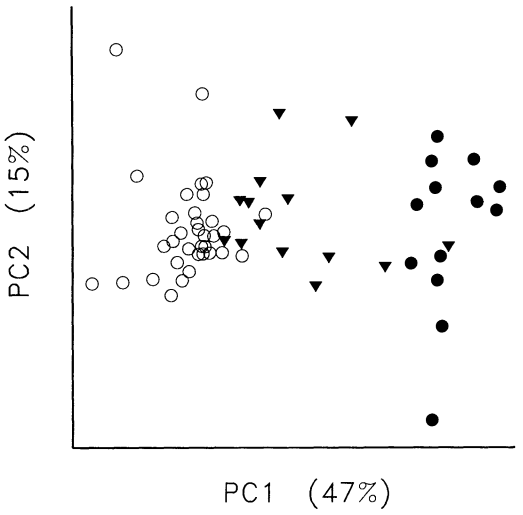

FIG. 2. Scatterplot of the first (PC1) and second (PC2) principal components of the allele frequencies at the five polymorphic loci. Each point represents a different willow. Hollow circles refer to BP populations, filled triangles refer to SL populations, and filled circles refer to the RC populations. The percentages refer to the proportion of the variance explained by the principal component.

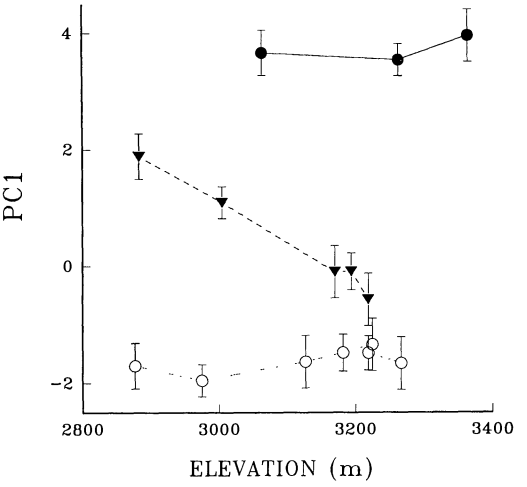

FIG. 3. Plot of PC1 versus elevation. Each point represents the least-squares mean for that locality and the error bars represent the standard error. The symbols refer to the different drainages as in Figure 2.

there is no Wahlund effect resulting from genetic structure below the lowest level of the sampling design.

In the principal components analysis, the first principal component (PC1) explained 47% of the variation in the 11 allele frequencies, and the second principal component (PC2) explained an additional 15% of the variation. However, a plot of PC1 versus PC2 shows that PC1 accounts for much of the variation among drainages (Fig. 2). The drainages differ in their PC1 scores (Nested ANOVA with drainages and localities as random factors $F_{2,14} = 102.8$, $P = 0.0001$), but PC1 also varied significantly among localities within a drainage ($F_{12,46} = 2.0$, $P = 0.04$). PC1 is most closely correlated to the two most common alleles at *pgi-1* ($r = 0.41$ for allele 1 and -0.42 for allele 2). Nevertheless, it is also fairly highly correlated to alleles 1 and 6 at *pgm-1* ($r = -0.41$ and 0.35, respectively) and to allele 1 at *mpi-1* ($r = -0.31$). Of the alleles included in the PC analysis, these five differ the most in frequency among the drainages.

There was a linear relationship between PC1 and elevation in drainage SL, but not in the other two drainages (Fig. 3). Linear regressions showed that there was no significant relationship in BP ($m = 0.001 \pm 0.001$, $r^2 = 0.05$, $P = 0.22$) or in RC ($m = 0.001 \pm 0.002$, $r^2 = 0.01$, $P = 0.70$), but the relationship between elevation and PC1 was highly significant in SL ($m = -0.007 \pm 0.002$, $r^2 = 0.58$, $P = 0.002$). Thus, the upper-elevation populations in SL were more genetically similar to the BP populations than the lower-elevation populations in SL. These populations are the closest to the BP populations (Fig. 1).

Differentiation among Willow Species.— At one of the localities in Big Pine Creek (BPf), I collected *C. aeneicollis* individuals from four neighboring willows of two species: two *S. boothi* individuals and two *S. orestera* individuals. To determine whether *C. aeneicollis* populations on different species were genetically differentiated, I used a different hierarchical F statistic (F_{SL}). First, I calculated F_{WL} for each species separately, then I pooled the genotype frequencies for each species and obtained F_{SL} for the pooled estimate. Although the sample size for any comparison of among-willow differentiation versus among-species differentiation is small, this technique would detect substantial genetic divergence. For the beetles collected on *S. boothi,* the jackknife estimate of F_{WL} was 0.053 ± 0.010, and for the beetles collected on *S. orestera,* the estimate

was 0.000 ± 0.003. The hierarchical F_{SL} was 0.040 ± 0.009, which is smaller than the jackknife estimate of F_{WL} for *S. boothi* alone. If populations on different species were subdivided, one would expect the hierarchical F_{SL} values to exceed the values for F_{WL} within each species (McPheron et al., 1988). Thus, my results indicated no differentiation among host species for *C. aeneicollis.*

Discussion

Chrysomela aeneicollis populations are indeed subdivided among drainages. The level of genetic subdivision (F_{DT} = 0.043) among these nearby drainages is higher than the F_{ST} values across broad geographic scales for many flying insects, including bark beetles, *Drosophila* spp., and several lepidopterans (McCauley and Eanes, 1987). The F_{ST} value for *Plagiodera versicolor* populations from Virginia to Illinois is 0.057 (McCauley et al., 1988). *Chrysomela aeneicollis*'s relatively higher levels of differentiation may result from the fact that this is a native species that has presumably been in the eastern Sierra Nevada for much longer than the length of time that the introduced *P. versicolor* has been in North America. However, it is also clear that *C. aeneicollis*'s population subdivision is also greatly affected by the topographic relief of the eastern Sierra Nevada.

Despite the among-drainage differentiation, the PC analysis suggested that there is gene flow over the Sierra crest. Although the streams are connected by nearly continuous stands of willow at the lower elevations, these areas appear to be very unsuitable habitats for *C. aeneicollis*; over seven years of research I never discovered a population below 2,300 meters. Thus, it appears that most of the gene flow among drainages occurs over the ridges rather than along the streams. This gene flow has another result; it is probably responsible for the greater F_{LD} values observed in the SL drainage than in the other two drainages. If this drainage had been examined alone, the high F_{LD} among the SL populations may have been erroneously attributed to genetic drift. Finally, these results illustrate that gene flow can be a "creative force" as described by Slatkin (1987). The variability at the *pgi-1* locus is highest in the SL populations because both of the common alleles are present at high frequencies there.

Previous studies have indicated that natural selection is acting on enzyme loci in herbivorous insects; Slatkin (1987) discusses an example in the butterfly *Euphydryas editha,* where F_{ST} at *hexokinase* was substantially greater than at other loci. Additionally, natural selection on *pgi* has been demonstrated for *Colias* butterflies (Watt, 1977; Watt et al., 1983, 1985). However, this is the first observation that natural selection is probably acting on *pgi* in a beetle. In the case of *Colias,* differential temperature sensitivities of the different *pgi* enzymes form the basis for natural selection. The *pgi* genotype affects the timing of flight activity. It is possible that different *pgi-1* genotypes vary in their temperature sensitivity for *C. aeneicollis.* Further investigation is required to determine the significance of this unusual pattern of differentiation at *pgi-1.*

Differentiation among localities within a drainage has implications for local adaptation in *C. aeneicollis.* Environmental conditions vary with elevation along all three of these drainages. For example, Smiley and Rank (1986) documented a reduction in levels of predation on *C. aeneicollis* with increasing elevation along Big Pine Creek. In addition, Smiley and Rank (1986) reported phenotypic differences in larval growth rates among populations from high versus low elevations. Similarly, McCauley et al. (1988) demonstrated spatial and temporal differences in average relatedness among populations of *P. versicolor.* The results of this study suggest that *C. aeneicollis* populations are sufficiently isolated to allow for local adaptation to this environmental variation. Genetic divergence among these populations at other loci that are under natural selection may be even greater than divergence at the relatively neutral allozyme loci (Slatkin, 1987).

At the lowest level of population structure, differentiation among trees within a locality, these results parallel those of McCauley et al. (1988), who observed significant differentiation among trees in two of eight cases for *P. versicolor.* In both studies, the among-tree F_{ST} varied considerably

among localities. It is possible that among-tree differentiation may reflect differentiation at an even smaller scale, i.e., within small groups of trees in a locality. This seems unlikely however, because there was no relationship between the distance between willows in a locality and F_{WL}. It is also unlikely that the among-tree differentiation reflects among-branch differentiation as Guttman et al. (1989) observed for treehoppers, or a Wahlund effect resulting from the combination of samples of several breeding colonies, or inbreeding, as Crouau-Roy (1988) suggested for cave-dwelling beetles. Among-branch differentiation is unlikely because the willows are relatively small, and because *C. aeneicollis* individuals move about considerably within a willow. A Wahlund effect is unlikely because most of the willows appear to be in HWE, and inbreeding appears to be rare in *C. aeneicollis.*

In some cases, the among-tree differentiation may have been based on sampling a few families on individual willows. As noted above, the willows sampled were occasionally relatively small, and because females lay eggs in clutches of 30 eggs, some trees may have consisted of only a few families. This was apparently the case on willow RV2 at locality SLe, where one locus indicated a deficit of heterozygotes and another indicated an excess, which would occur if many of the individuals came from an individual family. Family group sampling may also have occurred at localities (BPb, BPf, RCb, RCc) where some of the willows were relatvely small and beetle population sizes were low. On the other hand, other localities with among-tree differentiation (BPd, BPf, SLc) consisted of larger willows with larger population sizes. In these cases, it is most likely that the among-tree differentiation reflects genetic drift among *C. aeneicollis* populations on different willows. Thus, overall genetic subdivision at this scale may be influenced by founder effects as well as some genetic drift following establishment, as McCauley and Eanes (1987) concluded for the milkweed beetle *Tetraopes tetraophthalmus.*

In conclusion, these results represent yet another example of an herbivorous insect that shows significant genetic subdivision at small spatial scales down to individual host plants. This phenomenon has been observed over a broad range of taxonomic groups, including treehoppers (Guttman and Weigt, 1989; Guttman et al., 1989), milkweed beetles (McCauley and Eanes, 1987), willow leaf beetles (McCauley et al., 1988), and *Rhagoletis* fruit flies (McPheron et al., 1988; Feder et al., 1990*a,* 1990*b*). It appears that the association with patchily distributed host plants has important consequences for the subsequent evolution of herbivores whose vagility is limited. Indeed, if a subdivided population structure is combined with phenological differences among host plants, it may lead to host-race formation and sympatric speciation (Courtney Smith, 1988; Feder et al., 1990*a,* 1990*b*; Wood and Keese, 1990; Wood et al., 1990). In the absence of differences in willow phenology and host-preference among *C. aeneicollis* populations that occur on different hosts (Rank, 1990), host-race formation appears unlikely in this species despite its pronounced genetic subdivision.

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APPENDIX 1. Allele frequency data for all populations. Locality abbreviations are given in Table 1 and locus abbreviations are given in the text. Because only two alleles were present at *ak-1*, the frequency of the most common one is shown. The sample size for each locus is given on the first line.

Locus and Allele	Locality											
	BPa						BPb			BPc		
	BLT1	BLT2	BLT3	BLT4	BLT5	Willow BLT6	FL1	FL2	FL3	HB1	HB2	HB3
ak-1	*25*	*20*	*37*	*24*	*49*	*13*	*21*	*18*	*7*	*22*	*33*	*27*
2	0.88	0.9	0.89	0.9	0.88	0.73	0.86	0.72	1	0.98	0.94	0.85
idh-2	*27*	*20*	*38*	*24*	*49*	*19*	*21*	*18*	*7*	*22*	*34*	*30*
1	0.5	0.48	0.37	0.4	0.5	0.53	0.83	0.75	0.21	0.41	0.47	0.3
2	—	—	—	—	—	—	—	—	—	—	—	—
3	0.22	0.23	0.25	0.29	0.17	0.08	0.17	—	0.43	0.18	0.16	0.27
4	0.28	0.3	0.38	0.31	0.33	0.4	—	0.25	0.36	0.41	0.37	0.43
mpi-1	*27*	*20*	*38*	*20*	*48*	*18*	*21*	*18*	*7*	*22*	*34*	*30*
1	0.57	0.5	0.46	0.5	0.53	0.53	0.31	0.19	0.36	0.48	0.52	0.38
2	0.43	0.5	0.54	0.5	0.47	0.47	0.69	0.81	0.64	0.52	0.49	0.62
3	—	—	—	—	—	—	—	—	—	—	—	—
pgi-1	*27*	*20*	*39*	*24*	*50*	*19*	*21*	*18*	*7*	*22*	*34*	*30*
1	0.26	0.25	0.19	0.21	0.28	0.32	0.21	0.03	0.21	0.23	0.12	0.07
2	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—
4	0.74	0.75	0.81	0.79	0.72	0.68	0.79	0.97	0.79	0.77	0.88	0.93
5	—	—	—	—	—	—	—	—	—	—	—	—
pgm-1	*27*	*20*	*39*	*24*	*50*	*19*	*21*	*18*	*7*	*22*	*34*	*30*
1	0.24	0.33	0.27	0.23	0.26	0.29	0.6	0.31	0.21	0.52	0.15	0.3
2	—	—	0.06	0.04	—	—	0.02	—	—	—	—	—
3	0.02	—	0.01	—	—	—	—	—	—	0.09	—	—
4	0.65	0.63	0.63	0.54	0.61	0.66	0.31	0.44	0.71	0.36	0.79	0.62
5	0.02	0.03	0.01	0.02	—	—	0.07	0.25	—	—	—	0.03
6	0.04	—	0.01	0.06	0.04	0.03	—	—	—	—	0.06	0.05
7	0.04	0.03	—	0.1	0.09	0.03	—	—	0.07	0.02	—	—

APPENDIX 1. Extended.

Locus and Allele	Locality											
	BPd							BPe			BPf	
	U1	U2	U3	U4	KB1	Willow KB2	KB3	PB10	PB11	PB13	FT1	FT2
ak-1	*38*	*30*	*33*	*22*	*21*	*28*	*51*	*37*	*35*	*23*	*52*	*8*
2	0.96	0.93	0.99	0.89	0.88	0.95	0.86	0.92	0.94	0.98	0.7	0.93
idh-2	*38*	*33*	*34*	*22*	*22*	*31*	*51*	*37*	*35*	*23*	*52*	*8*
1	0.62	0.55	0.49	0.52	0.59	0.32	0.28	0.61	0.46	0.52	0.4	0.55
2	—	—	—	—	—	—	—	—	—	—	—	—
3	0.24	0.24	0.28	0.34	0.16	0.27	0.35	0.12	0.23	0.15	0.2	0.36
4	0.15	0.21	0.24	0.14	0.25	0.4	0.36	0.27	0.31	0.33	0.4	0.09
mpi-1	*38*	*32*	*34*	*22*	*22*	*29*	*51*	*37*	*35*	*23*	*52*	*8*
1	0.5	0.55	0.52	0.46	0.75	0.28	0.57	0.51	0.5	0.46	0.6	0.45
2	0.5	0.45	0.49	0.55	0.25	0.72	0.43	0.49	0.5	0.54	0.4	0.55
3	—	—	—	—	—	—	—	—	—	—	—	—
pgi-1	*38*	*33*	*34*	*22*	*22*	*31*	*51*	*37*	*35*	*23*	*52*	*8*
1	0.16	0.23	0.38	0.34	0.09	0.39	0.19	0.31	0.2	0.02	—	0.21
2	—	—	—	—	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—	—	—	—	—
4	0.84	0.77	0.62	0.66	0.91	0.6	0.76	0.69	0.8	0.98	1	0.79
5	—	—	—	—	—	0.02	0.06	—	—	—	—	—
pgm-1	*38*	*33*	*34*	*22*	*22*	*31*	*51*	*37*	*35*	*23*	*52*	*8*
1	0.2	0.14	0.18	0.21	0.27	0.27	0.35	0.34	0.34	0.24	0.5	0.48
2	0.07	0.03	0.03	0.02	—	—	0.02	—	—	0.02	—	0.02
3	—	0.02	0.02	—	—	—	—	—	0.1	—	—	0.02
4	0.61	0.76	0.72	0.61	0.55	0.71	0.61	0.65	0.5	0.65	0.5	0.46
5	0.03	0.05	0.04	0.07	0.11	—	—	—	0.06	0.02	—	—
6	0.07	0.02	0.02	0.07	0.05	—	0.01	0.01	—	0.07	—	—
7	0.04	—	—	0.02	0.02	0.02	0.01	—	—	—	—	0.02

APPENDIX 1. Extended.

Locus and Allele	Locality												
	BPf						BPg				RCa		
	SO10	SO11	FW2	FW5	FW6	Willow FW8	FB1	FB2	FB3	FB4	RC1	RC2	RC3
ak-1	*18*	*25*	*31*	*50*	*11*	*35*	*5*	*9*	*15*	*24*	*10*	*14*	*13*
2	0.94	0.92	0.9	0.92	1	0.94	0.9	1	0.93	0.81	0.95	0.96	1
idh-2	*18*	*25*	*31*	*50*	*11*	*35*	*5*	*9*	*15*	*24*	*10*	*14*	*14*
1	0.53	0.56	0.57	0.5	0.36	0.49	0.6	0.33	0.37	0.4	0.45	0.39	0.57
2	—	—	—	—	—	—	—	—	—	—	—	—	—
3	0.06	0.1	0.32	0.22	0.5	0.2	0.1	0.28	0.23	0.29	—	0.29	0.04
4	0.42	0.34	0.11	0.28	0.14	0.31	0.3	0.39	0.4	0.31	0.55	0.32	0.39
mpi-1	*18*	*25*	*31*	*50*	*11*	*35*	*4*	*9*	*15*	*23*	*10*	*14*	*14*
1	0.58	0.46	0.48	0.66	0.5	0.47	0.38	0.5	0.5	0.48	0.1	0.04	0.18
2	0.58	0.54	0.52	0.34	0.5	0.53	0.63	0.5	0.5	0.52	0.75	0.86	0.82
3	—	—	—	—	—	—	—	—	—	—	0.15	0.11	—
pgi-1	*18*	*25*	*31*	*50*	*11*	*35*	*4*	*9*	*15*	*24*	*10*	*14*	*14*
1	0.08	0.04	0.48	0.15	0.14	0.14	0.13	0.22	0.13	0.1	0.85	0.86	1
2	—	—	—	—	—	—	—	—	—	—	0.15	0.04	—
3	—	—	—	—	—	—	—	—	—	—	—	—	—
4	0.92	0.96	0.5	0.85	0.86	0.84	0.88	0.72	0.87	0.88	—	0.11	—
5	—	—	0.02	—	—	0.01	—	0.06	—	0.02	—	—	—
pgm-1	*18*	*25*	*31*	*50*	*11*	*35*	*4*	*9*	*15*	*24*	*10*	*14*	*14*
1	0.25	0.28	0.23	0.34	0.14	0.23	0.13	0.5	0.33	0.38	—	—	—
2	—	0.02	0.05	0.07	0.05	0.04	—	—	0.03	0.04	—	—	—
3	0.03	0.02	—	—	—	—	—	—	0.03	0.04	—	—	—
4	0.72	0.58	0.66	0.5	0.77	0.56	0.5	0.39	0.53	0.5	0.55	0.71	0.68
5	—	0.02	0.05	—	—	0.03	0.38	0.11	0.03	—	—	—	—
6	—	—	0.02	0.09	0.05	0.14	—	—	0.03	0.04	0.45	0.29	0.32
7	—	0.08	—	—	—	—	—	—	—	—	—	—	—

APPENDIX 1. Extended.

Locus and Allele	Locality												
	RCb							RCc			SLa		
	RC4	RC5	RC6	RC7	RC8	Willow RC9	RC11	RC12	RC13	RC14	PL1	PL2	PL3
ak-1	*51*	*5*	*21*	*15*	*14*	*25*	*20*	*14*	*7*	*28*	*46*	*53*	*54*
2	0.97	1	0.67	1	0.93	0.78	1	0.89	1	1	0.99	0.96	0.93
idh-2	*51*	*6*	*23*	*16*	*18*	*33*	*21*	*14*	*8*	*30*	*48*	*55*	*54*
1	0.25	0.33	0.37	0.22	0.39	0.49	0.52	0.36	0.25	0.37	0.55	0.29	0.44
2	0.11	—	—	—	—	0.02	—	—	—	—	0.02	0.05	—
3	0.11	0.08	—	0.03	—	0.09	0.17	0.25	—	0.05	0.22	0.29	0.3
4	0.54	0.58	0.63	0.75	0.61	0.41	0.31	0.39	0.75	0.58	0.21	0.37	0.27
mpi-1	*46*	*5*	*22*	*15*	*18*	*32*	*21*	*14*	*8*	*30*	*46*	*55*	*54*
1	0.35	0.1	0.32	0.3	0.28	0.17	0.24	0.18	0.69	0.17	0.41	0.39	0.4
2	0.6	0.9	0.61	0.6	0.64	0.83	0.76	0.61	0.31	0.83	0.51	0.6	0.52
3	0.05	—	0.07	0.1	0.08	—	—	0.21	—	—	0.08	0.01	0.08
pgi-1	*51*	*6*	*23*	*16*	*18*	*33*	*21*	*14*	*8*	*30*	*48*	*56*	*54*
1	1	0.92	0.91	0.84	0.89	0.97	0.98	1	1	1	0.44	0.34	0.68
2	—	0.08	—	0.16	0.08	—	—	—	—	—	0.01	0.02	—
3	—	—	—	—	—	—	—	—	—	—	—	0.01	—
4	—	—	0.09	—	0.03	0.03	0.02	—	—	—	0.55	0.63	0.32
5	—	—	—	—	—	—	—	—	—	—	—	0.01	—
pgm-1	*51*	*6*	*23*	*16*	*18*	*33*	*21*	*14*	*8*	*30*	*48*	*55*	*54*
1	—	—	—	0.06	—	—	—	—	—	—	0.14	0.08	0.2
2	—	—	—	—	—	—	—	—	—	—	—	0.02	0.02
3	—	—	—	—	—	—	—	—	—	—	0.03	—	—
4	0.88	0.83	0.8	0.53	0.75	0.7	0.33	0.96	0.38	0.77	0.71	0.8	0.69
5	—	—	—	—	—	—	—	—	—	—	0.01	—	—
6	0.12	0.17	0.2	0.41	0.25	0.3	0.67	0.04	0.63	0.23	0.04	0.06	0.04
7	—	—	—	—	—	—	—	—	—	—	0.07	0.05	0.05

APPENDIX 1. Extended.

Locus and Allele	Locality										
	SLb			SLc		SLd		SLe			
	SC1	SC2	SC3	HSC1	Willow HSC2	SL1	SL2	PC1	PC4	RV1	RV2
ak-1	*50*	*36*	*49*	*27*	*50*	*20*	*23*	*31*	*33*	*10*	*21*
2	0.92	1	1	0.96	0.99	1	0.94	0.98	0.91	1	1
idh-2	*51*	*36*	*51*	*27*	*52*	*20*	*24*	*31*	*37*	*10*	*21*
1	0.42	0.42	0.78	0.59	0.71	0.4	0.33	0.68	0.51	0.4	0.31
2	—	—	—	—	0.01	—	—	0.15	—	0.1	0.48
3	0.26	0.35	0.13	0.13	0.08	0.1	0.08	0.11	0.22	0.05	—
4	0.32	0.24	0.1	0.28	0.2	0.5	0.58	0.07	0.27	0.45	0.21
mpi-1	*51*	*36*	*51*	*27*	*52*	*19*	*24*	*31*	*37*	*10*	*21*
1	0.47	0.31	0.35	0.57	0.49	0.4	0.44	0.26	0.31	0.35	0.45
2	0.53	0.67	0.64	0.43	0.42	0.61	0.56	0.66	0.68	0.65	0.55
3	—	0.03	0.01	—	0.09	—	—	0.08	0.01	—	—
pgi-1	*51*	*36*	*51*	*27*	*52*	*20*	*24*	*31*	*37*	*10*	*21*
1	0.37	0.47	0.54	0.32	0.4	0.6	0.65	0.87	0.49	1	0.74
2	—	—	—	—	—	—	—	—	0.01	—	—
3	—	—	—	—	—	—	—	—	—	—	—
4	0.63	0.53	0.46	0.69	0.59	0.4	0.35	0.13	0.5	—	0.26
5	—	—	—	—	0.01	—	—	—	—	—	—
pgm-1	*51*	*36*	*51*	*27*	*52*	*20*	*24*	*31*	*37*	*10*	*21*
1	0.21	0.1	0.08	0.06	0.08	0.13	0.13	0.02	0.3	—	—
2	0.11	0.13	0.03	—	0.14	0.05	0.08	—	—	—	—
3	0.01	0.03	—	0.07	0.04	—	0.02	—	—	—	—
4	0.65	0.57	0.86	0.59	0.61	0.73	0.71	0.73	0.58	0.7	0.55
5	—	—	—	0.04	—	—	—	—	—	—	—
6	0.02	0.11	0.02	0.2	0.08	0.1	0.06	0.26	0.1	0.3	0.45
7	0.01	0.07	0.01	0.04	0.06	—	—	—	0.03	—	—