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Paul C. Johnson
Arthur Temple College of Forestry and Agriculture, Stephen F. Austin State University

Jack E. Coster

L. Zack Florence

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Behavioral and Genetic Diversity during Dispersal: Analysis of a Polymorphic Esterase Locus in Southern Pine Beetle, *Dendroctonus frontalis*[1,2]

L. ZACK FLORENCE,[3] PAUL C. JOHNSON,[4] AND JACK E. COSTER[5]

School of Forestry, Stephen F. Austin State University, Nacogdoches, Texas 75962

ABSTRACT

Dispersal behavior of the southern pine beetle, *Dendroctonus frontalis* Zimmermann (Coleoptera: Scolytidae), in response to synthetic aggregating pheromone (frontalin) indicates an exponential decrease in response with distance from the site of emergence. Differential response during dispersal among genotypes at a polymorphic esterase locus was found by using starch gel electrophoresis. These differences produced allelic distributions that predict increased genetic diversity concurrent with increasing dispersal distance from the source population.

The southern pine beetle (SPB), *Dendroctonus frontalis* Zimmerman (Coleoptera: Scolytidae), is regarded as a major insect pest among *Pinus* spp. of the southern United States, Mexico, Guatemala, Honduras, and Arizona. Though total population numbers are known to fluctuate drastically from year to year, epidemic levels have been maintained in some forests, such as in eastern Texas, for up to 20 years (Texas Forest Service 1978).

Dispersing populations of SPB offer a unique opportunity for studying variability during active dispersal episodes. Aggregation of populations to new breeding habitats (i.e., pine host phloem) are regulated by a pheromone system composed of Frontalin (SPB produced) and α-pinene (host tree produced) (Kinzer et al. 1969).

Considerable research effort has been devoted to studying the population dynamics and economic impact of the SPB in the southern United States; only recently has attention been given to the genetic variation maintained by pine beetle populations (Anderson et al. 1979, Namkoong et al. 1979).

The principle objectives of this study were: (1) to characterize SPB dispersal behavior in response to a series of synthetic aggregation pheromone sources aligned in a distance-gradient, (2) to analyze the distribution of genetic variation within and among the responding populations, and (3) to study possible relationships which may exist between dispersal behavior and the maintenance of genetic variation in *D. frontalis*. This study represents the first attempt to simultaneously observe pine beetle behavior during active, pheromone-mediated dispersal and the genetic variation allocated among the dispersed population.

Materials and Methods

A series of six sleeve olfactometers (Gara 1967) were set up along a transect extending from an active SPB infestation near Nacogdoches, Tex. An aerial survey of the research area during the experiment confirmed that no other active SPB populations were nearby to act as alternate sources of dispersing beetles. The host habitat was a naturally regenerated stand of loblolly, *Pinus taeda* L., and shortleaf, *P. echinata* Mill., pines and mixed hardwood species. The transect was aligned in the direction of anticipated infestation growth, and each olfactometer was baited with a mixture of synthetic Frontalin and α-pinene (a 1:6 ratio) placed in two open vials in the base of the olfactometer. The combined elution rate was determined gravimetrically to be 160 mg/h. Initially, the olfactometers were at 15-m intervals, the first being in the area of SPB emergence from host trees. The interval was subsequently increased to 30 m (18 November 1977) to include a greater dispersal range with trapping extending to a maximum of 150 m from the infestation.

The olfactometers were operated from 1200 to 1700 h to coincide with the period of maximum SPB emergence (Kinn 1978), flight (Vitè et al. 1964), and response to host trees (Coster et al. 1977). Trapping was restricted to clear, warm days when flight activity was high. The dispersal experiment was conducted between December 1977 and April 1978; however, the genetic data presented here were limited to catch occuring between 15 March and 1 April 1978. Each olfactometer was cleared of SPB at ca. 30-min intervals, and the number of beetles was recorded. The beetles were placed in vials, labeled, and transported to the laboratory in a cooler and stored at −57°C (−70°F) for subsequent genetic analysis.

During the course of the experiment, any trees which came under attack by SPB, either natural or induced by proximity to an olfactometer, were cut down and removed from the study site. Several days before and after each attack were deleted from the analyses to avoid including beetles which may have responded to the attacked trees.

Random samples ($n \geq 30$ per trap) of undamaged SPB were subjected to horizontal starch gel electrophoresis. Individual beetles were ground in 0.10 ml of cold gel buffer, and the extract was absorbed onto Whatmann 3MM paper wicks. Electrophoresis was performed in a 12% starch gel (wt/vol) at ca. 5 to 6°C and 3.5 mA/cm

for 3.5 to 4.0 h (migration front at ca. 7 to 8 cm). Gel and tray buffers were prepared by the method of Namkoong et al. (1979).

Esterases were assayed by using a 0.2 M phosphate buffer pH 7.0 containing α-napthyl propionate as the substrate (Selander et al. 1971). Esterase activity in the starch gel was made visible by adding 50 mg of fast blue RR salt per 100 ml of phosphate buffer.

Nonspecific esterases were chosen for study because preliminary surveys showed them to be polymorphic and interpretable for at least one region of activity. Though the functional role of the esterase is uncertain, it is generally believed that insect esterases are involved in food digestion and fat metabolism (Cohen et al. 1977).

Trap catch, gene frequencies, genotype frequencies, and genetic diversities were entered into simple linear regression analysis to determine their relationships along the distance gradient. Gene frequencies, genotype frequencies, and genetic diversity estimates were transformed to angular arcsin $\sqrt{\%}$ before regression. Genetic diversity was measured by using a statistic proposed by Nei (1973, 1975).

Results

A total of 14 days of olfactometer catch are reported. Due to daily fluctuation in the flight activity of the beetles, proportionate catch (olfactometer catch per total catch) was calculated on a daily basis for each olfactometer. Table 1 provides both total catch per day and proportionate catch for each trapping distance along the transect. The unequal number of observations per distance results from the shift in the distance interval and from missing data for some olfactometers. Mean olfactometer catch per day declined from a high of 26.71 (29%) in the area of SPB emergence to a low of 6.63 (9%) at the most distant olfactometer (150 m).

A linear regression was fitted to the mean proportionate catch data by using a log transformation of distance, $x' = \ln(x + 1)$. The regression model shown in Fig. 1 accounted for 97% of the observed variation ($r^2 = 0.97$) in mean proportionate catch. The data plotted in Fig. 1 show the observed mean proportionate catch against an untransformed distance axis.
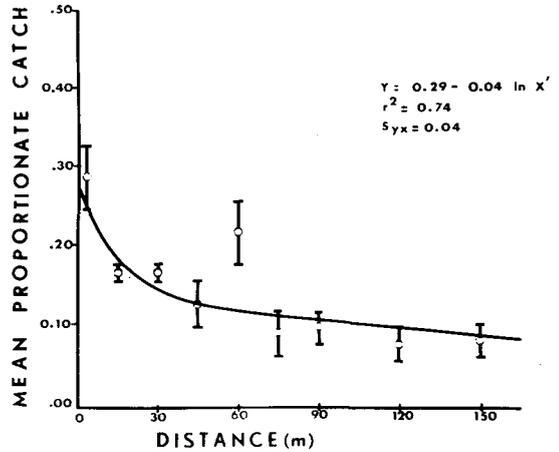

FIG. 1.—Regression of mean proportionate catch on natural log of distance. Observed mean proportionate catch and SE are included (I).
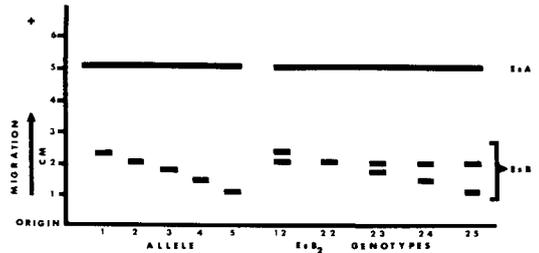

FIG. 2.—Schematic representation of the esterase gel banding patterns in the southern pine beetle. Examples of the monomeric, diploid genotypes are shown for the esB$_2$ allele only (variation at esA was observed, but not interpretable).

Two polymorphic, anodal zones of SPB esterase activity were observed, but only the slower-migrating region (designated esB) conformed to the gel phenotypes anticipated for a Mendelian, monomeric gene locus. Our interpretations are consistent with those noted by Namkoong et al. (1979).

Five allelic electromorphs were identified among the SPB sampled, designated esB_1 - esB_5, in descending order of their anodal migration distances from the origin, respectively (Fig. 2). Of the 15 electrophoretic genotypes possible, only 11 were observed. The four missing genotypes were associated with esB_1, the rarest allele at the locus having frequencies varying between 0.0 and 0.01. Therefore, the decision was made to pool esB_1 with esB_3 (the most common overall), yielding a locus with effectively four alleles and potentially four homozygous (esB_{22}, $-B_{33}$, $-B_{44}$, $-B_{55}$) and six heterozygous (esB_{23}, $-B_{24}$, $-B_{25}$, $-B_{34}$, $-B_{35}$, $-B_{45}$) genotype classes among the dispersed populations. A total of 200 individual SPB were assayed (ca. 25% of the total catch) from among the five olfactometer positions at 0, 60, 90, 120, and 150 m.[6] Table 2 shows the allele frequencies

Table 1.—Average total catch per day and proportionate catch per olfactometer per day at various distances from an infestation

Distance (m)	Avg. catch/day ($\bar{x} \pm SD$)	Proportionate catch ($\bar{x} \pm SE$)	Observations (days)
0	26.71 ± 6.13	0.29 ± 0.04	14
15	14.67 ± 4.69	0.17 ± 0.01	6
30	14.50 ± 2.44	0.17 ± 0.01	14
45	10.83 ± 2.59	0.13 ± 0.03	6
60	16.50 ± 2.71	0.22 ± 0.04	14
75	9.00 ± 3.71	0.10 ± 0.03	5
90	9.63 ± 1.79	0.11 ± 0.02	8
120	7.87 ± 2.44	0.08 ± 0.02	8
150	6.63 ± 2.10	0.09 ± 0.02	8
Overall	14.45 ± 1.46	0.17 ± 0.01	83

[6]No SPB were available for genetic analysis at the 30-m trap due to a mishap after collection and transport to the laboratory. The 150-m trap sample size was $n = 27$, ca. 40% of its total catch. All other trap samples were ≥ 30.

Table 2.—Electrophoretic allele frequencies calculated for the esB locus among the five olfactometers; also shown are estimates of diversity (h) or expected total heterozygosity for the esterase locus (note: expected heterozygosity in the next generation, assuming Hardy-Weinberg conditions)

Distance (m)	N^a	$SE_p{}^b$	B_2	$B_3{}^c$	B_4	B_5	h^d	$V(h)^d$
0	64	0.061	0.359	0.402	0.188	0.051	0.672	0.020
60	144	0.040	0.326	0.347	0.264	0.063	0.700	0.008
90	78	0.055	0.295	0.385	0.218	0.102	0.707	0.015
120	60	0.062	0.317	0.350	0.233	0.100	0.713	0.019
150	54	0.062	0.278	0.296	0.259	0.167	0.740	0.018

[a] Total gene sample.

[b] $\sqrt{\dfrac{p(1-p)}{N}}$; p = allele 3 + allele 1.

[c] Allele 3 + allele 1.

[d] See chapter 6 in Nei (1975).

calculated among the five dispersed populations. Males and females were not assayed separately, though subsequent analyses (Florence and Johnson, unpublished data) have indicated esB to be coded within one of the seven pairs of autosomes (Lanier 1981).[7] We have assumed that the emerging brood adults caught during the trapping experiment were in a ca. 1:1 sex ratio, based upon previous studies of emerging SPB populations (e.g., Osgood and Clark 1963).

Results from the regression of transformed allele frequencies against dispersal distances (Fig. 3) indicate a trend toward rapid convergence of the electromorph frequencies with distance from the source population. This trend proceeds to a frequency of ca. 0.25 (the transformed value $P' = 0.30$) in Fig. 3. Significant regressions were observed for both esB$_2$ and esB$_5$ ($P \leq 0.05$), whereas esB$_3$ was only significant at $P \leq 0.10$. The variation in frequencies explained by regressing over dispersal distance were $r^2 = 0.81$ and 0.86 for esB$_2$ and esB$_5$, respectively.

The contributions among the electrophoretic genotypes to the observed allelic convergence patterns were tested by regressing transformed genotype frequencies against distance. Figure 4 shows the regression results for three of the genotypes which strongly influenced the allelic distributions among the olfactometers in Fig. 3. Only genotype esB$_{22}$ demonstrated a highly significant relationship with distance ($0.01 \leq P \leq 0.025$), whereas esB$_{33}$ and esB$_{55}$ were marginally significant ($0.05 < P \leq 0.10$). Therefore, excepting genotypes esB$_{22}$, and possibly esB$_{33}$ and esB$_{55}$, all remaining electrophoretic genotypes were concluded to randomly disperse with distance among the five olfactometers.

The statistic, $h = 1 - \sum_{i}^{k} p_i{}^2$, where k = total alleles at a gene locus and p_i = frequency of the ith allele (Table 2), was selected as a measure of genetic diversity (Nei
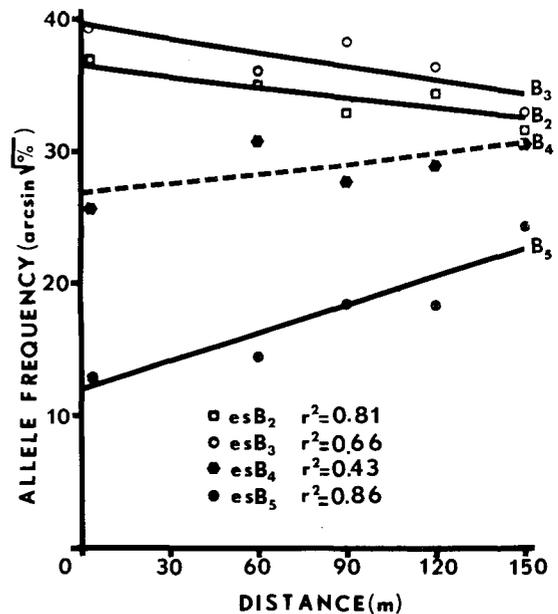

FIG. 3.—Regression of transformed esB allele frequencies (arcsin $\sqrt{\%}$) on distance dispersed among the five olfactometers. Significant ($P \leq 0.05$) regressions are indicated by solid lines.

1973, 1975). In our experiment, the values for h at each olfactometer would represent the predicted heterozygosity among the next generation's progeny. Our data are for only one gene locus representing a class of enzymes (i.e., esterases) known to exhibit above-average levels of heterozygosity (Selander 1976). Therefore, we do not consider our estimates of esterase variation to be indicative of average genetic variation in the SPB. However, the codominant gene products (electrophoretic genotypes) observed may assist in revealing underlying mechanisms in SPB populations which affect numerous gene loci.

In Fig. 5, the calculations of h are shown regressed against distance after their angular arcsin transformation ($h' = $ arcsin $\sqrt{\%}$). The increasing positive relationship

[7] Karyotypes of *D. frontalis* indicate the species to have seven pairs of autosomes and the males to be the heterogametic (XY) sex. Therefore, a sex-linked electrophoretic gene locus would negate the expression of a heterozygous phenotype in males. We have identified heterozygotes in both sexes at the esterase locus.
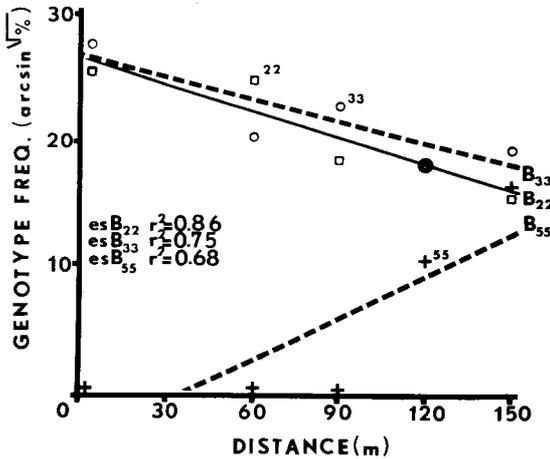
FIG. 4.—Regression of transformed esB genotype frequencies (arcsin $\sqrt{\%}$) on distance. Significant ($P \leqslant 0.05$) regressions are indicated by solid lines.
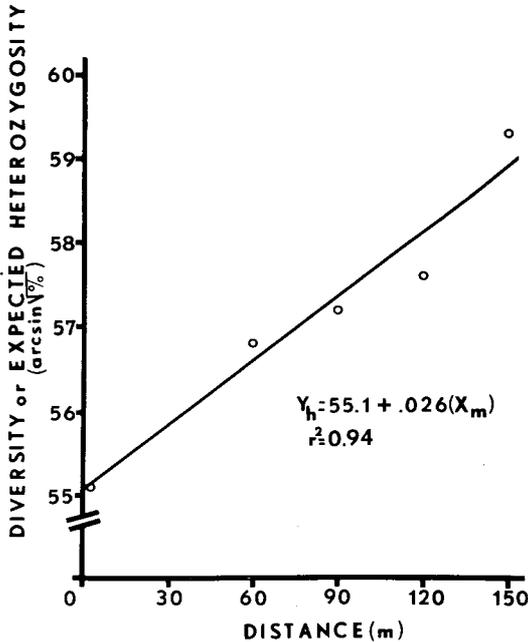

FIG. 5.—Regression of esB diversity (h) or expected total heterozygosity on distance. Expected heterozygosity is that anticipated in the next generation, given Hardy-Weinberg assumptions.

with distance is very significant, $P \leqslant 0.01$. It should be noted that the maximum h associated with a gene locus having four alleles would be 0.75 ($h' = 60$).[8] The results plotted in Fig. 5 show h to be approaching the

theoretical maximum with increasing distance in response to the convergence of the allele frequencies for the esB locus (Fig. 3). Implications are that these patterns of genetic variation resulted from the differential dispersal among the genotypes identified by this esterase locus (Fig. 4).

Discussion

Two important observations from this experiment are: (a) the esB gene locus proved to be a genetic marker of differential response among electrophoretic genotypes to an aggregating pheromone distance gradient, and (b) greater average genetic diversity is predicted with dispersal distance among increasingly smaller colonizing populations.

Patterns exhibited by the SPB esB locus may reflect the evolution of a multiallelic gene locus in response to the demands imposed by a heterogeneous pine-host habitat (Lewontin et al. 1978, Powell and Taylor 1979). Data in Fig. 3 suggest a system whereby genetic variation at a locus, and possibly other loci, could be maximized by equalizing allele frequencies via behavioral or selection intensity differences between genotypes. This system would also tend to equally distribute sampling probabilities among alleles at a locus, retard the rate of probable loss in variability due to inbreeding in smaller founding populations and provide the new populations with large amounts of genetic variation when confronted with potentially differing selection regimes. This conceptual model, although suggested by our data, has not been adequately tested in this study.

It is likely that the SPB sampled during our experiment (March and April) were generally larger, had higher than average lipid reserves, and were more disposed to active dispersal than SPB occuring at other times of the year (Hedden and Billings 1977). Whether the esB locus is truly reflective of differential selection among genotypes in their ability to store and metabolize flight energy reserves remains a topic for future investigation.

Any conclusions from our study implicating the esterases as direct behavioral modifiers in SPB are unwarranted at this time, but the codominant gene products analyzed offer possible markers for more research coupling beetle population dynamics and the apportionment of genetic variability.

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[8]Potential maximum genetic diversity at a gene locus having four alleles in Hardy-Weinberg equilibrium exists when allele frequencies are equal, e.g., $h = 1 - \sum\limits_1^4 (0.25)^2 = 0.75$.

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