Macrogeographic Genetic Variation in Populations of the Webbing Coneworm (Lepidoptera: Pyralidae)[1]

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ABSTRACT Genetic variation among 14 populations of *Dioryctria disclusa* Heinrich adults was examined using starch gel electophoresis. The average number of alleles per locus exceeded 2.0 in all populations. The number of polymorphic loci exceeded 70% in 11 populations. Genetic structure data suggest moderate differentiation (average F_{st}, 0.111) among the populations. Most of the differentiation is attributable to three of the eight loci (MDH, ME, and IDH). Nei's genetic identity ranged from 0.77-1.00 between populations. A phenogram based on genetic identity and unweighted pair-group method of analysis (UPGMA) clustered five of six populations in North Carolina closely together. With a cophenetic correlation of 0.96 the phenogram constructed is acceptable.

KEY WORDS Pine coneworm, populations, genetic similarity.

Larvae of *Dioryctria disclusa* Heinrich attack the cones of trees in the genus *Pinus* (Hedlin et al. 1981). The number of cones damaged or lost varies from year to year in natural and managed stands. Fluctuation in damage levels is a reflection of the size of the insect population. Dynamics of populations are affected by environmental factors and evolutionary forces (Nei 1987).

Assuming moderate vagility and limited migration, contact among widely separated populations of *D. disclusa* is unlikely. Restriction of gene flow among populations and random genetic drift within populations can produce genetically differentiated organisms (Hartl and Clark 1989). The genetic structure of subdivided populations can be measured by analyzing the differences in gene frequencies of organisms in patches or regions with imaginary boundaries (Nei 1977, Wright 1965).

The objective of this study was to describe the amount of genetic variation within and genetic similarity among 14 populations of *D. disclusa* in the eastern United States.

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Materials and Methods

Adult males of *D. disclusa* populations were collected in 1985 during their single annual flight period, on sticky traps baited with a synthetic pheromone. Traps were deployed at 13 locations in five eastern states (Table 1, Fig. 1). Moribund moths were counted and discarded. Only fresh and active moths were stored for analysis. Pupae collected from *P. sylvestris* cones near Lindenwood (LIN), Illinois were held for eclosion. Twenty-two of 24 moths from the LIN location were used in this study.

Stored for up to 12 months, the insects were maintained at -60C until horizontal starch gel electrophoresis was conducted. Individual insects were placed in a shallow plexiglas well; 1-2 drops of slightly modified extraction buffer II (Cheliak and Pitel 1984) were added; and the specimen was ground with a glass rod. Filter paper wicks, used to absorb the sample from the shallow well, were placed in slits in 12% starch gels.

Electrophoresis and histochemical staining followed standard techniques (Shaw and Prasad 1970, Harris and Hopkinson 1976), except as noted. Seven enzyme systems were resolved using Tris-citrate buffer, pH 6.2. The results are reported for the enzymes at eight loci: Leucine aminopeptidase (LAP-2, LAP-3), aspartate aminotransferase (AAT-1), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), glucose-3-phosphate dehydrogenase (G3PDH), esterase (EST-2), and malic enzyme (ME-1). Other enzyme systems surveyed are not reported because results were inconsistent or surveys were not run for all populations.

Analysis of single individual genotype data was performed with BIOSYS-1 computer program developed by Swofford and Selander (1981). Rare alleles with frequencies less than 0.010 (Hartl and Clark 1989) were pooled. The program generated the following output: allelic frequencies table; genetic varability as mean number of alleles per locus, percentage of polymorphic loci, and mean heterozygosity; contingency chi-square across loci; F-statistics; genetic similarity (I); and a phenogram using genetic similarity and the unweighted pair-group method of analysis (UPGMA).

Results and Discussion

Allele frequencies for the eight loci analyzed are listed in Table 2. Alleles were fixed at four loci (LAP-2, LAP-3, AAT-1, and IDH) in the small outlier population in Illinois. Alleles were fixed at the AAT-1 locus in four additional populations, at the MDH locus in three populations and at the ME-1 locus in nine populations.

The mean number of alleles per locus, percent polymorphic loci, and mean heterozygosity are presented in Table 3. Genetic variability was substantial within the populations. The average number of alleles ranged from 2.1 per locus in the isolated Illinois population to 3.5 per locus in the RAL-2 population in NC. Ten of the populations had 75-100% polymorphic loci. The average heterozygosity exceeded 0.20 in 12 of the 14 populations. Genetic variability within several populations was significantly reduced by fixation of alleles at the ME locus.

A comparison of allele frequencies at the loci among all populations indicates heterogeneity across the geographic range of *D. disclusa* (Table 4). The contingency chi-square values are highly significant at all loci.

Table 1. Number of moths caught on sticky traps at 13 locations.

States	Townships	Sites*	No. caught
NC	Murfreeboro (MUR)	Union Camp	135
	Research Triangle Park (RTP)	Triangle	242
	Raleigh (RAL-1)	Dix	311
	Raleigh (RAL-2)	Schenck	251
	Goldsboro (GOL)	Piedmont	184
	Lumberton (LUM)	Federal	31
SC	Newberry (NEW)	Champion	116
GA	Athens (ATH)	Baldwin	59
	Milledgeville (MIL)	Georgia Kraft	67
AL	Butler (BUT)	Reid	86
	Eutaw (EUT)	Weyerhaeuser	67
	Tuscaloosa (TUS)	Gulf States	57
MS	Roxie (ROX)	McNair	157

*Triangle and Dix are natural mixed pine-hardwood stands; Schenck is managed natural pine stand; and all others are managed seed orchards.

Fig. 1. Collection sites of *Dioryctria disclusa* adults used for macrogeographic genetic variation study.

Table 2. Allele frequencies in populations of *Dioryctria disclusa*.

Locus	Population 1 RAL-1 NC	2 RAL-2 NC	3 RTP NC	4 LUM NC	5 MUR NC	6 GOL NC	7 NEW SC	8 EUT AL	9 BUT AL	10 TUS AL	11 ROX MS	12 ATH GA	13 MIL GA	14 LIN IL
LAP-2														
(N)*	188	223	154	30	126	101	68	64	68	34	128	30	34	22
1	.979	.874	.756	.500	.933	.950	.868	.883	.971	.985	.973	.900	.912	1.000
2	.021	.126	.224	.500	.067	.050	.132	.117	.029	.015	.027	.100	.088	.000
3	.000	.000	.019	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
LAP-3														
(N)	188	223	153	30	126	101	68	65	68	.34	127	.30	34	22
1	.452	.112	.101	.017	.060	.104	.066	.085	.169	.147	.087	.133	.118	.000
2	.481	.780	.866	.583	.786	.743	.824	.708	.794	.735	.783	.867	.765	1.000
3	.066	.108	.033	.400	.155	.153	.110	.208	.037	.118	.130	.000	.118	.000
AAT-1														
(N)	188	223	154	30	126	102	68	65	68	34	128	30	34	22
1	.976	.984	.990	.967	.972	.931	.956	.962	.971	1.000	.973	1.000	1.000	1.000
2	.013	.016	.010	.033	.028	.054	.044	.038	.029	.000	.012	.000	.000	.000
3	.011	.000	.000	.000	.000	.015	.000	.000	.000	.000	.016	.000	.000	.000
IDH														
(N)	217	223	154	30	126	102	68	65	68	34	128	30	34	22
1	.159	.094	.032	.500	.060	.029	.015	.308	.051	.500	.059	.183	.015	.000
2	.841	.888	.955	.500	.929	.971	.985	.677	.949	.500	.930	.800	.985	1.000
3	.000	.018	.013	.000	.012	.000	.000	.015	.000	.000	.012	.017	.000	.000
4	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
MDH														
(N)	188	223	154	30	126	102	68	65	68	34	128	30	34	22
1	.000	.025	.029	.000	.107	.015	.000	.231	.074	1.000	.016	.000	.015	.000
2	.979	.975	.955	1.000	.893	.956	.985	.746	.926	.000	.973	1.000	.985	.977
3	.021	.000	.016	.000	.000	.029	.015	.023	.000	.000	.012	.000	.000	.023
4	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000

Table 2. Continued.

Locus	Population													
	1 RAL-1 NC	2 RAL-2 NC	3 RTP NC	4 LUM NC	5 MUR NC	6 GOL NC	7 NEW SC	8 EUT AL	9 BUT AL	10 TUS AL	11 ROX MS	12 ATH GA	13 MIL GA	14 LIN IL
G3PDH														
(N)*	180	221	94	28	120	68	68	31	68	27	124	30	32	22
1	.097	.016	.011	.000	.000	.015	.000	.032	.000	.000	.069	.000	.109	.091
2	.447	.670	.686	.482	.683	.757	.743	.532	.728	.704	.440	.900	.688	.864
3	.303	.145	.181	.196	.204	.059	.096	.194	.096	.111	.226	.017	.078	.045
4	.153	.152	.122	.321	.112	.169	.162	.242	.176	.185	.266	.083	.125	.000
5	.000	.018	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
EST-2														
(N)	180	219	148	26	114	97	68	57	68	30	118	27	34	22
1	.228	.267	.257	.115	.039	.062	.074	.088	.103	.067	.068	.000	.103	.250
2	.403	.288	.328	.135	.030	.057	.029	.061	.081	.067	.068	.056	.044	.136
3	.114	.116	.193	.058	.246	.160	.228	.175	.176	.167	.169	.111	.147	.091
4	.197	.256	.206	.308	.478	.670	.544	.614	.434	.483	.521	.611	.632	.159
5	.047	.073	.017	.288	.206	.052	.110	.061	.206	.217	.174	.167	.044	.341
6	.011	.000	.000	.096	.000	.000	.015	.000	.000	.000	.000	.056	.029	.023
ME-1														
(N)	127	223	154	30	126	.102	68	65	68	34	128	30	34	22
1	.988	1.000	1.000	1.000	1.000	1.000	.985	1.000	.971	1.000	1.000	1.000	.956	.523
2	.012	.000	.000	.000	.000	.000	.015	.000	.022	.000	.000	.000	.044	.477
3	.000	.000	.000	.000	.000	`.000	.000	.000	.007	.000	.000	.000	.000	.000

* (N) Refers to the number of individuals analyzed at each locus for each population.

Table 3. Genetic variability at gene loci in populations of *Dioryctria disclusa* (standard errors in parentheses).

Population	Mean sample size per locus	Mean no. of alleles per locus	Percentage of loci polymorphic*	Mean heterozygosity: Hardy-Weinberg expected
1. RAL-1-NC	182.0	3.0	100.0	0.299
	(8.9)	(0.5)		(0.110)
2. RAL-2-NC	222.3	2.9	100.0	0.268
	(0.5)	(0.5)		(0.094)
3. RTP-NC	145.6	3.0	100.0	0.256
	(7.4)	(0.4)		(0.093)
4. LUM-NC	29.3	2.5	75.0	0.378
	(0.5)	(0.6)		(0.110)
5. MUR-NC	123.8	2.6	87.5	0.252
	(1.6)	(0.4)		(0.082)
6. GOL-NC	96.9	2.9	100.0	0.212
	(4.2)	(0.4)		(0.070)
7. NEW-SC	68.0	2.8	100.0	0.221
	(0.0)	(0.5)		(0.079)
8 EUT-AL	59.6	2.9	87.5	0.349
	(4.2)	(0.4)		(0.082)
9 BUT-AL	68.0	2.6	100.0	0.239
	(0.0)	(0.4)		(0.086)
10 TUS-AL	32.6	2.3	62.5	0.266
	(0.9)	(0.5)		(0.102)
11. ROX-MS	126.1	3.0	100.0	0.250
	(1.3)	(0.4)		(0.100)
12. ATH-GA	29.6	2.3	62.5	0.191
	(0.4)	(0.5)		(0.072)
13. MIL-GA	33.8	2.8	87.5	0.222
	(0.3)	(0.6)		(0.082)
14 LIN-IL	22.0	2.1	50.0	0.199
	(0.0)	(0.6)		(0.106)

* A locus is polymorphic at the 0.99 criterion level.

The genetic structure of the populations is summarized by F-statistics in Table 5. The average F_{st} is 0.111 which indicates moderate genetic differentiation among the populations (Hartl 1980). The F_{st} value suggests that a high proportion (85.3%) of differences in allelic frequencies among the populations is due to variation among individuals within populations. The average F_{is} is 0.253 which indicates substantial variation within individuals in the populations. Although the F_{is} values are negative for three loci, random mating is assumed within the populations.

Genetic similarities and distances between populations are shown in Table 6. Using genetic similarity and UPGMA, a phenogram (Fig. 2) was constructed. Goodness-of-fit statistics (cophenetic correlation, 0.96; percent standard deviation,

Table 4. Contingency chi-square analysis for testing homogeneity across populations.

Locus	No. of alleles	Chi-square	df	*P*
LAP-2	3	283.138	26	.00000
LAP-3	3	449.008	26	.00000
AAT-1	3	51.393	26	.00214
IDH	3	363.208	26	.00000
MDH	3	1171.174	26	.00000
G3PDH	5	313.158	52	.00000
EST-2	6	859.616	65	.00000
ME-1	2	735.740	13	.00000
Totals		4226.335	260	.00000

Table 5. F-statistics of loci examined for population genetic structure among 14 populations of *Dioryctria disclusa*.

Locus	F_{is}	F_{it}	F_{st}
LAP-2	-0.061	0.108	0.160
LAP-3	0.477	0.523	0.089
AAT-1	0.785	0.788	0.016
IDH	-0.169	0.084	0.216
MDH	0.520	0.564	0.091
G3PDH	0.488	0.521	0.064
EST-2	0.159	0.231	0.086
ME-1	-0.445	0.071	0.357
Total	0.253	0.336	0.111

Table 6. Matrix of genetic similarity and distance of populations of *Dioryctria disclusa*.

	Population													
Population	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 RAL-1-NC	***	.028	.041	.115	.058	.064	.065	.066	.045	.266	.045	.075	.056	.116
2 RAL-2-NC	.972	***	.004	.079	.020	.025	.018	.037	.013	.228	.022	.027	.019	.059
3 RTP-NC	.960	.996	***	.090	.028	.036	.023	.053	.022	.248	.035	.037	.028	.064
4 LUM-NC	.891	.924	.913	***	.092	.108	.094	.065	.103	.276	.090	.091	.102	.189
5 MUR-NC	.944	.981	.972	.912	***	.008	.004	.018	.004	.182	.009	.014	.007	.066
6 GOL-NC	.938	.976	.965	.898	.992	***	.002	.023	.007	.217	.014	.010	.000	.078
7 NEW-SC	.937	.983	.978	.910	.996	.998	***	.027	.004	.228	.012	.009	.001	.063
8 EUT-AL	.936	.964	.949	.937	.982	.977	.973	***	.026	.120	.020	.028	.024	.121
9 BUT-AL	.956	.988	.978	.902	.996	.993	.996	.974	***	.193	.010	.010	.006	.052
10 TUS-AL	.766	.796	.781	.759	.834	.805	.796	.887	.825	***	.222	.201	.225	.299
11 ROX-MS	.956	.978	.966	.914	.991	.986	.988	.980	.990	.801	***	.028	.010	.081
12 ATH-GA	.928	.973	.963	.913	.986	.990	.991	.973	.990	.818	.973	***	.010	.068
13 MIL-GA	.946	.981	.972	.903	.993	1.000	.999	.977	.994	.799	.990	.990	***	.066
14 LIN-IL	.890	.943	.938	.828	.936	.925	.939	.886	.950	.741	.922	.934	.936	***

Below diagonal: Nei (1978) unbiased genetic identity.
Above diagonal: Nei (1978) unbiased genetic distance.

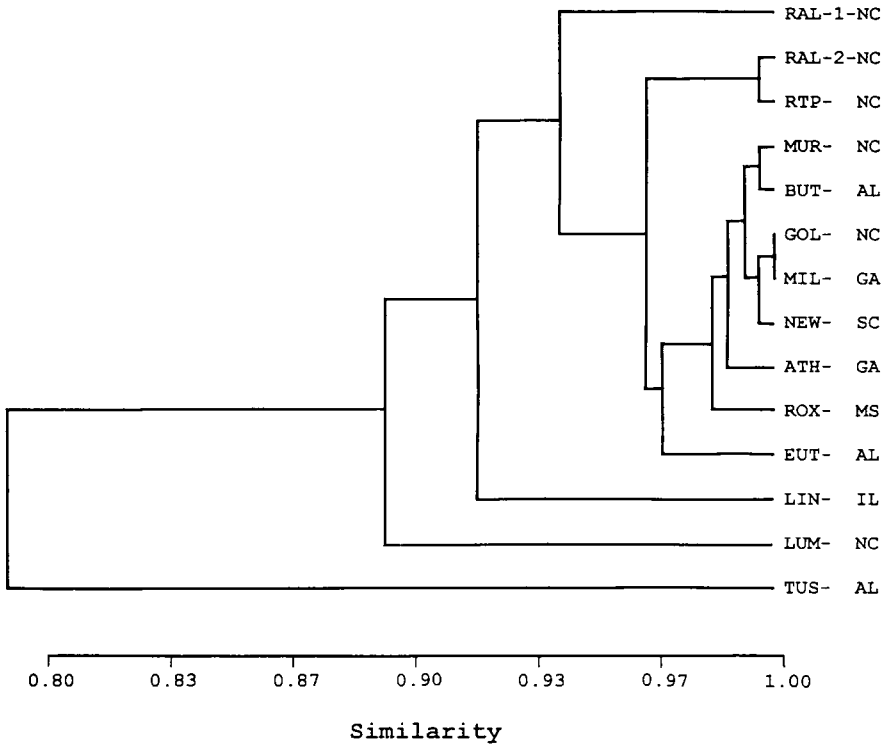

Fig. 2. Phenogram of 14 *Dioryctria disclusa* populations constructed using Nei's (1978) genetic similarity estimates and unweighted pair-group method with arithmetic averaging. (Cophenetic correlation = 0.958).

2.22) determined that this measure produced the best tree. A clustering level value > 0.95 was found between five of the six North Carolina (NC) populations; the LUM population was the exception. The clustering level value was highest between RAL-2-RTP, MUR-GOL, and RAL-1-RAL-2. Although the NC populations were more often closely clustered, both the greatest similarity (I = 1.0) between GOL-MIL and least similarity (I = 0.766) between RAL-1-TUS occurred among widely separated populations.

The genetic similarity among the three NC populations in the same area (RAL-1, RTP, RAL-2) suggests that gene flow may be occurring among them. Only a few migrants per generation are needed to maintain genetic variability within and genetic similarity among populations. Indeed, among subpopulations, migration is a potent force acting against genetic divergence that results from random genetic drift (Hartl and Clark 1989).

Geographically separated populations are exposed to different environments to which individuals adapt. This adaptation promotes change in the allele frequencies through natural selection and random genetic drift (Hartl and Clark

1989). There was no pattern of a geographic cline in allele frequencies across the north-south range of *D. disclusa* from North Carolina to Mississippi. There was no evidence of the Wahlund effect (Hartl and Clark 1989) which would reduce homozygosity in the relatively close populations (RAL-1, RTP, RAL-2) in NC.

Conclusions derived from the data are: (1) genetic variation is low in the population from Illinois due to a founder effect or bottleneck or sampling technique; (2) gene flow maintains genetic variation within and genetic similarity among the North Carolina populations; and (3) random mating occurs in the populations. Conceivably, the population genetic structure of *D. disclusa* is diverse because pine seed orchards are intensively managed to protect cones by using insecticides and increase tree vigor by applying fertilizers, and because natural stands are fragmented by urbanization.

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References Cited

Cheliak, W. M., and J. A. Pitel. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Can. For. Serv. Info. Report,PI-X-42. 49 pp.

Harris, H., and D. A. Hopkinson. 1976. Handbook of enzyme electrophoresis in human genetics. North-Holland Publ. Amsterdam. 473 pp.

Hartl, D. L. 1980. Principles of populations genetics. Sinauer Assoc., Inc., Sunderland, MA. 488 pp.

Hartl, D. L., and A. G. Clark. 1989. Principles of population genetics. Sinauer Assoc., Inc., Sunderland, MA. 682 pp.

Hedlin, A. F., H. O. Yates III, D. Cibrian-Tovar, B. H. Ebel, and E. P. Merkel. 1981. Cone and seed insects of North America conifers. Can. For. Serv., USDA For. Serv., and Secr. Agric. Recur., Hidraul, Mexico. 122 pp.

Nei, M. 1977. F-statistics and analysis of gene diversity in subdivided populations. Ann. Human Genet. 31: 225-233.

1987. Molecular evolutionary genetics. Columbia Univ. Press. NY. 512 pp.

Shaw, C. R., and R. Prasad. 1970. Starch gel electrophoresis of enzymes: A compilation of recipes. Biochem. Genet. 4: 297-320.

Swofford, D. L., and R. B. Selander. 1981. BIOSYS-1: A FORTRAN program for comprehensive analysis of electophoresis data in population genetics and systematics. J. Hered. 72: 281-283.

Wright, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19: 395-420.