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Abstract

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Comments

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Genetic Heterozygosity and Gene Flow in *Coleomegilla maculata* De Geer (Coleoptera: Coccinellidae)

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Coleomegilla maculata, an abundant, widely distributed, North American polyphagous beetle, is a predator of soft-bodied insects including aphids. Gene diversity and breeding structure of 12 C. maculata populations was investigated by using polyacrylamide gel electrophoresis. Thirty-five of 44 putative loci were polymorphic (79.5%). Genic heterozygosity was estimated at 28 polymorphic loci. Average heterozygosity among the 37 scorable loci was 18.3 ± 3.5% and was 24.2 ± 5.0% among only polymorphic loci. The average number of alleles was 4.00 ± 3.01 and the effective number of alleles was 1.61 ± 1.65 among the 37 loci. No null alleles were recognized. Forty-five putative alleles segregating at 7 presumptive loci were used to study breeding structure. A significant deficiency of heterozygotes was detected within populations; this deficiency was a result our failure to identify heterozygotes for alleles of similar mobilities. No significant differentiation was found among populations within fields, among populations within states, or among states. Gene frequencies did not significantly differ between beetles sampled in autumn nor among their progeny the next spring. There were no significant departures from random mating among populations. © 1995 Academic Press, Inc.

KEY WORDS: **Coleomegilla maculata; isozyme variation; breeding structure; gene flow.**

INTRODUCTION

Many coccinellid beetles are aposematically colored and demonstrate Müllerian mimicry which is believed to be related to their production of unpalatable alkaloids and histamine (reviewed by Brakefield, 1985). *Coleomegilla maculata* (De Geer) is an aposematically colored, abundant beetle that feeds on pollen, fungi, insect eggs, and several aphid species (Groden *et al.*, 1990; Hodek, 1973; Hagen, 1962). This beetle is indigenous to and widely distributed in the Nearctic (Obrycki and Tauber, 1978), where three *C. maculata* subspecies are recognized (Gordon 1985). No breeding studies, however, have been conducted to substantiate these subspecific designations.

The possibility exists that postpleistocene radiation may have led to local adaptation of *C. maculata* or possibly to speciation. If so, then a measure of reproductive isolation would occur between populations, and the degree of isolation would be reflected by differing gene frequencies at selectively neutral loci. Furthermore, the prey of *C. maculata* are patchily distributed both temporally and spatially. Local selective pressures may have led to a measure of genetic differentiation between beetle populations in different prey habitats. If so, there would be a degree of nonrandom mating among populations. For genetic loci that are selectively neutral, genetic drift would insure that different populations had different gene frequencies. Relative magnitudes of selection and gene flow among populations will govern their degree of genetic differentiation.

A good measure of reproductive isolation is provided by Wright's F_{st} statistic, defined as the correlation of random gametes within populations (Wright, 1951). F_{st} also is defined as "the ratio of the actual variance in allele frequencies among subpopulations to the maximum possible variance under complete isolation of subpopulations" (Wright, 1965). Steiner and Grasela (1993) found extraordinary departures from random mating both within and among 15 *C. maculata* populations sampled in Missouri, Illinois, Arkansas, Oklahoma, and Texas. Moreover, they claimed a longitudinal cline in which heterozygosity increased southerly. Coll *et al.* (1994), on the other hand, found no significant departures from random mating among 6 Maryland *C. maculata* populations sampled in a altitudinal transect extending from mountainous Washington Co. to Anne Arundal Co. on the Chesapeake Bay.

In this paper, we present data on gene diversity and its spatial components in *C. maculata*. We sampled 12 beetle populations from Delaware and Iowa to estimate gene flow between closely and distantly located populations. We show that there is a great deal of genetic heterozygosity in *C. maculata* and that this diversity is shared

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TABLE 1

Genetic Diversity at Electrophoretic Loci in *Coleomegilla maculata*

Enzyme	Enzyme commission	Symbol	Buffer system	No. loci	No. polymorphic
Acid phosphatase	EC 3.1.3.2	*Acph*	NAM	1	1
Aconotate hydratase	EC 4.2.1.3	*Aco*	OD	1	0
Adenylate kinase	EC 2.7.4.3	*Adk*	NAM	2	2
Aldehyde oxidase	EC 2.6.1.1	*Aox*	NAM	2	2
Alkaline phosphatase	EC 3.1.3.1	*Aph*	NAM	1	0
Arginine kinase	EC 2.7.3.3	*Argk*	NAM	1	0
Aspartate aminotransferase	EC 2.6.1.1	*Aat*	NAM	1	1
Diaphorase	EC 1.6.2.2	*Dia*	TBE	2	1
Enolase	EC 4.2.1.11	*Eno*	NAM, TBE	1	0
Esterase	EC 3.1.1.—	*Est*	NAM	4	4
Fructose biphosphatase	EC 3.1.3.11	*Fbp*	NAM	1	1
Fumarate hydratase	EC 4.2.1.2	*Fum*	NAM	1	1
α-Glycerophosphate dehydrogenase	EC 1.1.1.8	*α-Gpd*	NAM	1	1
Glycogen phosphorylase	EC 2.4.1.1	*Phos*	TBE	1	1
Glucose oxidase	EC 1.1.3.4	*Go*	NAM	1	1
Glucose-6-phosphate dehydrogenase	EC 1.1.1.49	*G6pdh*	NAM	1	1
Hexokinase	EC 2.7.1.1	*Hk*	NAM	1	1
Hydroxy acid dehydrogenase	EC 1.1.1.30	*Had*	NAM, MOPS	2	2
Isocitrate dehydrogenase	EC 1.1.1.42	*Idh*	NAM	2	2
Leucine aminopeptidase	EC 3.4.1.1	*Lap*	NAM, TBE	1	1
Malate dehydrogenase	EC 1.1.1.37	*Mdh*	NAM, MOPS	3	3
NADP-dependent MDH	EC 1.1.1.40	*Me*	OD	1	1
Mannose-6-P-dehydrogenase	EC 5.3.1.8	*Mpi*	NAM	1	1
Peptidase[a]	EC 3.4.—	*Pep*	—	1	1
Phosphoglucoisomerase	EC 5.3.1.9	*Pgi*	OD, NAM	1	1
Phosphoglucomutase	EC 5.4.2.2	*Pgm*	NAM	1	1
6-Phosphogluconate dehydrogenase	EC 1.1.1.44	*6pgdh*	NAM	1	1
Sorbitol dehydrogenase	EC 1.1.1.14	*Sdh*	NAM	1	0
Superoxide dismutase	EC 1.15.11	*Sod*	NAM, TBE	3	1
Trehalase	EC 3.2.1.28	*Tre*	NAM	1	1
Triose phosphate isomerase	EC 5.3.1.1	*Tpi*	NAM	2	1
Xanthine dehydrogenase	EC 1.1.1.37	*Xdh*	OD	1	0

[a] Coll *et al.* (1994).

among all of the sampled populations. Moreover, we show that mating is essentially random within and between populations.

METHODS

Biological Material

Beetles were field collected by using 35-cm sweep nets, chilled, and shipped to Ames, Iowa, where they were killed by freezing at −75°C. The beetles were homogenized in 1.5-ml microfuge tubes each containing 150 μl of grinding buffer. Beetle collections were made in 1991 and 1992 from three locations near Ames, Iowa; from Ankeny, Knoxville, Chariton, Napier, and Muscatine, Iowa; and from Kent Co., Delaware. Beetles were thus sampled from nine locations, but from three locations in successive years to provide a total of 12 populations.

To examine the breeding structure of local populations, discrete aggregations of adults were sampled separately in a single, large red clover field in Smyrna, Kent Co., Delaware. In Knoxville, Iowa, a single alfalfa field was arbitrarily divided into quarters, and separate collections were made by sweep nets from each subsection.

To examine the possibility that genetic selection operated on overwintered beetles, collections were made of overwintering adults in late autumn and of adults in spring from fields adjacent to the overwintering sites.

Electrophoresis

Beetles were ground individually in a pH 8.6 grinding buffer (Black and Krafsur, 1985a). Vertical slab acrylamide gels consisted of 6.18% acrylamide plus 0.325% bis-acrylamide, 0.05% ammonium persulfate, and 0.15% TEMED in gel buffer. Resolution of phosphoglucoisomerase (*Pgi*) was maximized by using 8.5% gels. We adopted 5% gels in efforts to resolve aldehyde oxidase (*Aox*), leucine aminopeptidase (*Lap*), glucose-6-phosphate dehydrogenase (*G6pdh*), and fumarase (*Fum*).

TABLE 2

Genetic Statistics Estimated from Alleles at 37 Putative Genetic Loci in *C. maculata*

Locus	N	Number alleles	Subunit structure	Genetic statistics			
				n_e[a]	h_0[b]	h_e[c]	F[d]
Adk-1	461	7	Monomer	1.05	0.039	0.045	0.13
Adk+2	32	2	Monomer	1.03	0.31	0.031	−0.02
Aat	56	3	Dimer	1.11	0.107	0.102	−0.05
Dia-2	113	6	Dimer	1.09	0.088	0.086	0.02
Est-4	236	15	Monomer	10.61	0.780	0.906	0.14
Fbp	56	5	Dimer	1.40	0.250	0.286	0.12
Fum	40	2	Dimer	1.05	0.050	0.049	−0.02
α-Gpdh	56	3	Dimer	1.04	0.035	0.03	−0.03
Go	36	6	Tetramer	2.57	0.667	0.611	−0.09
6pgdh	491	8	Dimer	1.13	0.098	0.117	0.16
Had-1	55	3	Dimer	1.08	0.073	0.071	−0.03
Had+2	56	5	Dimer	1.14	0.125	0.120	−0.04
Hk	56	2	Monomer	1.05	0.054	0.052	−0.04
Idh-1	498	6	Dimer	1.13	0.104	0.118	0.12
Idh-2	498	5	Dimer	1.09	0.084	0.083	−0.01
Mdh-1	56	3	Dimer	1.09	0.089	0.086	−0.03
Mdh+2	256	2	Dimer	1.17	0.154	0.142	−0.08
Mdh+3	56	7	Dimer	1.12	0.107	0.104	−0.03
Me	490	4	Tetramer	1.03	0.024	0.029	0.17
Mpi	467	6	Monomer	1.27	0.210	0.214	0.02
Pep[e]	123	8	—	2.52	—	0.604	—
Pgi	143	7	Dimer	3.68	0.532	0.728	0.27
Pgm	474	8	Monomer	2.63	0.511	0.619	0.17
Phos	56	2	Dimer	1.02	0.018	0.018	0
Sod-1	56	4	Dimer	1.48	0.250	0.325	0.23
Tpi-2	56	3	Monomer	2.14	0.500	0.532	0.06
Tre	43	5	Monomer	2.80	0.410	0.642	0.35
			Polymorphic loci (n = 28)				
Means	4.96			1.806	2.003	0.2419	0.058
SD	2.85			1.865		0.0499	0.009
			All loci (n = 37)				
Means	4.00			1.610	0.1502	0.1831	
SD	3.01			1.653		0.0352	

[a] n_e is the efective number of alleles, $1/\sum p_i^2$.
[b] h_0 is the observed heterozygosity.
[c] h_e is the heterozygosity expected on Hardy–Weinberg criteria, $1 - \sum p_i^2$.
[d] $F = 1 - (h_0/h_e)$, measuring overall departures from random mating.
[e] Estimated from Coll *et al.* (1994) Appendix A.

The buffer systems included the Ornstein–Davis system (Hames, 1981) (without the stacking gel or riboflavin), Tris–borate–EDTA, pH 9 (TBE) (Munstermann, 1985), and NAM–citrate, pH 6.5 (Clayton and Tretiak, 1972). Composition of the TBE electrode and gel buffer was 81 m*M* Tris, 20 m*M* borate, and 1.5 m*M* disodium EDTA. The NAM–citrate gel buffer was 2.7 m*M* citrate, and the electrode buffer was 5.35 m*M* citrate, both adjusted to pH 6.5 with *N*-(3-aminopropyl)morpholine. The MOPS system of Thomas and Hodes (1981) was used to resolve cationic proteins; we did not use the stacking gel.

Electrophoresis was performed in Hoefer SE600 gel boxes at 0–4°C. Only 1.2–2.5 μl of sample homogenate was applied to each well, and homogenates from 28 beetles were run on each gel. NAM gels were run at 30 mA per gel for 4 h and TBE and OD gels at 350 V for 2.5 h. MOPS gels were run at 30 mA per gel for 6 h.

Staining methods generally followed those of Murphy *et al.*, 1990). Agar overlays were used to resolve coupled reactions [e.g., adenylate kinase (*Ak*), glycogen phosphorylase (*Phos*), hexokinase (*Hk*), phosphoglucomutase (Pgm), trehalase (Tre)], as well as diaphorase (*Dia*) and isocitrate dehydrogenase (*Idh*).

Analysis of Data

Nei's (1987) prescriptions were used to compute gene diversities and variances. Gene diversity at a locus was measured by the statistic $h_e = 1 - \sum p_i^2$,, where p_i is each putative allele at the locus. Gene diversity for n loci is $H_E = \sum(h_e)/n$, with variance $= \sum(h_e\text{-}H_E)^2/[n(n\text{-}1)]$. H_E and H_e are the expected heterozygosities when mating is random and other Hardy–Weinberg assumptions apply. The effective number of alleles, n_e is $1/\sum p_i^2$ (Nei, 1987); it is equal to the observed number of alleles only when their frequencies are equal which would be the case if an ideal Hardy–Weinberg population were at equilibrium. Gene frequency data were analyzed by using BIOSYS-1 (Swofford and Selander, 1981) and GENESTATS (Black and Krafsur, 1985b). The procedures of Weir and Cockerham (1984) were used to calculate F -statistics because their methods weight for variable sample sizes, number of alleles, and populations and provide standard errors. The F statistics were tested for significance by using the χ^2 tests of Li and Horvitz (1953) and Workman and Niswander (1970). Wright's (1978) methods were used to partition variance in gene frequencies into two components, within and among beetle collections. χ^2 tests of homogeneity of gene frequencies were done by using the methods of Workman and Niswander (1970).

RESULTS

Of 37 presumptive loci for which adequate resolution was obtained, 28 (75.7%) were polymorphic (Table 1). We found substantial levels of heterozygosity in *C. maculata:* mean heterozygosity was 0.242 ± 0.050 among the polymorphic loci (Table 2) and 0.183 ± 0.035 among all loci at which gene frequencies were estimated. The distribution of single-locus heterozygosities (Fig. 1) is skewed to the right and roughly conforms to an infinite alleles model (Kimura, 1991).

There were some loci that demonstrated polymorphic banding patterns the heterozygotes of which did not show clear subunit structures and therefore could not be scored unambiguously; these loci included *Aph*, *G6pdh*, and *Lap*.

Many of the polymorphic loci were difficult to score consistently from gel to gel because of technical problems; e.g., *Aox* alleles were too close to identify clearly some heterozygotes; *Dia* activity varied from gel to gel; *Est* simply had too many well-resolved alleles to allow consistently objective scoring; *Pgi* was an extremely active locus and became overstained before all lanes could be scored; *Mdh* was frequently smeared and obscured by *Sod* activity; and the subunit structure of *G6pdh* "heterozygotes" could not be clearly resolved. *G6pd* is dimeric in other species (Murphy *et al.*, 1990). The foregoing loci therefore were not used in studies of gene flow among populations.

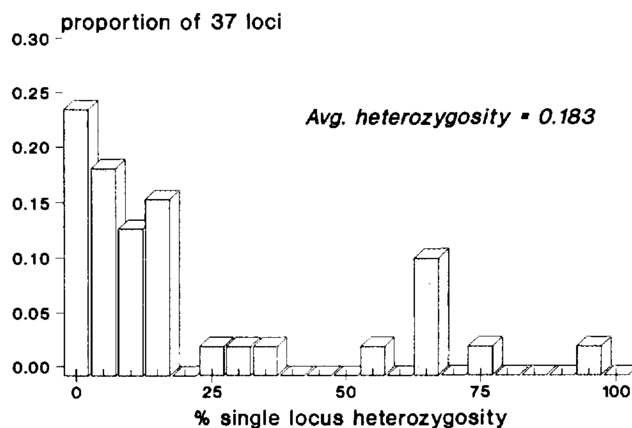

FIG. 1. Histogram of single-locus heterozygosities in *C. maculata.*

Gene diversity pooled from all samples, in the form of allelic frequencies, is provided in Table 3. How was this gene diversity partitioned among the 12 populations? Contingency χ^2 analysis showed that gene frequencies were heterogeneous among populations at *Mpi* and *Pgm* (Table 4). Wright's F statistics suggested statistically significant deficiencies of heterozygotes at *Mpi, 6pgdh,* and *Pgm* (Table 5). Furthermore, most of the foregoing departures from random mating were detected within populations (F_{is}) but not between populations (F_{st}). Heterozygosities at the remaining loci did not differ significantly from Hardy–Weinberg expectations, so mating was random at these loci.

F_{st} is the correlation of gametes between individuals within populations relative to gametes in the overall population and is a measure of reproductive isolation among populations. In *C. maculata*, F_{st} statistically differed from zero at *Mpi* and *Pgm* (Table 5). The jackknife means and SD suggest that the three mean F estimates differed significantly from zero (Table 5).

Nonrandom mating among individuals within populations is measured by F_{is}; this is a correlation of gametes within individuals relative to a correlation of gametes for their own population (Wright, 1951). Large values of F_{is} were observed at *Mpi, 6pgdh,* and *Pgm.* These statistics could be explained by selection against certain heterozygotes, positive assortative mating, null alleles, or technical problems in scoring gels.

The positive F_{is} estimates could also have been caused by sampling among subpopulations that differed in their gene frequencies, demonstrating the "Wahlund effect" (Hartl and Clark, 1989). This hypothesis was tested by recording the gene frequencies of six beetle clusters from a single field in Delaware. An additional test of the Wahlund principle was made by comparing gene frequencies among three subpopulations in a field in Knoxville, Iowa. χ^2 tests showed that gene frequencies were homogeneous among clusters in both fields (Table 6) and that

TABLE 3

Enzyme Polymorphisms Scored for Gene Flow in *C. maculata*

Locus	N	Subunit structure	Allele	Rd[a]	Freq.
Adk	704	Monomer	A	0.79	0.0053
			B	0.84	0.0060
			C	0.92	0.0022
			D	1.00	0.9772
			E	1.09	0.0062
			F	1.14	0.0023
			G	1.21	0.0008
Idh-1	701	Dimer	A	0.64	0.0036
			B	0.71	0.0254
			C	1.00	0.9399
			D	1.25	0.0123
			E	1.40	0.0142
			F	1.60	0.0047
Idh-2	734	Dimer	A	0.64	0.0033
			B	0.70	0.0105
			C	1.00	0.9558
			D	1.22	0.0246
			E	1.45	0.0059
Me	718	Tetramer	A	0.74	0.0025
			B	0.80	0.0109
			C	1.00	0.9849
			D	1.14	0.0016
Mpi	678	Monomer	A	0.74	0.0033
			B	0.82	0.0063
			C	0.90	0.0515
			D	1.00	0.8808
			E	1.07	0.0291
			F	1.18	0.0290
6pgdh	709	Dimer	A	0.67	0.0007
			B	0.74	0.0048
			C	0.86	0.0301
			D	1.00	0.9353
			E	1.20	0.0112
			F	1.26	0.0105
			G	1.34	0.0051
			H	1.43	0.0024
Pgm	684	Monomer	A	0.44	0.0003
			B	0.67	0.0080
			C	0.76	0.0318
			D	0.84	0.0570
			E	1.00	0.4838
			F	1.12	0.3639
			G	1.22	0.0237
			H	1.29	0.0233
			I	1.38	0.0022

[a] Distance migrated relative to the most common allele.

the overall deficiency of heterozygotes persisted even in subpopulations as shown by the jackknife estimates (Table 7); none of the individual F_{st} and F_{it} estimates differed significantly from zero, however. Heterozygotes of alleles *B, C,* and *D* at *Idh, C* and *D* at *Mpi, C* and *D* at *6pgdh,* and *D* and *E* at the *Pgm* locus were scored less frequently (Table 8) than expected because these alleles were too close together (see Table 3 for relative distances) to distinguish heterozygotes unambiguously.

TABLE 4

Contingency χ^2 Analysis of Loci in 12 *C. maculata* Populations

Locus	N	No. alleles	χ^2	df	P
Adk	694	7	81.56	66	0.094
Idh-1	739	6	42.41	55	0.893
Idh-2	734	5	57.75	44	0.080
Me	718	4	20.24	33	0.960
Mpi	678	6	155.53	55	<0.001
6pgdh	709	8	96.88	77	0.062
Pgm	684	9	211.67	88	<0.001
Totals		45	666.05	418	<0.001

Thus, the excess of homozygotes in populations was most probably the result of technical difficulties in scoring gels, a common, if often unacknowleged, problem. A nested analysis of variance supports the contention: variance among clusters nested in fields was 0.0063, accounting for all of the variance. Thus, the residual variance between fields in Iowa and Delaware, approximately 2000 km apart, was essentially zero.

C. maculata, like many coccinellid species, overwinter as adults in aggregations (Hagen, 1962). We compared gene frequencies among beetles collected in early autumn from overwintering sites with the first generation beetles sampled in adjacent fields during the next June. There were three independent comparisons (Table 9) and the overall test of homogeneity for the six samples was, $\chi^2_{[180]} = 245.2$, $P = 0.0001$. Only in Knoxville was

TABLE 5

F Statistics for 12 Populations of *C. maculata*

Locus	N	F_{is}[a]	F_{st}[b]	F_{it}[c]
Adk	694	0.0204	0.0047	0.0250
Idh-1	739	−0.0139	−0.0010	−0.0149
Idh-2	734	0.0374	0.0056	0.0427
Me	718	−0.0089	−0.0020	−0.0109
Mpi	678	0.0707	0.0128*	0.0826
6pgdh	709	0.1077	0.0007	0.1083[d]
Pgm	684	0.1425	0.0149*	0.1553**
Mean		0.0966	0.0103	0.1059
Jackknife estimates over loci				
Mean		0.1138	0.0121	0.1248
SD		0.0433	0.0045	0.0469

[a] Mean reduction in heterozygosity of individuals in subpopulations.
[b] Departures from random mating among subpopulations.
[c] Departures from random mating from all causes.
[d] $\chi^2[56] = 58.21$, $P = 0.39$.
* $P = 0.004$.
** $P < 0.001$.

TABLE 6

Contingency χ^2 Analysis of Allele Frequencies in *C. maculata* Subpopulations

	Delaware[a]				Knoxville[b]				Total[c]			
Locus	No. alleles	χ^2	*df*	*P*	No. alleles	χ^2	*df*	*P*	No. alleles	χ^2	*df*	*P*
Adk	2	8.50	5	0.13	4	6.19	6	0.40	4	22.28	24	0.56
Idh-1	6	21.02	25	0.69	6	7.61	10	0.67	6	30.98	40	0.85
Idh-2	4	11.07	15	0.75	5	16.51	8	0.04	5	36.24	32	0.28
Me	4	20.10	15	0.17	4	4.03	6	0.40	4	31.66	24	0.14
Mpi	6	26.62	25	0.38	5	5.87	8	0.66	6	44.92	40	0.27
6pgdh	7	19.35	30	0.93	6	12.44	10	0.26	7	45.98	48	0.56
Pgm	8	44.45	35	0.13	8	19.76	14	0.14	8	78.64	56	0.02
Totals		151.11	150	0.46		72.41	60	0.13		290.60	264	0.12

[a] $N \approx 142$ per locus.
[b] $N \approx 130$ per locus.
[c] $N \approx 272$ per locus.

there a significant difference, however, and this was caused by *Pgm*. The six samples were homogeneous when *Pgm* was removed from consideration ($\chi^2_{[145]}$ = 149.0, $P = 0.392$. Analysis of variance showed that all of the variance was attributable to samples nested in fields ($s^2 = 0.01337$), and the variance between fields was essentially zero. When *Pgm* was dropped from consideration, the between-generation (i.e., within field) variance became 0.00254. These statistics indicate that the change in gene frequencies from one generation to the next, although small, was greater than the variation from field to field. Jackknife estimates over the seven loci (Weir and Cockerham 1984) indicate that departures from random mating in fields were significant ($F_{is} = 0.119 \pm 0.037$) but not so among fields ($F_{st} = 0.0167 \pm 0.013$). Most of the variance to F was contributed by *Pgm*, and when this locus was dropped F_{is} became 0.070 $\pm$ 0.025 and F_{st} became -0.002 ± 0.003. A Darwinian advantage for a particular *Pgm* genotype would seem unlikely because only in one of three populations did the allele frequencies show significant change from one generation to the next.

TABLE 7

F Statistics for *C. maculata* Subpopulations in Delaware and Knoxville

Locus	*n*	F_{is}[a,*]	F_{st}[b,*]	F_{it}[c,*]
Adk	272	−0.0036	−0.0026	−0.0062
Idh-1	267	−0.0186	−0.0029	−0.0216
Idh-2	263	0.1344	0.0032	0.1371
Me	270	−0.0191	0.0043	−0.0147
Mpi	260	0.1147	−0.0029	0.1122
6pgdh	274	0.1253	−0.0053	0.1206
Pgm	254	0.1202	0.0053	0.1249
Mean		0.0982	0.0016	0.0996
Jacknife estimates over loci				
Mean		0.1059	0.0028	0.1084
SD		0.0242	0.0034	0.0266

[a] Mean reduction in heterozygosity of individuals in subpopulations.
[b] Departures from random mating among subpopulations.
[c] Departures from random mating from all causes.
* No single locus estimate differs significantly from zero.

DISCUSSION

Our estimate of mean gene diversity in *C. maculata* ($H_e = 0.183$) is greater than an estimate of gene diversity in another predacious ladybird, *Coccinella septempunctata* L. ($H_e = 0.160$; Krafsur *et al.*, 1992); indeed, many of the same loci are polymorphic. There were a few exceptions: *Mpi* and *Pgi* were monomorphic in *C. septempunc-*

TABLE 8

Heterozygote Deficiencies in Nine Subpopulations from Delaware and Knoxville, Iowa, as Measured by the F_{is} Statistic

	F_{is} estimate			
Allele	*Idh-2*	*Mpi*	*6pgdh*	*Pgm*
A	0.0057	−0.0031	−0.0084	0.0155
B	0.1632	−0.0009	0.1051	0.0521
C	0.1526	0.0835	0.2153	−0.0186
D	0.1674	0.1630	−0.0089	0.1426
E	−0.0134	−0.0762	−0.0003	0.1491
F		−0.0167	−0.0133	−0.0193
G			−0.0013	−0.0215
H				−0.0091

TABLE 9

Contingency χ^2 Tests of Homogensity for Autumn–Late Spring (Parent–Offspring) *C. maculata* Populations

	Ames[a]				Ankeny[b]				Knoxville[c]			
Locus	No. alleles	χ^2	*df*	*P*	No. alleles	χ^2	*df*	*P*	No. alleles	χ^2	*df*	*P*
Adk	5	6.40	4	0.17	3	1.04	2	0.59	5	4.41	4	0.35*
Idh-1	5	2.74	4	0.60	4	0.00	3	0.99	6	3.56	5	0.61
Idh-2	3	2.82	2	0.24	3	5.53	2	0.06	5	3.78	4	0.44
Me	3	1.01	2	0.61	4	0	0	—	4	2.72	3	0.44
Mpi	5	2.51	4	0.64	4	3.72	3	0.29	6	8.04	5	0.15
6pgdh	5	2.69	4	0.32	5	6.58	4	0.16	7	6.18	6	0.40
Pgm	7	4.75	6	0.58	7	3.03	6	0.81	8	33.52	7	<0.001
Totals		24.91	26	0.52		19.91	20	0.46		62.21	34	0.002

[a] $N = 112$ per locus.
[b] $N = 112$ per locus.
[c] $N = 185$ per locus.

tata but highly polymorphic in *C. maculata.* The two species are assigned to different coccinellid subfamilies (Sasaji, 1968) and such differences are to be expected.

An independent study of genic variation in *C. maculata* by using starch gel electrophoresis showed 16 of 23 (70%) putative loci (15 stains) to be polymorphic and $H_e = 0.11$ (Steiner and Grasela, 1993). We confirm their estimate of polymorphic loci by finding 35 of 44 (79%) putative loci (31 stains) polymorphic in Iowa beetles. But we cannot test the hypothesis that the two estimates of mean genic heterozygosity were independent because Steiner and Grasela (1993) did not provide the standard error of their estimate. They reported gene frequencies for *G3pdh* and *G6pdh,* for which we were unable to detect clear heterozygotes in our samples. Moreover, Steiner and Grasela (1993) scored 3 *Adk,* 2 *Aat,* 3 *Hk,* and 2 *Pgm* loci, but we observed only 2, 1, 1, and 1 loci, respectively. We found three zones of activity in *Hk* gels, but the slowest was tetrameric *GO,* and the next two zones were identical, one being a conformational isozyme of the other (Richardson *et al.,* 1986). Steiner and Grasela (1993) also observed null alleles at five loci and numerous rare alleles. We were unable to confirm the presence of null alleles in our samples and attribute most differences in results of our studies to the matrix in which electrophoresis was done (i.e., starch versus acrylamide) and to sampling plans and sample sizes.

We encountered technical problems in scoring some loci consistently. These problems were twofold: too many segregating alleles, e.g., *Pgm, Est,* and some pairs of alleles too close together on gels to distinguish unambiguously when heterozygous, e.g., *6pgdh* and *Mpi.* One highly polymorphic locus, *Pgi,* was not used in the population survey because it overstained and diffused before one could finish scoring the gel.

Gene diversity, as measured by the mean number of alleles segregating at loci, was 4.00 alleles per locus, an estimate identical to Steiner and Grasela's (1993). Coll *et al.* (1994) detected more alleles than we did at *Idh, Fum, Mdh, Pgi,* and *Pgm,* which may testify to the resolving power of cellulose acetate electrophoresis. But the frequencies of the most common alleles at the foregoing loci were quite similar in the Maryland, Delaware, and Iowa samples.

The effective number of segregating alleles (n_e) was much smaller than their arithmetic mean. For selectively neutral loci at equilibrium, theory predicts that the arithmetic mean and n_e would be the same because each allele frequency would be nearly equal (Nei, 1987).

Steiner and Grasela (1993) recorded substantial (though statistically untested) departures from random mating within (F_{is}) and among (F_{st}) the 15 populations they sampled. The overall departures from random mating, F_{it}, were very great and from their Table 4, we estimate an arithmetic mean $F_{it} = 0.268$ (range, -0.22 to 0.71) and a mean $F_{st} = 0.143$ (range, 0.04 to 0.68). Steiner and Grasela's (1993) F_{st} estimates were >0.1 at 13 of 21 loci. Coll *et al.* (1994) obtained a mean F_{st} for nine loci of 0.0015 ± 0.0006 from six Maryland *C. maculata* populations. We calculated a mean $F_{it} = 0.034$ from their Table 3, making our data consistent with theirs: *C. maculata* populations are essentially panmictic, at least in the middle tier of states. Departures from random mating, according to our analysis, are artifacts arising from technical problems in scoring some heterozygotes on gels in which two allelomorphs are so close together as to appear as a single, thick band. It seems to us possible that Steiner and Grasela's (1993) high F_{st} estimates may have arisen from an uneven designation of the same allele as heterologous across samples, but uneven sampling may also have contributed to their unweighted estimates.

We conclude that, despite spatial and temporal het-

erogeneities in climate and prey throughout its range, *C. maculata* maintains high rates of gene flow among populations and displays no clearly identifiable genetic differentiation.

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