Gene Flow in Populations of the Seven-Spotted Lady Beetle, *Coccinella septempunctata*

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Polyacrylamide gel electrophoresis was used to reveal gene diversity in exotic North American *Coccinella septempunctata* populations. This lady beetle recently spread across the northern Nearctic. Sixteen of 28 putative loci were polymorphic; average gene diversity for all loci was 0.1598 ± 0.041. Gene frequencies were estimated at eight polymorphic loci in natural North American beetles from Arkansas, Delaware, Iowa, Kansas, New York, Oregon, and Michigan. Also studied were F_2 beetles from four laboratory colonies that originated in Eurasia, along with field-collected beetles from France, Greece, and Sicily. Gene diversity among the Nearctic beetles was as great as that among the Palearctic beetles. Analysis of variance by Wright's method showed that only 29% of the variance in gene frequencies was between USDA cultures, Palearctic, and Nearctic beetles; 71% of the genetic variance was shared by beetles within the 21 subpopulations. No evidence of bottlenecks or drift was detected among the Nearctic subpopulations, and gene flow was essentially unrestricted.

In the Palearctic region, the seven-spotted lady beetle, *Coccinella septempunctata* (Coleoptera: Coccinellidae), is a predator of numerous herbivorous insects, particularly aphids. Both larvae and adults are predacious, and large quantities of prey may be consumed (Hodek 1973). Because of its large size and voracious appetite, the seven-spotted lady beetle, called C7 for short, was released in California in 1957 in the hope that it would become established and act as a control agent of insect pests. Nearctic *C. septempunctata* was first recorded in New Jersey and Quebec in 1973, and an increase in detections since 1973 reflects its numerical increase and geographic spread.

Between 1958 and 1973, the progeny of beetles from France, India, Italy, Norway, and Sweden were released sporadically in Arizona, Connecticut, Delaware, Florida, Maine, Maryland, New Jersey, Ohio, Pennsylvania, Virginia, and Washington. From 1974 to 1978, about 500,000 adult C7 were collected from New Jersey populations and released in 21 states across the United States (Angalet et al. 1979). It was not demonstrated that any of these releases established self-sustaining C7 populations. A large, rapid increase in C7 range occurred in the 1980s, but it is not clear if the distribution of cultured beetles throughout much of North America was responsible (Schaefer et al. 1987). Indeed, the origins of the originally detected Nearctic C7 are unclear. Schaefer et al. (1987) pointed out that the Quebec and New Jersey populations were near major waterways where transoceanic freighters are common and that C7 may have been introduced accidentally.

Did the putative "explosion" in C7 distribution have a genetic basis? Was it the result of a single introduction of particularly fit genotypes? Was it the result of recombination of two or more released forms to generate a race with superior performance in North America? Or was it the slow, unobserved increase and spread of progeny of a few pioneers until numbers became great enough to call attention to themselves? Allozyme electrophoresis was used to measure gene diversity, to examine its distribution among and within Nearctic C7 subpopulations, and to compare it with the gene diversity among selected Palearctic populations.

Materials and Methods

Biological Material

Adult North American beetles were field-collected, chilled, and shipped to Ames, Iowa, where they were killed by freezing at −75°C. Living, colonized beetles, originally from Eurasia, were sent to Ames from

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the USDA-APHIS-PPQ laboratory at Niles, Michigan; these colonies, composed of F_2 beetles, were maintained in diapause, and the beetles were many months old. Field-collected European beetles were maintained in diapause in USDA quarantine in Newark, Delaware, and shipped in liquid nitrogen to Ames.

We made the Nearctic collections in Arkansas in 1990 and 1991; in Delaware in 1990; in four locations in Iowa in 1989 and 1990; in Kansas in 1991; in two locations in Michigan in 1990; in New York in 1990 and 1991; and in Oregon in 1991, for a total of 14. These collections were made principally in alfalfa fields by sweeping beetles with insect nets. The colonized C7 from the USDA Niles laboratory were the F_2 progeny of beetles collected in France in 1989; progeny of four females from Syria in 1990; from Moldavia S.S.R., in 1989; and from the Crimea, Ukraine, in 1989, for a total of four. The feral Palearctic material included beetles from southeastern France in 1990, from Greece in 1990, and from Sicily in 1990, for a total of three.

We removed the elytra (wing covers) and wings before beetles were homogenized in microfuge tubes, each containing 200 μl of grinding buffer. Grinding buffer composition was the same as given in Black and Krafsur (1985).

Table 1. Genetic diversity at electrophoretic loci of *Coccinella septempunctata*

Enzyme	Locus	EC number	Buffer system	Expected heterozygosity[a] (h_e)
Aldehyde oxidase	*Aox*	EC 2.6.1.1	NAM	0.6931
Adenylate kinase	*Adk*	EC 2.7.4.3	NAM	0.1356
Arginine kinase		EC 2.7.3.3	NAM	0
Aspartate aminotransferase		EC 2.6.1.1	OD	0
Catalase		EC 1.11.1.6	NAM	0
Diaphorase	*Dia*	EC 1.6.2.2	NAM	0.4979
Fructose biphosphatase		EC 3.1.3.11	NAM	0.0950
Fumarate hydratase		EC 4.2.1.2	OD	0.1479
α-glycerophosphate dehydrogenase		EC 1.1.1.8	OD	0
Hexokinase-1	*Hk-1*	EC 2.7.1.1	TBE	0
Hexokinase-2	*Hk-2*			0
Hexokinase-3	*Hk-3*		TBE	0.1938
Hydroxy acid dehydrogenase		EC 1.1.1.30	OD	0.3727
Isocitrate dehydrogenase-1	*Idh-1*	EC 1.1.1.42	NAM	0.0886
Isocitrate dehydrogenase-2	*Idh-2*		NAM	0.1112
Lactate dehydrogenase	*Ldh*	EC 1.1.1.27	NAM	0.3231
Malate dehydrogenase	*Mdh*	EC 1.1.1.37	OD	0.0753
NADP-dependent MDH		EC 1.1.1.40	OD	0
Mannose-6-P-dehydrogenase		EC 5.3.1.8	NAM	0
Phosphoglucoisomerase		EC 5.3.1.9	OD	0
Phosphoglucomutase	*Pgm*	EC 5.4.2.2	OD	0.1884
6-phosphogluconate dehydrogenase	*6-Pgdh*	EC 1.1.1.44	NAM	0.3538
Sorbitol dehydrogenase-1		EC 1.1.1.14	NAM	0.7147
Sorbitol dehydrogenase-2				0
Superoxide dismutase	*Sod*	EC 1.15.1.1	OD	0.0304
Trehalase	*Tre*	EC 3.2.1.28	NAM	0.4523
Triose phosphate isomerase		EC 5.3.1.1	NAM	0
Xanthine dehydrogenase		EC 1.1.1.37	OD	0
		Mean of 16 polymorphic loci: H_e = 0.2796		
		Variance = 0.00296		
		Mean of all loci (n = 28): H_e = 0.1598		
		Variance = 0.00165		

[a] Expected proportions heterozygous when mating is random.

Electrophoresis

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.

The buffer systems included the Ornstein-Davis system (Hames 1981) without the stacking gel or riboflavin, tris-borate EDTA pH 9 (TBE), and NAM-citrate pH 6.5 (Clayton and Tretiak 1972). Composition of the TBE electrode and gel buffer was 81 mM Tris, 20 mM borate, and 1.5 mM disodium EDTA. The NAM-citrate gel buffer was 2.7 mM citrate, and the electrode buffer was 5.35 mM citrate, both adjusted to pH 6.5 with N-(3-aminopropyl)-morpholine.

Electrophoresis was performed in Hoefer SE600 gel boxes at 0°–4°C using 4 μl of sample homogenate. NAM gels were run at 25 mA per gel for 4 h and TBE and OD gels at 350 V for 2.5 h. We used agar overlays to resolve coupled reactions (Adenylate kinase, Hexokinase, Phosphoglucomutase, Trehalase, and other loci), as well as Diaphorase and Isocitrate dehydrogenase, for which we used the prescriptions of Wendel and Weeden (1989), except that concentrations of coupling enzymes were 25%–50% of those recommended.

Aldehyde oxidase in C7 was extremely active; the staining solution consisted of 2 μl of benzaldehyde, 5 mg MTT, and 1 mg PMS, all in 100 ml of 50 mM, pH 8, tris-HCl buffer. *Aox* also appeared on gels stained in solutions that contained any monosaccharide.

Analysis of Data

We calculated gene diversities and variances according to Nei (1987). Gene diversity at a locus was measured by the statistic $h_e = \Sigma\ (1 - p_i^2)$, where p_i is the frequency of each putative allele at the locus. Gene diversity for n loci is $H_E = \Sigma\ (h_e)/n$, with variance $= \Sigma\ (h_e - H_E)^2/[n(n-1)]$. H_E and h_e represent the heterozygosity expected when mating is random and other Hardy-Weinberg conditions apply. To analyze the gene frequency data we used BIOSYS-1 (Swofford and Selander 1981), and to partition variance in gene frequencies into two components, within and among collections, we used Wright's (1978) methods. We used the procedures of Weir and Cockerham (1984) to calculate F statistics because their methods weight for variable sample sizes, number of alleles, and populations and provide standard errors.

Results

The survey of genetic variation at enzyme loci is shown in Table 1 with the buffer systems that best resolved them. Of 28 presumptive loci, 16 were polymorphic (57%). The mean genetic heterozygosity (h_e) for 28 loci was 0.1598 ± 0.041. There were an additional two or three *esterase* loci that were polymorphic, but alleles at these loci overlapped, and careful breeding work would be required to demonstrate inheritance patterns.

Presumptive loci used to compare C7 populations are provided in Table 2, and the gene frequencies are summed by origin in Table 3. Deviations from Hardy-Weinberg expectations, at $P \leq .01$, included six instances in *Aox* and *Idh-2*. The *Aox* locus was dropped from consideration because it was hard to score objectively. Deviations in *Idh-2* occurred in the central Iowa subpopulations and were not due to problems in gel interpretation.

Variance in gene frequencies was partitioned into within regions and between

Table 2. Enzyme polymorphisms in *Coccinella septempunctata*

Enzyme	Locus	Subunit structure	Allele	Rf[a]
Adenylate kinase	*Adk*	monomer	*A*	0.88
			B	1.00
			C	1.15
			D	1.25
Diaphorase	*Dia*	dimer	*A*	0.85
			B	1.00
			C	1.15
Hydroxy acid dehydrogenase	*Had*	dimer	*A*	0.79
			B	1.00
			C	1.16
			D	1.21
			E	1.39
			F	1.50
			G	1.63
Isocitrate dehydrogenase	*Idh-1*	dimer	*A*	0.85
			B	1.00
			C	1.19
			D	1.26
	Idh-2	dimer	*A*	0.90
			B	1.00
			C	1.12
Malate dehydrogenase	*Mdh*	dimer	*A*	0.76
			B	1.00
			C	1.19
			D	1.29
			E	1.35
Phosphoglucomutase	*Pgm*	monomer	*A*	0.66
			B	1.00
			C	1.13
			D	1.40
			E	1.50
Superoxide dismutase	*Sod-2*	tetramer	*A*	0.84
			B	1.00
			C	1.13

[a] Distance migrated from origin relative to the distance migrated by the most common allele (1.00).

regions, where "regions" refer to USDA colonies, feral Nearctic (North American) beetle populations, and feral Palearctic (European) beetle populations. Clearly, most (71%) genetic variation in C7 was shared within the 21 subpopulations, and only 29% was between regions (Table 4).

Gene diversity and mean alleles per locus were similar among the North American and European beetles (Table 5). There were three alleles among the feral European collections that were not found among feral North American beetles, and three alleles segregating among North American populations that were not found in the European samples. Gene diversity was least among the C7 colonies maintained at the USDA laboratory, probably because of small founding numbers and subsequent drift.

Application of Workman and Niswander's (1970) chi-square tests to allele frequencies among beetle populations grouped by geographic criteria showed that only the three field-collected European beetle samples were homogeneous (Table 6). The four laboratory colonies were highly differentiated genetically. Patterns of genetic variation differed significantly among North American subpopulations.

Wright's *F* statistics estimate departures from random mating. Among the four APHIS colonies, $F_{st} = 0.10$, not an extraordinary value, considering the small parental numbers used to establish the original lines and the great geographic distances between the colony sources (Table 7). A statistically valid surplus of heterozygotes within colonies was suggested by $F_{is} \approx -0.09 \pm 0.02$. Avoidance of sib matings or selection favoring heterozygotes seems to be indicated.

No excess of heterozygotes was detected among field-collected beetles in Europe or in North America (Tables 8 and 9), and none of the *F* statistics from European beetles were statistically significant (Table 8).

Among the U.S. subpopulations, correlations among uniting gametes averaged $F_{it} = 0.12 \pm 0.03$, but most of this existed within populations ($F_{is} = 0.11 \pm 0.03$). The between-population component was $F_{st} = 0.014 \pm 0.006$, not significantly different from zero. Thus, there seems to be little restriction on gene flow among North American populations and no evidence of local adaptation.

Table 3. Gene frequencies among *Coccinella septempunctata* samples pooled according to origin

Locus	Allele	Origin: Nearctic (North American)	Origin: Palearctic (European)	Origin: USDA lab cultures
Adk	(*n*)	697	140	255
	A	0.0027	0.0066	0.0224
	B	0.9356	0.8981	0.9752
	C	0.0617	0.0867	0.0024
	D	—	0.0086	—
Dia	(*n*)	698	136	267
	A	—	0.0043	—
	B	0.5380	0.6075	0.7967
	C	0.4620	0.3859	0.2033
Had	(*n*)	678	83	240
	A	0.0056	0.0123	0.0149
	B	0.0099	0.0069	0.0235
	C	0.7910	0.7303	0.7802
	D	0.0695	0.0455	0.0749
	E	0.0812	0.1244	0.0477
	F	0.0291	0.0316	0.0589
	G	0.0138	0.0455	—
	H	—	0.0035	—
Idh-1	(*n*)	582	134	164
	A	0.0072	0.0087	—
	B	0.9614	0.9795	0.9868
	C	0.0210	0.0097	0.0132
	D	0.0104	0.0022	—
Idh-2	(*n*)	574	139	168
	A	0.0215	0.0022	0.0060
	B	0.9415	0.9689	0.9911
	C	0.0293	0.0245	0.0030
Mdh	(*n*)	686	139	168
	A	0.0021	0.0022	—
	B	0.0094	0.0067	—
	C	0.9559	0.9627	0.9285
	D	0.0304	0.0284	0.0588
	E	0.0022	—	0.0128
Pgm	(*n*)	692	161	247
	A	0.0034	—	—
	B	0.0486	0.0315	0.0015
	C	0.9115	0.9176	0.9078
	D	0.0317	0.0508	0.0907
	E	0.0049	—	—
Sod	(*n*)	694	83	252
	A	0.0232	—	0.0190
	B	0.9768	1.000	0.9663
	C	—	—	0.0147

Table 4. Analysis of variance in gene frequencies at eight loci in 21 populations of *Coccinella septempunctata*

Source	Variance	Percentage of variance
Within regions[a]	0.03964	71.0
Between regions	0.01619	29.0
Total	0.05583	

[a] There were three regions: USDA lab cultures, Nearctic, and Palearctic populations.

Table 5. Mean gene diversity and mean numbers of alleles per locus in *Coccinella septempunctata* origins

Sample origin	Heterozygosity	Alleles
Nearctic	0.1799 ± 0.0640	2.726 ± 0.419
Palearctic	0.1807 ± 0.0678	2.877 ± 0.535
USDA lab cultures	0.1355 ± 0.0566	1.973 ± 0.337

Table 6. Chi-square contingency tests for homogeneity of gene frequencies at eight loci among *Coccinella septempunctata* subpopulations

Origin	*n*	df	Chi-square	Probability
Nearctic (North American)	14	312	665.24	<.0001
Iowa	5	80	151.29	<.0001
New York	2	17	37.35	.003
Michigan	2	19	43.60	.001
Arkansas	2	22	35.46	.035
USDA laboratory cultures	4	51	395.55	<.0001
Palearctic (European)	3	48	52.01	.320

Table 7. *F* statistics for four *Coccinella septempunctata* USDA laboratory colonies

Locus	F_{is}	F_{st}	F_{it}
Adk	.044	.042	.085
Dia	−.104	.148	.059
Had	−.064	.067	.007
Idh-1	−.017	.015	−.001
Idh-2	−.006	.007	.001
Mdh	−.200	.146	−.024
Pgm	−.108	.069	−.032
Sod	−.090	.086	.003
Mean	−.0918	.0970	.0141
Jackknife estimates			
Mean	−.0889	.0961	.0153
SD	.0210	.0246	.0182

Table 8. *F* statistics for three subpopulations of European (Palearctic) field-collected *Coccinella*

Locus	F_{is}	F_{st}	F_{it}
Adk	.001	.035	.036
Dia	−.081	−.001	−.082
Had	.041	−.005	.037
Idh-1	−.021	.004	−.016
Idh-2	.253	−.001	.252
Mdh	−.019	−.010	−.029
Pgm	.077	.011	.087
Sod	.000	.000	.000
Mean	.0078	.0030	.0108
Jackknife estimates:			
Mean	.0012	.0020	.0033
SD	.0452	.0051	.0460

Table 9. *F* statistics for 14 subpopulations of North American (Nearctic) *Coccinella*

Locus	F_{is}	F_{st}	F_{it}
Adk	.086	.012	.097
Dia	.088	.005	.093
Had	.079	.021	.098
Idh-1	.085	.007	.091
Idh-2	.390	.010	.396
Mdh	.068	.007	.074
Pgm	.192	.048	.230
Sod	−.037	.019	−.017
Mean	.1149	.0148	.1280
Jackknife estimates:			
Mean	.1097	.0140	.1223
SD	.0296	.0061	.0312

Discussion

There is much genetic diversity in C7. Among the 16 loci of 28 (57%) that could be scored on the basis of simple Mendelian inheritance patterns, the mean heterozygosity (H_e) was 28% ± 5%; among all scorable loci, the estimated mean heterozygosity was 16% ± 5%. Estimates of gene diversity among five species of Coleoptera vary from 0.131 in the bark beetle, *Dendroctonus,* to 0.31 in the weevil, *Otiorrhynchus,* and the mean for the five Coleoptera spp. was $H_e = 0.191 \pm 0.032$ (Graur 1985). Among electrophoretic loci at least, gene diversity seems to be no greater in this widely distributed species rapidly expanding its range than in species of more restricted distributions.

It is clear that Nearctic C7 populations share a common gene pool that is more diverse than the combined gene pools of the four F_2 Palearctic cultures maintained by the Niles laboratory. The small number of founding beetles is responsible for the large F_{st} estimates. It is interesting that the laboratory beetles showed heterozygote frequencies that were significantly greater than Hardy-Weinberg expectations, giving negative F_{is} estimates. In this instance, selection favoring heterozygotes is a tenable hypothesis.

The 14 Nearctic and three Palearctic C7 gene pools each showed more variance within subpopulations than variance among subpopulations. One of the European collections (Sicily) represented an island population, and it might be expected that drift would have been operating.

Nearctic beetles northwest of the Mississippi seem to have become established within the past seven years (Obrycki et al. 1987). The data show no evidence of genetic drift, even where beetles were very recently established, as in Oregon and Arkansas. But the large F_{is} estimates for the 14 Nearctic populations suggest local departures from random mating, especially at the putative *Idh-2* and *Pgm* loci. This is puzzling; there was no evidence that a silent allele obscured heterozygotes, and the dimeric *Idh* seemed easy to score. C7 colonies show patchy, discontinuous distributions even within fields, and samples from a field were pooled. The patchy distribution arises from the oviposition behavior of single females, which tend to place their eggs in discrete clusters of 50 or more (Hodek 1973). The Nearctic C7 F_{is} estimates are best explained by Wahlund's principle: pooling samples from subpopulations that differ in gene frequencies creates an artificial deficiency of heterozygotes (Hartl and Clark 1989).

Few estimates are available of the numbers of C7 collected in Europe whose progeny were released in North America. It is unknown if any such releases were effective. Unfortunately, our data do not allow discrimination between hypotheses about the origin of the Nearctic beetles. Accidental colonization by shipborne C7 remains a valid hypothesis because of the tendency of overwintering adults to cluster (Hodek 1973). Dispersal of a large cluster of overwintering C7 from shipboard probably would preserve such variation as existed in the original population because bottleneck effects rapidly diminish when effective population numbers exceed 10 (Nei et al. 1975).

Genic diversity of Nearctic C7 is approximately the same as in Eurasia, and there is no evidence for genetic differentiation of any kind in North America. North American beetles demonstrated more genetic variation than the laboratory colonies and are not yet genetically differentiated from European beetles. The low F_{st} and high diversity values for North American beetles suggest that this species encountered no stringent bottlenecks in becoming established. The available genetic data would not seem to justify further introductions of exotic material.

There is some controversy in the literature about the best tactics to adopt for selecting and propagating desirable insects for biological control projects and for genetic manipulation of pest insect species (Mackauer 1976; Remington 1968; Roush 1990; Whitten and Foster 1975). The paucity of genetic data makes such controversies diffuse and sterile. Gene diversity among C7 subpopulations of this colonizing species is consistent with the notion that successful establishment requires a broad genetic basis. Large amounts of gene diversity seem to characterize structural loci in other species that have colonized North America, such as horn flies (McDonald et al. 1987), house

flies (Krafsur et al. 1992), and face flies (Krafsur and Black, unpublished).

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