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Gene Flow in the Exotic Colonizing Ladybeetle *Harmonia axyridis* in North America

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Gene flow was studied in *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), an exotic, arboreal ladybeetle predator that recently spread rapidly throughout North America. A survey of isozyme polymorphisms showed 30 of 52 resolved putative loci were polymorphic (58%), and the mean heterozygosity was 16.75 ± 2.98% among all loci and 26.31 ± 4.37% at only the polymorphic loci. The mean number of alleles at the 52 loci was 2.01 ± 1.97. Gene frequencies were estimated in populations from Georgia, Virginia, Delaware, Rhode Island, Arkansas, Illinois, Iowa, and Oregon and differed significantly ($P < 0.02$) at 11 of 16 loci. Three of 16 loci (*Fbp, Est-1, Tre*) were not in Hardy–Weinberg equilibrium in all populations and are not included in the *F* statistics. Random mating was indicated within populations ($F_{IS} = -0.005 \pm 0.014$) but not among populations ($F_{ST} = 0.025 \pm 0.005$). According to Wright's island model, F_{ST} estimates the average number of reproducing immigrants to be equivalent to ca. 10 beetles per population per generation. Thus there was a measure of genetic differentiation caused by drift, but this differentiation was small with respect to the large geographical distances among the sampled populations. The genetic data suggest that the founding North American population(s) was substantial. © 1997 Academic Press

KEY WORDS: ***Harmonia axyridis;* isozyme variation; breeding structure; gene flow; colonizing species.**

INTRODUCTION

Harmonia axyridis (Pallas) is native to eastern Asia (Chapin and Brou, 1991) and has attracted attention from geneticists because its color and spotting patterns are among the most variable of the Coccinellidae (Dobzhansky, 1933; Ayala, 1978). *H. axyridis* is a predator of aphids, but it also feeds on scales and coleopteran and lepidopteran eggs (Hodek, 1973); this ladybeetle is typically arboreal, but it has been found often in herbaceous habitats in the United States (Tedders and Schaefer, 1994; T. Kring, unpublished data).

Numerous attempts have been made to add *H. axyridis* to the North American fauna, the first of which was in 1916 in California (Gordon, 1985). From 1978 to 1986, 11 *H. axyridis* collections, ranging from 35 to 438 adults (mean 167), were made in Japan, Korea, and the former USSR for propagation and release in the United States. Fifty to 75 adults were used to begin cultures in USDA quarantine. In many cases, eggs from the founding beetles were transferred from quarantine and shipped to the cooperating agencies for propagation. The number and life stages of *H. axyridis* released have varied. In 1981, releases were made in Maryland, Maine, and Washington of eggs, larvae, or adults from cultures established from Japanese and Russian beetles. More releases were made in Maryland and Washington in 1982. Coulson (1992), Coulson *et al.* (1988), and Tedders and Schaefer (1994) should be consulted for a more comprehensive list of releases. No *H. axyridis* were detected, despite repeated sampling, after the foregoing releases were made. In 1979, 32 cultured *H. axyridis* were released in Louisiana, and in 1980, there were eight releases totaling 3781 specimens in Mississippi; these releases were descendants of beetles collected in Japan (Chapin and Brou, 1991). About 1000 cultured *H. axyridis* were released in Connecticut pine forests in 1985 and 1986 (McClure, 1987), and nearly 87,000 larvae were release in Byron, Georgia, pecan orchards from 1978 to 1981 (Tedders and Schaefer, 1994). Intensive sampling from 1981 to 1991 failed to show any postrelease recoveries of *H. axyridis* in Georgia (Tedders and Schaefer, 1994).

The first detections of *H. axyridis* in North America were made in 1988 in Louisiana, 1990 in Mississippi, and 1991 in Georgia (Tedders and Schaefer, 1994), hundreds of kilometers from the sites where they had been released 8 to 10 years earlier. These reports suggest a sudden appearance and explosive increase in

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population density together with a rapid spread of the ladybeetle. Tedders and Schaefer (1994) suggested that two independent populations of *H. axyridis* were established, one in Louisiana and the other in Georgia. In contrast, Day *et al.* (1994) argued that *H. axyridis* establishment in the United States likely resulted from accidental introductions occurring at seaports. We record here the abrupt appearance of *H. axyridis* in Arkansas (1992), Iowa (1994), Illinois (1994), and Oregon (1993). This is the second recent instance of a surprising, explosive colonization of North America by ladybeetles. The first such was the exotic *Coccinella septempunctata* L., which spread rapidly over the United States after first being detected in 1973, despite extensive earlier releases of cultured beetles (Schaefer *et al.*, 1987).

The rapid spread of *H. axyridis* raises interesting and important questions about the roles of selection and genetic drift among populations. What effect has the expansion in geographic range had on genetic differentiation? If the distribution of *H. axyridis* is patchy, can we expect to observe local effects of genetic drift and natural selection? We also wished to address the question of *H. axyridis* origins in North America by the use of genetic analysis. The basic hypotheses we tested were whether (1) gene frequencies were homogeneous among the sampled populations and (2) mating was random within and among these populations.

METHODS

Biological Material

Adult ladybeetles were collected in Little River County, Arkansas, in July 1994. Delaware beetles were swept from shrubs on 1–3 June 1994 in Newark, New Castle County. *Harmonia axyridis* from Vienna, Dooly County, Georgia, were collected in crimson clover in April 1994. Adults were collected in Champaign, Illinois, in late September 1994. Beetles were swept from trees in Ames, Story County, Iowa, in September 1994. Virginia *H. axyridis* were sampled from an overwintering aggregation on 28 October 1993 in McDowell, Highland County. *H. axyridis* were collected from trees on the campus of Oregon State University, Corvallis, Benton County, in June 1994. Rhode Island ladybeetles were collected in Kent County in August 1995.

Sample Preparation, Electrophoresis, and Staining

The beetles were homogenized individually in 200 µl of grinding buffer containing 200 g sucrose, 50 mg bromophenol blue, 20 mg basic fuschsin (tracking dyes), 770 mg dithiothreotol, and 186 mg ethylenediaminetetracetic acid (EDTA) in 54 m*M* pH 8.9 Tris–glycine buffer. Two microliters of homogenized sample was applied to each well in 6.5% polyacrylamide gels.

Three buffer systems were used: Tris–borate EDTA, pH 9 (TBE), NAM-citrate, 6.5 (Clayton and Tretiak, 1972), and, for cationic proteins, the Mops system (Thomas and Hodes, 1981). The TBE electrode and gel buffer consisted of 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 included 3-(*N*-morpholino)propanesulfonic acid (Mops) titrated with KOH to pH 6.8.

Staining recipes were as prescribed by Murphy *et al.* (1990), and agar overlays were used for all stains requiring coupling enzymes.

Genetic Statistics

Gene diversity (the expected frequency of heterozygotes under Hardy–Weinberg assumptions) at a locus was estimated as $h_e = 1 - \Sigma p_i^2$, where p is the frequency of allele i and the mean over n loci is $H_E = \Sigma h_e/n$ with variance $\Sigma(h_e - H_E)^2/ [n(n - 1)]$; the effective number of alleles n_e is $1/\Sigma p_i^2$ (Nei, 1987). H_E and h_e represent the heterozygosity expected when mating is random and other Hardy–Weinberg assumptions apply. Departures from random mating were estimated in the first instance by the simple statistic, $F = 1 - h_0/h_e$, where h_0 is the heterozygosity observed at a locus. F is an inbreeding coefficient and measures reduction in heterozygosity. F can vary, in theory, from -1 to $+1$ and approaches 0 when mating is random. BIOSYS-1 (Swofford and Selander, 1981) was used to analyze the gene frequency data. Tests of homogeneity of allele frequencies in beetle samples were made by using the methods of Workman and Niswander (1970). 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. F_{IS} is the reduction, in heterozygosity, caused by nonrandom mating within populations and may also be defined as the correlation of uniting gametes in individual beetles in populations. F_{ST} is a measure of nonrandom mating of beetles among populations and is defined as the average correlation of gametes in a population relative to the gametes in the entire gene pool. Reproductively isolated populations give a positive F_{ST} because gametes randomly chosen from a population have alleles more often derived from a common ancestor than do gametes from the entire gene pool. Nonrandom mating from all causes is expressed by F_{IT}. The relationship between these Fs is: $(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$. Formulae for calculating the foregoing statistics are given in Black and Krafsur (1985).

Wright (1969) showed that, for independently assorting, selectively neutral alleles in "island" populations at equilibrium, the relationship between F_{ST}, local popula-

tion size N, and average rate of immigration m is $F_{ST} \approx (1 + 4Nm)^{-1}$. This equation can be solved for Nm, the mean number of reproducing immigrants per population per generation (Slatkin, 1987, 1993). Our ladybeetle populations were probably not at equilibrium, but F_{ST} rapidly approaches equilibrium where strong founder effects do not occur (Crow and Aoki, 1984).

RESULTS

Gene Diversity

Thirty-nine stains were applied to *H. axyridis* homogenates of which 35 showed enzyme activity (Table 1). No lactate, octanol, succinic, or xanthine dehydrogenase activity was demonstrated. A sum of 52 putative loci were resolved adequately, of which 32 (61.5%) showed polymorphic banding patterns and 29 could be scored on at least one gel (Table 1). Glucose oxidase, clearly polymorphic, could not be interpreted by simple Mendelian criteria and was therefore not included in the estimate of gene diversity. There were additional activity zones that seemed polymorphic on gels stained for *Acph* and *Pep*, but resolution was too poor to score genotypes. Presumptive *Hk* loci were polymorphic, but conformational isozymes and variation in expression from gel to gel made interpretation problematical.

Of the 29 presumptive loci that were scored (Table 2), 4 showed expected heterozygosities of ≤1% and 6 showed heterozygosities of ≤5%. *Acph*, *Amy*, *Est-1*, *Est-3*, and *Tre* showed heterozygosities that exceeded 60%. The distribution of single-locus heterozygosities was unimodal (Fig. 1). The mean number of alleles per

TABLE 1

Enzymes and Buffer Systems Used for Studying *Harmonia axyridis* Gene Diversity

Enzyme	Locus	E.C. number	Buffer system	No. loci	No. polymorphic
Acid phosphatase	*Acph*	3.1.3.2	NAM	2	2
Aconitase	*Aco*	4.2.1.3	OD	1	1
Aldehyde oxidase	*Aox*	1.2.3.1	NAM	1	1
Adenylate kinase	*Adk*	2.7.4.3	NAM, MOPS	3	0
Aldolase	*Ald*	4.1.2.13	NAM	2	1
Amylase	*Amy*	3.2.1.1	OD	2	2
Arginine kinase	*Argk*	2.7.3.3	NAM	1	1
Aspartate aminotransferase	*Aat*	2.6.1.1	NAM	1	0
Catalase	*Cat*	1.11.1.6	NAM	1	0
Diaphorase	*Dia*	1.8.1.4	NAM	2	1
Esterase	*Est*	3.1.1.-	TBE	3	3
Formaldehyde dehydrogenase	*Form*	1.2.1.46	NAM	1	1
Fructose biphosphatase	*Fbp*	3.1.3.11	NAM	2	1
Fumarate hydratase	*Fum*	4.2.1.2	OD	1	0
Glucose oxidase	*Go*	1.1.3.4	NAM	1	1
Glucose-6-phosphatase dehydrogenase	*G6pd*	1.1.1.49	NAM	1	0
Glutamate dehydrogenase	*Gdh*	1.4.1.2	TBE	1	0
Glyceraldehyde-3-phosphate dehydrogenase	*G3pd*	1.2.1.12	NAM	1	1
α-Glycerophosphate dehydrogenase	*α-Gpd*	1.1.1.8	TBE, OD	2	1
Glycogen phosphorylase	*Phos*	2.4.1.1	NAM	1	0
Hexokinase	*Hk*	2.7.1.1.	TBE, MOPS	3	2
Hydroxy acid dehydrogenase	*Had*	1.1.99.6	NAM, MOPS	2	2
Isocitrate dehydrogenase	*Idh-1*	1.1.1.42	NAM	2	1
Lactate dehydrogenase	*Ldh*	1.1.1.27	NAM	0	0
Leucine aminopeptidase	*Lap*	3.4.1.1	NAM	2	1
Malate dehydrogenase	*Mdh*	1.1.1.37	NAM, OD	1	0
Malic enzyme	*Me-1*	1.1.1.40	OD	2	1
Mannose-6-P-dehydrogenase	*Mpi*	5.3.1.8	NAM	1	1
Octanol dehydrogenase	*Odh*	1.1.1.73	NAM	0	—
Peptidase	*Pep*	3.4.-.-	NAM	2	1
Phosphoglucoisomerase	*Pgi*	5.3.1.9	OD	1	0
Phosphoglucomutase	*Pgm*	5.4.2.2	NAM, OD	1	1
6-Phosphogluconate dehydrogenase	*6pgd*	1.1.1.44	NAM	1	1
Sorbitol dehydrogenase	*Sdh*	1.1.1.14	NAM	1	1
Succinic dehydrogenase	*Suc*	1.3.99.1	NAM, OD	0	—
Superoxide dismutase	*Sod*	1.15.1.1	TBE, OD	2	1
Trehalase	*Tre*	3.2.1.28	NAM	1	1
Triose-phosphate isomerase	*Tpi*	5.3.1.1	NAM	1	1
Xanthine dehydrogenase	*Xdh*	1.1.1.204	TBE	0	—
Totals and mean				52	32 (61.5%)

TABLE 2

Gene Diversity in Seven North American *H. axyridis* Populations

Locus	*N*	No. alleles	Subunit structure	n_e	h_0	h_e	No. populations	Fixation index (F)
Aco	343	5	Monomer	1.52	0.3201	0.3400	7	0.058
Acph-2	55	5	Tetramer	3.14	0.6909	0.6812	1	−0.014
Ald	20	2	Monomer	1.27	0.1754	0.2154	2	0.186
Amy-1	101	5	Monomer	3.17	0.6139	0.6842	2	0.103
Amy-2	52	5	Monomer	3.11	0.7500	0.6781	1	−0.106
Aox	343	4	Dimer	1.32	0.2362	0.2424	7	0.023
Argk	37	4	Monomer	1.79	0.4595	0.4427	1	−0.038
Dia-2	344	3	Dimer	1.01	0.0116	0.0104	7	−0.115
Est-1	332	5	Monomer	2.59	0.4669	0.6112	7	0.236
Est-2	179	4	Monomer	1.17	0.1341	0.1478	3	0.093
Est-3	94	3	Monomer	2.82	0.5532	0.6450	2	0.142
Fbp	341	4	Dimer	1.10	0.0645	0.0891	7	0.276
Form	103	6	Dimer	1.53	0.3301	0.3477	2	0.048
aGpd	348	2	Dimer	1.06	0.0632	0.0591	7	−0.069
G3pd	348	2	Tetramer	1.01	0.0086	0.0076	7	−0.132
Had-1	312	3	Dimer	1.04	0.0419	0.0350	7	−0.197
Had+2	300	3	Dimer	1.13	0.1400	0.1177	7	−0.189
Hk-1	170	4	Monomer	2.47	0.2294	0.5959	3	0.615
Hk-2	112	2	Monomer	1.23	0.1696	0.1843	2	0.079
Idh-2	348	2	Dimer	1.00	0.0029	0.0026	7	−0.115
Lap	54	3	Monomer	2.45	0.5556	0.5919	1	0.061
Me	342	3	Tetramer	1.01	0.0117	0.0105	7	−0.114
Mpi	327	3	Monomer	1.02	0.0183	0.0185	7	0.011
Pep-1	49	4	Monomer	1.31	0.1837	0.2368	1	0.224
Pgm	342	3	Monomer	2.80	0.5234	0.5200	7	−0.006
Sdh	345	4	Dimer	1.08	0.0725	0.0720	7	−0.007
Sod-1	94	2	Dimer	1.12	0.0532	0.1102	3	0.517
6pgd	349	3	Dimer	1.24	0.1862	0.1964	7	0.052
Tre	291	5	Monomer	2.83	0.5636	0.6466	7	0.128
Polymorphic loci								
Means		3.55		1.70	0.2631	0.2945		0.1066
Standard deviation		1.15		0.79	0.0437	0.0470		
All loci								
Means		2.01		1.39	0.1496	0.1675		
Standard deviation		1.97		0.20	0.0290	0.0298		

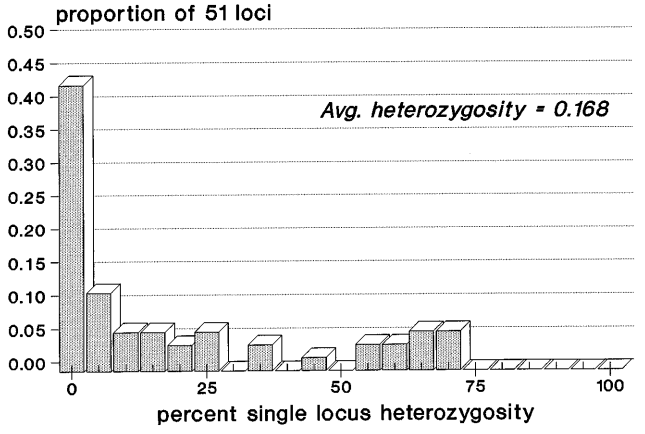

FIG. 1. Distribution of single-locus heterozygosities, h_e, in *Harmonia axyridis*.

polymorphic locus was 3.55 ± 1.15 and 2.01 ± 1.97 among all loci. Mean gene diversity among the 29 polymorphic loci was 29.45 ± 4.7%; among all 52 loci, diversity was 16.75 ± 2.98% (Table 2).

Deviations from random mating, estimated by the F statistic (Table 2), ranged from −0.197 at *Had-1* (excess of heterozygotes) to 0.517 at *Sod-1* (excess of homozygotes). The overall mean F was 0.107. F is a variance statistic that has several components. These components include departures from random mating within and among populations and deviations caused by demographic instability (Slatkin, 1987). In addition, the F statistic is affected by technical problems in assigning genotypes from banding patterns on gels.

Gene Frequency Analysis

To rationalize the foregoing components, 16 loci were examined in all populations. Significant deviations

from Hardy–Weinberg proportions were detected in 8 of 128 evaluations. Two of these 8 were at *Fpb*, 2 at *Est-1*, and 2 at *Aox*. At the 5% level, 6.4 significant deviations are expected in 128 independent trials, so the overall deviation was no greater than expected by chance ($\chi^2 = 0.74$, $df = 1$, $P = 0.39$). Gene frequencies at *Est-1*, *Fbp*, and *Tre* were thought to indicate technical problems and provide questionable estimates. There was a paucity of heterozygotes at *Fbp* in the one sample (Illinois) of high diversity ($h_0 = 0.226$, $h_e = 0.35$, $F = 0.35$). *Tre* resolution varied among collections so that its interpretation probably was uneven, increasing the F_{ST} statistic.

Within-population expected heterozygosity at 16 loci ranged from 13.6 ± 4.7% in Georgia beetles to 20.0 ± 5.9% in Oregon beetles (Table 3). The observed and expected mean heterozygosities were 0.1688 and 0.1743, respectively. When the 3 problematic loci were dropped, average expected and observed heterozygosities became 0.1228 and 0.1240, respectively. Overlapping standard errors suggest that the expected and observed heterozygote frequencies, summed over all loci, did not differ significantly. The mean F estimate for the eight populations was 0.0326 ± 0.0481; when the 3 problematic loci were eliminated from consideration F became 0.0135 ± 0.0657.

TABLE 3

Gene Diversity at 16 Polymorphic Loci in Geographical Samples of *H. axyridis*

Population	Mean: Sample size per locus	Mean: No. alleles per locus	Mean: Percentage of loci polymorphic[a]	Mean heterozygosity: Direct count	Mean heterozygosity: Hardy–Weinberg expected[b]
Georgia	33.7	1.8	50.0	0.125	0.136
	(2.3)	(0.3)		(0.043)	(0.047)
Virginia	51.8	2.6	68.8	0.158	0.158
	(3.0)	(0.4)		(0.050)	(0.048)
Delaware	55.4	2.5	68.8	0.186	0.190
	(0.2)	(0.4)		(0.055)	(0.056)
Rhode Island	55.9	2.6	68.8	0.172	0.194
	(0.1)	(0.4)		(0.053)	(0.061)
Oregon	54.2	2.7	93.8	0.199	0.200
	(0.7)	(0.2)		(0.060)	(0.056)
Arkansas	37.8	2.1	52.9	0.141	0.149
	(0.3)	(0.3)		(0.048)	(0.053)
Iowa	46.8	2.4	62.5	0.189	0.182
	(2.1)	(0.3)		(0.064)	(0.061)
Illinois	54.9	2.3	62.5	0.171	0.176
	(0.6)	(0.3)		(0.053)	(0.053)

Note. Parentheses show standard errors.

[a] A locus is considered polymorphic if more than one allele was detected.

[b] Unbiased estimate (Nei, 1978).

TABLE 4

Summary of F Statistics at 16 and 13 Putative Loci

Locus	F_{IS}	F_{ST}	F_{IT}
Aco	−0.0078	0.0291	0.0367
aGpd	−0.0869	0.0628	−0.0186
Aox	0.0602	0.0326	0.0908
Dia-1	−0.0029	−0.0007	−0.0036
Est-1	0.0981	0.1749	0.2558
Fbp	0.2810	0.0830	0.3407
G3pd	−0.0530	0.0437	−0.0070
Had-1	−0.0172	0.0009	−0.0163
Had+2	−0.0211	0.0046	−0.0164
Idh-2	−0.0051	0.0013	−0.0021
Me	−0.0184	0.0178	−0.0003
Mpi	−0.0409	0.0398	0.0005
Pgm	−0.0133	0.0227	0.0097
Sdh	0.0116	0.0820	0.0926
6pgd	0.0469	0.0063	0.0530
Tre	0.0200	0.1470	0.1640
Mean	0.0333	0.0841	0.1146
Jackknife estimates over loci			
Mean	0.0333	0.0886	0.1194
SD	0.0198	0.0325	0.0454
Estimates less *Est*, *Fbp*, and *Tre*			
Mean	−0.0039	0.0263	0.0225
Jackknife estimates over loci			
Mean	−0.0049	0.0252	0.0300
SD	0.0137	0.0044	0.0147

The χ^2 contingency tests showed that gene frequencies were homogeneous among the eight populations at *Dia*, *Had-1*, *Had-2*, *Idh-2*, and *6pgd* (data not shown), but the sum of deviations from homogeneity over all 16 loci was large ($\chi^2 = 1322$, $df = 280$, $P \approx 0$). When the greatest sources of heterogeneity, *Est-1*, *Fbp*, and *Tre*, were excluded from the analysis, the overall deviation remained highly significant ($\chi^2 = 552.9$, $df = 203$, $P \approx 0$). Clearly the eight beetle populations showed different allele frequencies.

Gene Flow among Populations

Departures from random mating within populations, F_{IS}, were marked at *6pgd*, *Est-1*, and especially *Fbp* (Table 4). Natural selection, combining samples from populations with different gene frequencies (the Wahlund effect), or technical problems in scoring gels could explain a positive F_{IS} estimate. If matings were assortative, we would expect all selectively equivalent loci to show positive F_{IS} estimates. The mean F_{IS} estimate of 0.0333 was barely significant and became −0.0049 ± 0.0137 when *Est-1*, *Fbp*, and *Tre* were excluded from the calculations (Table 4). Thus, excluding the problematic loci resulted in an estimate of random mating within populations.

Alleles segregating at *Est* and *Tre* showed significant departures from random mating among populations

(F_{ST}, Table 4), which could suggest the operation of natural selection in equilibrium populations. These departures from random mating more likely were caused by technical problems in scoring the gels, however. The mean F_{ST} was 0.0886 ± 0.0325, but this estimate became 0.0252 ± 0.0044 when *Est-1, Fbp,* and *Tre* were excluded. Thus, there was a small, but statistically significant, amount of reproductive isolation among the eight populations. Application of Wright's island model (1969) that relates F_{ST} to N_m, the mean number of reproducing migrants ($N_m = [(1/F_{ST}) - 1]/4$) suggests a level of gene flow equivalent to about 10 beetles per generation.

Genetic Distance Measures

Given the small degree of genetic differentiation among populations, can a geographical pattern be discerned? Nei's (1978) unbiased genetic distance was 0.005 or less among all populations except those found in Georgia. The geographically most distant population, Oregon, showed trivial genetic distances from the other populations. Cavalli-Sforza and Edward's arc distance (1967), advocated by Wright (1978), also showed no obvious pattern of geographic differentiation among *H. axyridis* populations (Table 5).

DISCUSSION

The fraction of polymorphic loci in *H. axyridis* of 58% is substantial and consistent with allozyme polymorphisms in other Coccinellidae. The mean gene diversity of 16.75 ± 2.98% also is consistent with estimates in other Coccinellidae, among which the mean was 19.1 ± 3.4% (Krafsur *et al.,* 1992, 1995). Such diversities are typical of the Coleoptera and thought to result from large effective population sizes and high rates of gene flow (Graur, 1985; Hsiao, 1989).

In principle, departures from random mating could indicate the operation of selection at some loci and genetic drift at others. If only drift were operating among neutral loci, we would expect much less variation among loci than we observed. In fact, large values of *F* often occur for technical reasons related to difficulties in interpreting banding patterns on gels. For example, heterozygotes can be more difficult to distinguish than homozygotes because they stain weakly or are otherwise ill resolved. Indeed, for technical reasons some clearly polymorphic loci were not suitable for analysis of gene flow. *Tre* resolution varied among beetle populations so that interpretation of gels varied among populations, thereby inflating F_{ST}. *Fbp* was not scored in Hardy–Weinberg proportions in two of eight populations and showed an unusual paucity of heterozygotes in the Illinois sample. Difficulty in scoring heterozygotes accurately is a common, if generally unacknowledged, problem in enzyme electrophoresis.

Questions we wished to address in analysis of North American *H. axyridis* populations were (a) what genetic changes may correlate with the geographic spread and population increase in *H. axyridis* and (b) the origins of North American *H. axyridis?*

In the Old World, *H. axyridis* shows great diversity in color and pattern. Both red and black morphs of *H. axyridis* were collected and reared initially in USDA quarantine facilities. In Oregon and the Pacific Northwest, the black morph is present. Only red morphs have been found elsewhere in the United States, and there seems to be little phenotypic variation in North American *H. axyridis.* Only red morphs were available to us. This apparent reduction in color and pattern variation could have been caused by genetic drift or by natural selection. How can we discriminate?

Theory predicts that gene diversity at neutral loci will vary inversely with founding population size (Nei *et al.,* 1975); indeed, the χ^2 tests of homogeneity suggest highly significant differences in allele frequencies among populations at 11 of 17 loci, consistent with genetic drift. The high level of gene diversity in each *H. axyridis* sample argues strongly that there were no substantial population bottlenecks in its establishment in North America. Any reduction in heterozygosity that occurred in the establishment of local colonies has been

TABLE 5

Matrix of Genetic Distance Coefficients in *H. axyridis*

Population	Georgia	Virginia	Delaware	Connecticut	Oregon	Arkansas	Iowa	Illinois
Georgia	—	0.010	0.011	0.007	0.009	0.006	0.011	0.008
Virginia	0.138	—	0.003	0.004	0.003	0.001	0.001	0.001
Delaware	0.127	0.117	—	0.002	0.005	0.001	0.002	0.002
Connecticut	0.117	0.117	0.108	—	0.004	0.002	0.003	0.002
Oregon	0.148	0.137	0.149	0.132	—	0.002	0.002	0.002
Arkansas	0.088	0.099	0.086	0.110	0.138	—	0.000	0.000
Iowa	0.119	0.106	0.099	0.101	0.128	0.081	—	0.000
Illinois	0.126	0.103	0.116	0.105	0.129	0.011	0.086	—

Note. Above diagonal: Nei's (1978) unbiased genetic distance. Below diagonal: Cavalli-Sforza and Edwards (1967) arc distance.

restored by gene flow among colonies, as is indicated by the low F_{ST} estimate, and there is now no evidence for much genetic drift. It may be that beetles in subpopulations disperse before mating, so that matings are essentially random over large areas. Some ladybeetle species are known to form migrating aggregations and there is long distance movement in a number of coccinellid species (Lee, 1980). This tendency to migrate must have a homogenizing effect on the spatial components of gene diversity. It is hard to imagine, however, that the effective dispersal distances are continental.

Much greater genetic differentiation in *H. axyridis* could be expected had one or more sampled populations been founded by only a few beetles and had the populations been isolated until they were detected in 1993 and 1994. Alternatively, it is possible that small, discontinuously distributed *H. axyridis* populations became established (and many extinguished) in the past 15 to 80 years. As these populations grew, gene flow among them increased to present levels and more or less simultaneously, *H. axyridis* became noticed. This last scenario strikes us as highly unlikely because this ladybeetle is large and conspicuous, and it is doubtful that its populations were undetected for very long. Indeed, *H. axyridis* has become something of a pest because it forms large overwintering aggregations in, among other places, human dwellings, thereby drawing much attention.

The low F_{ST} and small genetic distance estimates provide no evidence for regional selection and adaptation in *H. axyridis*. The microhabitats occupied by *H. axyridis* may be essentially similar throughout its range. It is probably too soon to evaluate the existence of regionally different selective regimes because the inferred high rates of gene flow and the short time in which putatively different selection regimes may have been operating could account for our results. Because there are only two generations annually, it may be some time before a balance occurs between selection and gene flow.

Are the North American *H. axyridis* adventive or are they the progenies of released, USDA ladybeetles? We have no estimate of allozyme gene diversity in Old World populations, nor in the discarded USDA cultures; therefore we cannot address the question directly. Examination of USDA cultures of three other ladybeetle species showed high levels of genetic differentiation among cultures of each species (Krafsur *et al.*, 1992, 1996a,b) and 11 cultures of *H. axyridis* were released in recent times; we therefore could expect genetic differentiation among these 11 cultures. The genetic similarities among the widely distributed North American *H. axyridis* populations argue instead for a single source but whether that source was a USDA culture or an invasion by natural means cannot be addressed on the basis of our data.

Whatever the history of *H. axyridis* in North America, widely distributed populations show high levels of gene flow, and mating seems nearly panmictic. A similar population structure was detected in the seven-spotted lady beetle, *Coccinella septempunctata*, which had rapidly spread throughout much of North America only a few generations before genetic work was performed (Krafsur *et al.*, 1992). *C. septempunctata* demonstrated transiently large populations only to become less abundant within 3–5 years. The same "boom and bust" population dynamics may now occur in *H. axyridis*.

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