Gene Flow and Diversity at Allozyme Loci in the Twospotted Lady Beetle (Coleoptera: Coccinellidae)

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ABSTRACT High levels of genetic variation in biological control agents are thought to be necessary to ensure their successful establishment and geographical spread. Gene diversity was studied in F_2 twospotted lady beetle, *Adalia bipunctata* (L.), descended from specimens collected in Uzbekistan and from beetles collected in Oregon, Iowa, and Lake Michigan at Chicago, IL. Twenty-four of 39 resolved putative loci were polymorphic and the mean observed heterozygosity was 22.7 ± 4.4% at the polymorphic loci. The average expected heterozygosity was 24.7 ± 4.9% at the polymorphic loci and 15.2 ± 3.3% at all loci. The mean number of alleles at the 39 loci was 1.9 ± 1.0, and the mean effective number of alleles was 1.3 ± 0.5. These are substantial levels of diversity. Significant departures from random mating were detected within (F_{IS} = 0.068 ± 0.042) and among (F_{ST} = 0.070 ± 0.012) the North American populations but matings were random within populations when problematic loci were excluded. Analysis of gene frequencies at 5 loci in 4 British and a French population showed the same magnitude of gene flow as the 3 North American populations. The fixation index between Iowa and Uzbekistan ladybirds was F_{ST} = 0.428 ± 0.128. Our data do not support an Old World origin for North American *A. bipunctata* in historical times.

KEY WORDS *Adalia bipunctata,* biocontrol, allozymes, gene diversity

Adalia bipunctata (L.), the twospotted lady beetle, is distributed in much of the Holarctic and in South America (Hodek 1973, Gordon 1985). Old World populations are polymorphic in color, and a variety of melanic forms with red spots occur together with orange beetles bearing 2 black spots. New World beetles are typically orange with black spots, although a melanic form was described early in the 20th century (Gordon 1985, Majerus 1994). The adaptive significance of the melanic and orange forms has been investigated in Europe and reviewed (Hodek 1973, Brakefield 1985, Majerus 1994). Historically, coccinellid beetles were favored for studying the ecological genetics of elytral pattern polymorphisms (Dobzhansky 1933, Muggleton 1978, Brakefield 1984, Majerus 1994). Clines were detected, inferring the operation of natural selection. It was suggested also that putative recessive lethals segregating in *A. bipunctata* provided a means of ensuring high levels of heterozygosity (see Hodek 1973 and Majerus 1994, for reviews). Breeding studies generally showed confounding environment–genotype interactions, multiple alleles, dominance, and, for some traits, continuous variation. In all the foregoing studies, therefore, the properties of color and pattern confound the unambiguous scoring of gene frequencies, making these polymorphisms less than ideal for population genetic work. Other genetic markers are necessary to undertake studies on gene flow and adaptation.

The origin of the North American *A. bipunctata* is unclear; they were first described here by Say in 1824 (Gordon 1985) and they show little, if any, of the color polymorphisms observed in Palearctic populations. Is the seeming absence of color polymorphisms a response to natural selection, or to a paucity of genetic variation? A paucity of genetic variation could be explained, in principle, by genetic drift that may have occurred during the establishment of *A. bipunctata* in North America by a small number of beetles and subsequent restriction of population size as the species became adapted to its new environments. It is often written that a biological control agent should have adequate genetic variation to ensure an initially successful colonization and later spread (e.g., Kennedy 1993). Roush (1990) and Hopper et al. (1993) comprehensively reviewed genetic theory as applied to biocontrol. For many years discussions in the literature focused on the relative merits of collecting natural enemies from marginal or central populations to maximize genetic variability. These controversies, which continue today (Roderick 1992), have little empirical and no experimental genetic data to discuss. The release of presumably preadapted "biotypes" of natural enemies is thought to have been critical for some established, exotic biological control agents (Tauber and Tauber 1975). Much importance has been placed on the problematic adaptability of introduced natural en-

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emies to their new environments (Messenger et al. 1976).

It has been argued as a matter of principle that a substantial amount of genetic variation is necessary to ensure the adaptability of an effective biocontrol species to the wide variety of habitats and prey it may encounter (e.g., Hopper et al. 1993) but no data were offered to support the idea. Is this generalization true? *A. bipunctata* is an effective biocontrol agent that may be an appropriate model. What levels of genetic variation exist in this species? What are the origins of North American *A. bipunctata* and how much gene flow occurs among populations? To answer the foregoing questions, we chose to use allozyme variation as genetic markers. To do so, first we assayed variation at allozyme loci, then we compared gene diversity in U.S. and Eurasian *A. bipunctata* populations, and finally we examined gene flow among disparate North American populations.

There are preliminary data that bear on the foregoing questions. Using starch gel electrophoresis, Eggington (1986) estimated gene frequencies in 4 *A. bipunctata* populations from the United Kingdom and a French population. Our analysis of her data suggested significant differentiation among populations at 4 of 5 polymorphic loci. Where mating is random within demes, the inbreeding coefficient of an average beetle relative to the total population was estimated to be F_{DT} = 0.061 (Wright 1978). This *F* statistic suggests only a moderate degree of differentiation, considering the wide (Glasgow to Paris) geographic separation between populations. According to Wright's (1969) infinite island model, assuming selectively equivalent alleles at all loci measured and equilibrium populations, F_{DT} of 0.061 provides an estimate of 4 reproducing migrants per population per generation. Evidence from the United Kingdom and western Europe, then, suggests a substantial level of gene flow among *A. bipunctata* populations.

Materials and Methods

Biological Material. We obtained 3 collections of North American *A. bipunctata.* In Ames, IA, beetles were collected as they emerged from overwintering in a large, 6 story brick and concrete building in April 1993. A collection was made in July 1994 of beetles washed up on the beach of Lake Michigan at Chicago, IL. An additional sample of *A. bipunctata* was obtained in August 1994 in Corvallis, OR. All the North American beetles were orange with 2 black spots on their elytra. These North American *A. bipunctata* were compared with beetles collected in Uzbekistan in 1990 and propagated by the U.S. Department of Agriculture Biological Introductions Research Laboratory in Newark, DE. About 90% of these USDA beetles were black with 2 orange spots on their elytra. Second-generation (F_2) beetles were killed by freezing at −80°C and stored at this temperature until used.

The numbers of *A. bipunctata* electrophoresed were 56 from Iowa, 14 from Chicago, 18 from Oregon, and 56 from the Uzbekistan culture, for a total of 288 haploid genomes. Voucher specimens are deposited in the collection of J. J. Obrycki in the Department of Entomology, Iowa State University, Ames.

Demonstrating Allozyme Variation. The electrophoretic and staining methods were as described for coccinellid beetles (Krafsur et al. 1992, 1995). Briefly, beetles were homogenized in 150 μl of grinding buffer (Black and Krafsur 1985a). Two microliters of homogenate were applied to each well in 6.5% acrylamide gels. Vertical polyacrylamide gel electrophoresis was performed at 1–4°C by using N-(3-aminopropyl)-morpholine-citrate pH 6.5 (NAM), tris-borate EDTA pH 8.9 (TBE), 3-(N-morpholino) propane sulfonic acid (MOPS), and Ornstein-Davis (OD) buffer systems. Enzyme activity was demonstrated by using 34 stains, 39 putative loci thereby being identified (Table 1).

Analysis of Data. Gene frequencies (see Appendices) were analyzed by using *Biosys-1* (Swofford and Selander 1981) and *Genestats* (Black and Krafsur 1985b). Details of the numerical procedures were set forth in earlier papers (Krafsur et al. 1992, 1995). In brief, only loci in Hardy–Weinberg equilibrium in samples were used for statistical analysis of populations. Workman and Niswander's (1970) formulae were used for contingency chi-square testing of hypotheses of homogeneity in allele frequencies. Methods of Weir and Cockerham (1984) were used to compute Wright's *F* statistics because they weight for sample sizes and provide jackknife estimates of standard errors. Three *F* statistics were estimated: F_{IS} for departures from random mating within populations, F_{ST} for departures from random mating among populations, and F_{IT} for departures from random mating as a result of all causes. The relationship among these statistics is $(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$ (Wright 1969). F_{ST} measures the effects of random drift and differential selection among subpopulations and for selectively equivalent variation at each locus, it measures only drift. The null hypothesis that $F_{ST} = 0$ can be tested by: $\chi^2 = 2N F_{ST}(k - 1)$ for $(k - 1)(s - 1)$ df where k is the number of alleles segregating at a locus and s subpopulations. F_{ST} can be used to estimate the average level gene flow in terms of the mean number of migrants per subpopulation per generation according to the island model of Wright (1969). Assuming that subpopulations are at equilibrium with respect to drift and migration, the model is, $F_{ST} \approx 1/(1 + 4Nm)$, from which the mean number of migrants is, $Nm \approx (1 - F_{ST})/4F_{ST}$. Although estimates from this model are based on an island model of population structure, they have been found to be reasonable approximations for the opposite extreme of stepping-stone arrays (Slatkin 1987).

Table 1. Enzyme and buffer systems for *A. bipunctata*

Enzyme	Enzyme commission no.	Symbol	Buffer system	No. loci	No. polymorphic
Aspartate aminotransferase	E.C. 2.6.1.1	*Aat*	NAM	1	0
Acid phosphatase	E.C. 3.1.3.2	*Acph*	NAM	2	1
Aconitate hydratase	E.C. 4.2.1.3	*Aco*	OD, TBE	1	0
Adenylate kinase	E.C. 2.7.4.3	*Adk*	TBE, MOPS	3	2
Alcohol dehydrogenase	E.C. 1.1.1.1	*Adh*	NAM, MOPS	0	—
Aldehyde oxidase	E.C. 1.2.3.1	*Aox*	NAM	1	1
Aldolase	E.C. 4.1.2.13	*Ald*	NAM	1	0
Alkaline phosphatase	E.C. 3.1.3.1	*Aph*	NAM	0	—
Amylase	E.C. 3.2.1.1	*Amy*	TBE	0	—
Arginine kinase	E.C. 2.7.3.3	*Argk*	NAM	1	1
Catalase	E.C. 1.11.1.6	*Cat*	OD, NAM	0	—
Diaphorase	E.C. 1.8.1.4	*Dia*	NAM, TBE	2	2
Fumarate hydratase	E.C. 4.2.1.2	*Fum*	TBE, OD	1	1
Formaldehyde dehydrogenase	E.C. 1.2.1.1	*Fdh*	NAM	0	—
Fructose biphosphatase	E.C. 3.1.3.11	*Fbp*	NAM	1	1
Glucose-6-phosphate dehydrogenase	E.C. 1.1.1.49	*G6pd*	NAM	1	1
Glutamate dehydrogenase	E.C. 1.4.1.2	*Gdh*	NAM, TBE	0	—
Glyceraldehyde-3-phosphate dehydrogenase	E.C. 1.2.1.12	*G3pd*	NAM	1	1
a-Glycerophosphate-dehydrogenase	E.C. 1.1.1.8	*aGpd*	TBE, OD	2	0
Glycogen phosphorylase	E.C. 2.4.1.1	*Phos*	NAM	1	1
Hydroxyacid dehydrogenase	E.C. 1.1.1.30	*Had*	TBE, MOPS	2	2
Hexokinase	E.C. 2.7.1.1	*Hk*	TBE	3	2
Isocitrate dehydrogenase	E.C. 1.1.1.42	*Idh*	NAM	2	2
Leucine aminopeptidase	E.C. 3.4.1.1	*Lap*	NAM	1	0
Malic dehydrogenase	E.C. 1.1.1.37	*Mdh*	NAM	2	2
Malic enzyme	E.C. 1.1.1.40	*Me*	TBE	1	0
Mannose phosphate isomerase	E.C. 5.3.1.8	*Mpi*	TBE	1	1
Phosphoglucoisomerase	E.C. 5.3.1.9	*Pgi*	TBE	1	0
6-Phosphoglucodehydrogenase	E.C. 1.1.1.44	*6pgd*	NAM	1	1
Phosphoglucomutase	E.C. 5.4.2.2	*Pgm*	NAM, TBE	1	1
Sorbitol dehydrogenase	E.C. 1.1.1.14	*Sdh*	NAM	0	—
Superoxide dismutase	E.C. 1.15.1.1	*Sod*	OD	2	0
Trehalase	E.C. 3.2.1.28	*Tre*	NAM	1	1
Triose-phosphate isomerase	E.C. 5.3.1.1	*Tpi*	NAM	1	0
Xanthine dehydrogenase	E.C. 1.1.1.37	*Xdh*	TBE	1	0
Totals				39	24

NAM, N-(3-aminopropyl)-morpholine-citrate pH 6.5; TBE, tris-borate EDTA pH 8.9, MOPS, 3-(N-morpholino)propane sulfonic acid; OD, Ornstein-Davis.

F_{ST} approaches its equilibrium value rapidly (Crow and Aoki 1984) unless pronounced founder effects caused the initial divergence among subpopulations.

There was difficulty in distinguishing some classes of heterozygotes at *Adk, Aox, Had+2,* and *Pgm,* and these technical problems resulted in an excess of homozygotes for certain allele combinations in populations (F_{IS}). The problem was that some alleles were too close together on gels to distinguish them in heterozygotes.

Results

Gene Diversity. Thirty-nine loci were resolved in North American *A. bipunctata,* of which 24 were polymorphic (61.5%), and mean diversity among these loci, on the basis of Hardy–Weinberg expectations, was H_E = 15.2 ± 3.3%. The mean number of alleles per locus was 1.9 ± 1.0 (Table 1). Gene diversity in the North American beetles was H_E = 17.3 ± 4.2% among the 31 loci used to compare populations (Table 2). Thus, there is plenty of variation with which to study gene flow.

The cultured Eurasian *A. bipunctata* were polymorphic for only 11 of 30 putative loci (37%); mean expected heterozygosity H_E = 11.4 ± 3.7%, and the mean number of alleles per locus was 1.5 ± 0. There was no significant difference in H_E between the Iowa and Eurasian samples as shown by their broadly overlapping standard errors.

Acid phosphatase-2 was not in Hardy–Weinberg equilibrium in Eurasian beetles (χ^2 = 18.41, df = 6, P = 0.005), but this locus was hard to score objectively because it was poorly resolved.

The distribution of single locus heterozygosities showed 2 modes, the 1st at 0–30%, and the 2nd at 46–70% (Fig. 1).

Gene Flow in North American *A. bipunctata*. Contingency chi-square analysis showed significant deviations from homogeneity among the 3 populations at 10 of 19 loci (Table 3) and differentiation was particularly marked at *Adk-1, Aox, G6pd,* and *Had+2.* Departures from random mating in pop-

Table 2. Gene diversity at polymorphic loci in North American *A. bipunctata*

Locus	*N*	No. alleles	Subunit structure	n_e[a]	h_o[b]	h_e[c]	F[d]
Acph-2	49	4	Tetramer	2.2	0.469	0.546	0.141
Adk-2	88	2	Monomer	1.1	0.045	0.066	−0.077[e]
Adk+3	56	2	Monomer	1.2	0.203	0.184	−0.094
Aox	88	4	Dimer	3.0	0.523	0.665	0.214
Argk-2	55	2	Monomer	1.1	0.055	0.054	−0.019
Dia-1	70	2	Monomer	1.0	0.029	0.029	0
Dia-2	56	2	Dimer	1.0	0.018	0.018	0
Fbp-2	41	3	Dimer	2.2	0.537	0.564	0.048
Fum	56	2	Dimer	1	0.018	0.018	0
G6pd	56	3	Dimer	2.3	0.544	0.559	0.027
G3pd	56	2	Tetramer	1.2	0.179	0.164	−0.091
Had-1	55	2	Dimer	2.0	0.418	0.502	0.167
Had+2	55	2	Dimer	1.9	0.400	0.484	0.174
Hk-1	88	2	Monomer	1.0	0.045	0.044	−0.024
Hk-2	88	2	Monomer	1.0	0.011	0.012	0.040
(Hk-2)[f]	56	2	Monomer	1.4	0.304	0.284	−0.070
Idh-1	56	2	Dimer	1.2	0.179	0.164	−0.091
Idh-2	88	3	Dimer	1.1	0.068	0.069	−0.019
Mdh-1	56	2	Dimer	1.4	0.304	0.284	−0.070
Mdh+2	52	2	Dimer	1.3	0.269	0.235	−0.145
Mpi	14	2	Monomer	1.2	0.143	0.133	−0.077
(Mpi)[f]	56	3	Monomer	2.6	0.574	0.620	0.074
Pgm	54	2	Monomer	1.2	0.148	0.199	0.256
Phos	14	3	Monomer	1.3	0.286	0.253	−0.132
6pgd	55	2	Dimer	1	0.036	0.036	0
Tre	55	5	Monomer	2.8	0.527	0.646	0.197
Polymorphic loci (n = 24):							
Means		2.46		1.49	0.227	0.247	0.018
SD		0.83		0.61	0.039	0.046	
All 39 loci:							
Means		1.90		1.30	0.140	0.152	
SD		0.97		0.53	0.030	0.033	

[a] Effective number of alleles, $n_e = 1/p_i$, where p_i is the frequency of allele I.
[b] Observed heterozygosity.
[c] Heterozygosity expected under random mating, $h_e = 1 - \Sigma\, p_i^2$.
[d] Inbreeding coefficient, $F = 1 - (h_o/h_e)$.
[e] $\chi^2 = 8.45$, $P < 0.004$ by test of Li and Horvitz (1953).
[f] Except when estimated from Uzbekistan beetles.

ulations, F_{IS}, were marked at *Adk-1, Aox, Had-1, Had+2,* and *Pgm* (Table 4). Ten loci showed more heterozygotes than expected in populations ($-F_{IS}$), however, suggesting that mating patterns alone were unlikely to have caused the paucity of heterozygotes. Mating patterns should affect all selectively neutral loci more or less equally (Wright 1978). When the problematic *Adk-2, Aox, Had+2,* and *Pgm* were dropped from consideration, the mean F_{IS} estimate no longer differed from zero (Table 4). Significant departures from random mating among the 3 North American populations (F_{ST}) were observed at 15 of 19 loci (Table 4). F_{ST} is the 'fixation index' and, for neutral alleles, is proportional to the amount of drift among populations. Its value, in ideal populations at equilibrium, should be nearly the same for all loci, but this clearly was not the case. We can obtain a 1st approximation of the mean number of reproducing migrants per generation Nm from F_{ST} and this is $Nm = [(F_{ST})^{-1}\text{-}1]/4 = [(0.068^{-1})\text{-}1]/4 = 3.4$ (Wright 1969). Thus, significant genetic differentiation was evident among widely scattered beetle populations, but the same evidence showed also a substantial level of gene flow among them.

North American Versus Eurasian *A. bipunctata*. Gene frequencies of Iowa and cultured F_2 Uzbekistan beetles were compared at the 17 polymorphic loci resolved in both collections. Contingency chi-square tests showed the 2 populations to differ significantly at 15 loci, and only *Fum* and *Idh-1* were homogeneous (Table 5). Wright's F statistics showed significant deficiencies of heterozygotes within populations at *Acph, Had-1, Pgm,* and *Tre*; and an excess of heterozygotes at *6pgd* (Table 6). As might be expected of geographically isolated populations, F_{ST} estimates were large, particularly at *Fbp, G6pd, Hk, Pgm,* and *Tre.* The mean F_{ST} value, 0.428 ± 0.128, demonstrates a substantial genetic distance between Iowa and the Uzbek *A. bipunctata* population.

Discussion

Gene Diversity. Gene diversity in North American *A. bipunctata,* at 15.2 ± 3.3%, is similar to that estimated in sevenspotted lady beetle, *Coccinella septempunctata* L., at 16.0 ± 4.1% (Krafsur et al. 1992), and in *Coleomegilla maculata* (De Geer), at 18.3 ± 3.5% (Krafsur et al. 1995). These

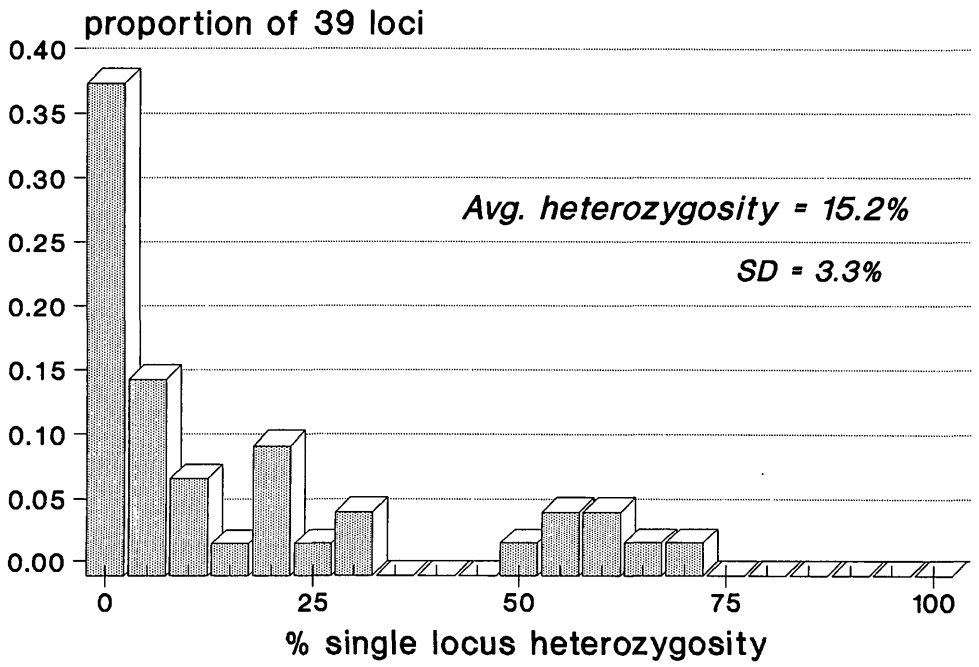

Fig. 1. Single locus heterozygosity (h_e) in *A. bipunctata* ($h_e = 1 - \Sigma p_i^2$, where p is the frequency of *allele I*).

predators maintain levels of gene diversity quite similar to the levels found in other beetle families (Hsaio 1989) and in many dipterans and lepidopterans (Graur 1985). It has been suggested that these high levels of diversity are maintained by large effective population sizes sustained over long time periods (Graur 1985). There is, as yet, no reason to believe that these diversities are directly related to the fitness of *A. bipunctata* to colonize habitats (cf., Hopper et al. 1993). Colonizing species must often go through short term genetic bottlenecks in which genetic diversity becomes greatly reduced until it is restored by immigration from other colonies. Nor is it clear that high levels of gene diversity at structural loci are necessary for the numerical and geographical expansion of a colonizing species; for example, the elm leaf beetle, *Xanthogaleruca luteola* (Muller) was first detected in North America in Baltimore in 1834 and has since spread throughout the continent. It shows a mean gene diversity of 0.0025 ± 0.0015 at 39 loci (Krafsur and Nariboli 1995).

Table 3. Contingency chi-square tests of homogeneity among North American *A. bipunctata* samples

Locus	No. alleles	χ^2	df	*P*
Adk-2	2	21.747	2	<0.001
Adk+3	2	5.841	2	0.054
Aox	4	30.725	6	<0.001
Argk	2	6.065	2	0.048
Dia-1	2	10.693	2	0.005
Dia-2	3	5.189	4	0.268
Fum	2	8.089	2	0.175
G6pd	4	30.914	6	<0.001
G3pd	2	2.695	2	0.260
Had-1	2	10.678	2	<0.005
Had+2	2	14.858	2	<0.006
Hk-1	2	8.771	2	0.012
Hk-2	2	5.316	2	0.070
Idh-1	2	5.609	2	0.061
Idh-2	3	4.469	4	0.346
Mdh-1	2	9.791	2	0.007
Mdh+2	2	1.678	2	0.432
Pgm	2	4.277	2	0.118
6pgd	2	4.158	2	0.125
Totals		191.561	50	≪0.001

The Uzbek *A. bipunctata*, at $H_E = 11.4 \pm 3.7\%$, showed less variation than the Ames population, but not significantly so. Some of the lesser diversity can be attributed to sampling and small effective population sizes in establishing the culture. We do not know how many beetles contributed to the culture, and genetic drift may have strongly affected allele frequencies.

There were some interesting differences in gene frequencies between Ames and Uzbek beetles. *Mpi* in Eurasian *A. bipunctata* was highly polymorphic, but monomorphic in Iowa. *Hk-2* was much more polymorphic in the Eurasian *A. bipunctata* than in the North American samples, but monomorphic loci in the cultured beetles originally may have been polymorphic, diversity becoming lost through drift. Eggington's (1986) western European beetles showed much greater diversities at *Idh-1, Idh-2, Me,* and *6pgd* than did the North American and cultured samples, data that tend to support the contention that the cultured Eurasian beetles had lost variation by drift. Additional sampling is necessary to confirm the possibility that

Table 4. *F* statistics for 3 North American *A. bipunctata* populations

Locus	F_{IS}	F_{ST}	F_{IT}
Adk-2	0.229	0.190***	0.376
Adk+3	−0.111	0.044	−0.062
Aox	0.159	0.064***	0.213
Argk	−0.082	0.045	−0.033
Dia-1	−0.060	0.091***	0.370
Dia-2	−0.014	0.012	−0.002
Fum	−0.056	0.066**	0.014
G6pd	−0.007	0.071***	0.064
G3pd	−0.080	0.009	−0.070
Had-1	0.148	0.087**	0.223
Had+2	0.243	0.132***	0.344
Hk-1	−0.060	0.072*	0.017
Hk-2	−0.020	0.036	0.017
Idh-1	−0.068	0.040	−0.025
Idh-2	−0.018	−0.008	−0.025
Mdh-1	−0.058	0.080**	0.029
Mdh+2	−0.121	−0.001	−0.122
Pgm	0.219	0.020	0.234
6pgd	−0.045	0.025	−0.018
Mean	0.063	0.069	0.127
Jackknife estimates over all loci			
Mean	0.067	0.070	0.133
SD	0.042	0.012	0.047
Estimates less *Adk-2, Aox, Had+2* and *Pgm:*			
Mean	−0.014	0.056	0.043
Jackknife estimates over all loci			
Mean	−0.008	0.058	0.051
SD	0.043	0.012	0.051

*, $P < 0.01$; **, $P < 0.001$; ***, $P < 0.0001$.

diversities are greater in Old World populations than among New World populations. The issue is important because of the much greater color polymorphisms in the Old World and with regard to the origin of New World *A. bipunctata.*

Breeding Structure. A deficiency of heterozygotes in populations (F_{IS}) was observed at some loci, but more loci showed a slight excess of heterozygotes. If forces of mutation, selection, drift, and migration (Hardy–Weinberg assumptions) were not operating on the loci studied, *F* statistics would have approached zero. To what forces may F_{IS} be attributed? It is hard to imagine that selection favored homozygotes. The Wahlund effect (Hartl and Clark 1989) could well explain some homozygote excess because the Ames beetles were taken from an overwintering aggregation derived from many breeding units and the Chicago collec-

Table 5. Contingency chi-square analysis for Ames and USDA Uzbek *A. bipunctata* populations

Locus	No. alleles	χ^2	df	*P*
Acph	4	26.242	3	0.0001
Adk+3	2	12.026	1	0.0005
Dia-2	2	6.699	1	0.0096
Fbp-2	3	56.307	2	0.0001
Fum	2	1.004	1	0.3162
G6pd	3	74.888	2	0.0001
G3pd	2	10.467	1	0.0012
Had-1	2	4.108	1	0.0427
Had+2	2	10.367	1	0.0013
Hk 2	1	59.023	1	0.0001
Idh-1	2	1.077	1	0.2994
Idh-2	2	3.041	1	0.0812
Mdh-1	2	6.266	1	0.0123
Mdh+2	2	3.195	1	0.0738
Pgm	3	220.000	2	0.0001
6pgd	2	9.468	1	0.0021
Tre	5	158.133	4	0.0001
Totals		762.312	25	<0.000001

Table 6. *F* statistics for Iowa and USDA Uzbekistan *A. bipunctata*

Locus	F_{IS}	F_{ST}	F_{IT}
Acph	0.1734	0.1056***	0.2607
Adk+3	−0.1047	0.0890***	−0.0064
Dia-2	−0.0700	0.0502***	−0.0163
Fbp-2	0.0467	0.3596***	0.3895
G6pd	0.0270	0.4389***	0.4540
G3pd	−0.0891	0.0818	0.0000
Had-1	0.1718	0.0263	0.1935
Had+2	0.0826	0.0803***	0.1562
Hk	−0.0686	0.8289	0.8172
Idh-1	−0.0732	0.0013	−0.0718
Idh-2	−0.0185	0.0182	0.0000
Mdh-1	−0.0654	0.0465**	−0.0159
Mdh+2	−0.1151	0.0212	−0.0915
Pgm	0.2586	0.9017***	0.9271
6pgd	−0.1167	0.0745***	−0.0336
Tre	0.1493	0.5603***	0.6259
Mean	0.0622	0.4139	0.4527
Jackknife estimates over loci:			
Mean	0.0714	0.4284	0.4685
SD	0.0338	0.1284	0.11857

**, $P < 0.001$; ***, $P < 0.0001$.

tion represented migrating beetles presumably derived from highly diverse locations (Lee 1980). But the largest factor contributing to the homozygote excess probably was difficulty in distinguishing some classes of heterozygotes at *Adk, Aox, Had+2,* and *Pgm.* In the foregoing loci, some allele combinations can be too close together on gels to distinguish visually. If these loci are dropped from consideration in the North American samples, the mean F_{IS} becomes −0.042 and mean F_{ST} becomes 0.045.

Spatial Components of Diversity. Clearly there was ample gene flow among *A. bipunctata* from Oregon, Iowa, and the Great Lakes. Analysis of Eggington's (1986) data showed a measure of genetic differentiation between 5 western European beetle collections (F_{DT} = 0.061), although this variation could not be partitioned within and among demes. There seems to be long distance movement in a number of coccinellids, including *A. bipunctata* (Lee 1980), and this tendency to migrate must have a homogenizing effect on the spatial components of gene diversity. Thus, genetic adaptation, if any, to environmental heterogeneities in climate and prey are constantly subject to dilution by immigrant genotypes.

Geographically more extensive sampling, subsampling of populations within regions, and larger sample sizes are necessary to examine gene flow within and among geographical regions in better detail. In particular, a large number of populations must be sampled to achieve more representative estimates of F_{ST}.

The genetic distance between the Eurasian *A. bipunctata* and Iowa samples, as measured by chi-square and F_{ST}, was particularly large. How much did genetic drift contribute to this? A small founding population size and subsequent drift during culture propagation may have contributed much to the difference observed between the 2 samples. On the other hand, the occurrence of variation at *Mpi* in the Eurasian ladybirds not found in the Ames beetles argues that much of the between-population variation is characteristic of the parental population. Eggington's data suggests rather different gene frequencies in western Europe than in North America. Is *A. bipunctata* an immigrant to North America that became established in colonial times, or has it been resident for much longer? We cannot yet answer the question with an acceptable degree of precision, but the allele frequency data support very many generations of separation of New World and Old World populations and no detectable recent infusion.

There are precedents for adventive introduction of lady beetles in historical times. Five coccinellid species have become established fortuitously in North America since 1900 (Gordon and Vandenberg 1991) and it is by no means certain that these are the only ones. We have examined numerous Old World and New World samples of the exotic coccinellid beetles *Hippodamia variegata* (Goeze) and *Propylea quatuordecimpunctata* (L.), and found genetic differences among populations (F_{ST}) of lesser magnitude than in *Adalia* (E.S.K., unpublished data). Sampling and analysis of additional Eurasian beetle populations is necessary to address adequately the question of genetic distance between New World and Old World populations.

Concluding Remarks. There is sufficient diversity in *A. bipunctata,* assuming selective equivalency of alleles, to examine in detail questions about gene flow among local, regional, and continental populations. Further studies are desirable to examine the effects of migrating swarms of beetles on breeding structure. Estimates of genetic distance (Wright 1978) should be made and these require additional sampling in the New and the Old World. It would be interesting also to examine the question of lady beetle population differentiation by prey species and by the prey's host plant. Are there races adapted for particular prey, or does natural selection favor a generalist approach, where beetles are able to switch to whatever prey species may be available?

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Appendix 1. Gene frequencies in Iowa *A. bipunctata*

Allele	Locus and sample size													
	Acph 49	*Adk-1* 56	*Adk-2* 56	*Adk+3* 54	*aGpd* 56	*Aox* 56	*Dia-1* 56	*Dia-2* 56	*Fbp-1* 56	*Fbp-2* 41	*Fum* 56	*G6pd* 57	*G3pd* 56	*Had-1* 55
A	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.061	0.089	0.464
B	0.061	1.000	1.000	0.102	0.000	0.339	0.000	0.991	0.000	0.585	0.009	0.500	0.911	0.536
C	0.622	0.000	0.000	0.898	0.000	0.411	0.000	0.009	0.000	0.122	0.991	0.439	0.000	0.000
D	0.255	0.000	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.293	0.000	0.000	0.000	0.000
E	0.061	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H_0	0.469	0.000	0.000	0.203	0.000	0.500	0.000	0.018	0.000	0.537	0.018	0.544	0.179	0.418

H_E = 0.190 ± 0.045, H_0 = 0.172 ± 0.039. Mean number of alleles per locus = 1.89 ± 0.19.

Appendix 2. Gene frequencies in Chicago *A. bipunctata*

Allele	Locus and sample size								
	Adk-2 14	*Adk+3* 14	*Aox* 14	*Argk* 14	*Dia-1* 14	*Dia-2* 14	*Fum* 14	*G6pd* 14	*G3pd* 14
A	0.179	0.000	0.107	0.143	0.929	0.000	0.000	0.000	0.000
B	0.821	1.000	0.286	0.857	0.071	1.000	1.000	0.429	1.000
C	0.000	0.000	0.536	0.000	0.000	0.000	0.000	0.571	0.000
D	0.000	0.000	0.071	0.000	0.000	0.000	0.000	0.000	0.000
H_0	0.214	0.000	0.643	0.286	0.143	0.000	0.000	0.571	0.000

H_E = 0.191 ± 0.049, H_0 = 0.177 ± 0.042. Mean number of alleles per locus = 1.89 ± 0.15.

Appendix 3. Gene frequencies in Oregon *A. bipunctata*

Allele	Locus and sample size								
	Adk-2 18	*Adk+3* 17	*Aox* 18	*Argk* 18	*Dia-1* 18	*Dia-2* 18	*Fum* 17	*G6pd* 10	*G3pd* 17
A	0.028	0.029	0.111	0.083	1.000	0.028	0.088	0.200	0.088
B	0.972	0.971	0.194	0.917	0.000	0.944	0.912	0.100	0.912
C	0.000	0.000	0.694	0.000	0.000	0.028	0.000	0.600	0.000
D	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.100	0.000
H_0	0.056	0.059	0.500	0.167	0.000	0.111	0.176	0.500	0.176

H_E = 0.186 ± 0.042, H_0 = 0.176 ± 0.037. Mean number of alleles per locus = 2.05 ± 0.18.

Appendix 4. Gene frequencies in cultured Uzbekistan *A. bipunctata*

Allele	Locus and sample size													
	Acph 48	*Adk-1* 56	*Adk-2* 56	*Adk+3* 56	*aGpd* 56	*Aox* 7	*Dia-1* 56	*Dia-2* 56	*Fbp-1* 56	*Fbp-2* 56	*Fum* 56	*G6pd* 56	*G3pd* 56	*Had-1* 55
A	0.000	1.000	1.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.600
B	0.156	0.000	0.000	0.000	0.000	0.500	0.000	0.920	0.000	1.000	0.000	1.000	1.000	0.200
C	0.271	0.000	0.000	1.000	0.000	0.214	0.000	0.080	0.000	0.000	1.000	0.000	0.000	0.200
D	0.385	0.000	0.000	0.000	0.000	0.286	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.188	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H_0	0.583	0.000	0.000	0.000	0.000	1.000	0.000	0.161	0.000	0.000	0.000	0.000	0.000	0.400

H_E = 0.127 ± 0.041, H_0 = 0.131 ± 0.044. Mean number of alleles per locus = 1.57 ± 0.16.

Appendix 1. Extended.

Locus and sample size													
Had+2 55	*Hk* 56	*Idh-1* 56	*Idh-2* 56	*Mdh-1* 56	*Mdh+2* 52	*Me* 56	*Pgi* 56	*Pgm* 54	*6pgd* 55	*Sod-1* 56	*Sod−2* 56	*Tpi* 56	*Tre* 55
0.600	0.000	0.000	0.000	0.170	0.865	1.000	1.000	0.000	0.000	1.000	1.000	1.000	0.009
0.400	1.000	0.911	0.973	0.830	0.135	0.000	0.000	0.111	0.982	0.000	0.000	0.000	0.127
0.000	0.000	0.089	0.027	0.000	0.000	0.000	0.000	0.889	0.018	0.000	0.000	0.000	0.409
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.418
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.036
0.400	0.000	0.179	0.054	0.304	0.269	0.000	0.000	0.148	0.036	0.000	0.000	0.000	0.527

Appendix 2. Extended.

Locus and sample size									
Had-1 14	*Had+2* 13	*Hk-1* 14	*Hk-2* 14	*Idh-1* 14	*Idh-2* 14	*Mdh-1* 14	*Mdh+2* 14	*Pgm* 14	*6pgd* 14
0.714	0.231	0.036	0.036	0.750	0.036	0.036	0.893	0.071	0.964
0.286	0.769	0.964	0.964	0.250	0.964	0.964	0.107	0.929	0.036
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.286	0.308	0.071	0.071	0.357	0.071	0.071	0.214	0.143	0.071

Appendix 3. Extended.

Locus and sample size									
Had-1 18	*Had+2* 17	*Hk-1* 18	*Hk-2* 18	*Idh-1* 18	*Idh-2* 17	*Mdh-1* 18	*Mdh+2* 18	*Pgm* 17	*6pgd* 16
0.722	0.353	0.083	0.000	0.833	0.029	0.000	0.944	0.000	0.906
0.278	0.647	0.917	1.000	0.167	0.941	1.000	0.056	1.000	0.094
0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.444	0.235	0.167	0.000	0.333	0.118	0.000	0.111	0.000	0.188

Appendix 4. Extended.

Locus and sample size													
Had+2 56	*Hk* 56	*Idh-1* 56	*Idh-2* 56	*Mdh-1* 56	*Mdh+2* 56	*Me* 56	*Pgi* 56	*Pgm* 56	*6pgd* 56	*Sod-1* 56	*Sod-2* 56	*Tpi* 56	*Tre* 56
0.384	0.000	0.000	0.000	0.063	0.938	1.000	1.000	1.000	0.000	1.000	1.000	1.000	0.036
0.616	0.170	0.946	1.000	0.938	0.063	0.000	0.000	0.000	0.875	0.000	0.000	0.000	0.938
0.000	0.830	0.054	0.000	0.000	0.000	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.027
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.482	0.304	0.107	0.000	0.125	0.125	0.000	0.000	0.000	0.250	0.000	0.000	0.000	0.125