Gene Flow in Colonizing *Hippodamia variegata* Ladybird Beetle Populations

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Genetic diversity and gene flow at allozyme loci was investigated in natural and laboratory-reared populations of the predatory ladybird species *Hippodamia variegata*, a recently naturalized beetle from the Palaearctic. Gene diversity was 24.9 ± 4.9% at 28 resolved loci and 31.6 ± 5.5% at 22 polymorphic loci. Average gene diversity at nine polymorphic loci was only slightly greater among field-collected beetles than among laboratory-cultured beetles. There were five alleles in cultured beetles not found in natural U.S. population samples. Wright's *F* statistics showed modest genetic differentiation among two field collections from the northeastern United States and one from Europe (F_{ST} = 0.107 ± 0.077). Analysis of variance in gene frequencies in these feral ladybirds showed that about 97% of the variance existed within populations of this colonizing species. A substantial level of genetic differentiation was detected among 10 laboratory populations descended from collections made in Europe, Asia, North Africa, and Chile (F_{ST} = 0.191 ± 0.021). Drift is the most likely explanation for this differentiation.

Hippodamia variegata (Goeze)(Coleoptera: Coccinellidae) is an Old World ladybird, occurring also in Africa. There is much variation in Old World *H. variegata* in color and pattern of the elytra (wing covers)(Dobzhansky 1933; Hodek 1973), but none has been noted among New World forms. The larvae and adults of *H. variegata* are important predators of aphids and other plant parasitic insects (Obrycki and Orr 1990). Both sexes disperse to search for prey, and the female ladybirds oviposit where aphids are abundant. Like most members of Coccinellidae, adult *H. variegata* are able to exploit habitats where prey exist and to move on to new, unexploited patches when local prey abundance dwindles. Thus *H. variegata* was considered to be a valuable agent for biological control of plant pests, and it was reasoned by the U.S. Department of Agriculture (USDA) that the species could add diversity to the endemic North American ladybird fauna of eight species (Wheeler 1993). The USDA-Animal Plant Health Inspection Service-Plant Pest and Quarantine (USDA-APHIS-PPQ) National Biological Control Laboratory at Niles, Michigan, propagated numerous cultures of *H. variegata* beetles descended from diverse worldwide populations. These reared beetles were distributed to cooperating agencies in the United States for release as biological control agents to supplement the endemic aphidophagous insect fauna. Attempts were made in 1957–1958 to establish *H. variegata* originating from India in Arizona, Florida, and Georgia. Further attempts were made with the same strain in 1983 in Louisiana, Maine, Maryland, Nebraska, New Jersey, Pennsylvania, South Carolina, and Texas (Gordon 1985). A strain of *H. variegata* originating in the Soviet Union was released in Delaware in 1981. Another round of attempts was made from 1987 to 1993, primarily in the western United States, but also in Maryland, Massachusetts, and Pennsylvania. In 1990, for example, more than 220,000 *H. variegata* were produced and released. Despite the massive numbers released in the western United States, no recoveries have been documented (Flanders RV, personal communication).

The first discovery of established *H. variegata* was made near Montreal, Quebec, Canada, in 1984 (Gordon 1987), and because there are no recorded attempts to introduce *H. variegata* there, its origin is unknown and is probably adventive [e.g., from ships transiting the St. Lawrence Seaway (Schaefer and Dysart 1988)]. Subsequent *H. variegata* findings include Vermont, New Hampshire, Massachusetts, Rhode Island, Connecticut, New Jersey, New York, and eastern Pennsylvania (Ellis

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Table 1. Geographical origins of *H. variegata* used in electrophoretic studies[a]

Origin	Population designation	Year of collection	No. collected	No. beetles to establish culture	Filial generation electrophoresed
Chile	Chile	1990	137	467	F_5
China	China	1992	203	99	F_5
Connecticut	CN	1993	51	—	P_0
Western Europe	Eur	1990	28	—	P_0
Greece	Greece	1993	25	11	F_3
Kazakhstan	KZ	1991	?	14	F_5
Kirghizia	KG	1989	580	138	F_5
Moldavia	Mold	1989	81	1,214	F_5
Morocco	Mor	1990	?	?	F_3
Quebec	Que	1987	165	221	F_3
Rhode Island	RI	1993	46	—	P_0
Spain	Spain	1992	35	142	F_2
Syria	Syria	1992	12	?	F_4

[a] Based on available USDA information.

and Adams 1993; Wheeler 1993). The possibility cannot be ruled out that at least some New World *H. variegata* originated from recorded and unrecorded releases generating populations that went undetected for years. With the assistance of USDA-APHIS-PPQ, we undertook studies on the population genetics of *H. variegata* to determine if there were distinguishing allozyme genetic markers that could be used to discriminate the strains of cultured beetles and gather evidence on the origin of naturalized populations in the United States. We found that all *H. variegata* sampled shared most of their detectable genetic variation.

Materials and Methods

Beetles

The countries of origin, founding numbers of cultured beetles, and the approximate cultured generation sampled for electrophoresis are set forth in Table 1. Field collected beetles that were obtained in the Old World were pooled and bred for at least one generation at the USDA European Parasite Laboratory in France. Progeny were sent to the USDA-ARS Quarantine Laboratory at Newark, Delaware, where there was at least one additional generation before progeny were shipped to the USDA National Biological Control Laboratory at Niles, Michigan. Connecticut and Rhode Island beetle collections were pooled from various fields in their respective states and therefore consisted of multiple breeding units. All beetles were chilled, shipped overnight, and received alive at Iowa State University, then were allowed to warm for about 4 h, and killed by freezing at −80°C. Beetles were stored at this temperature until use.

Electrophoresis and Staining

Laboratory procedures followed Krafsur et al. (1992). Acrylamide gels for anodally migrating enzymes 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 modified Ornstein-Davis (OD) (Black and Krafsur 1985a, system A) without the stacking gel, tris-borate EDTA pH 9 (TBE9), and NAM-citrate 6.5 (Clayton and Tretiak 1972). The TBE9 electrode and gel buffer consisted of 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.

Hoefer SE600 gel boxes were used for vertical slab electrophoresis. Sample homogenate (2 μl) was applied to each of 28 sample wells per gel. Ornstein-Davis and NAM gels were run at a constant 25 mA per gel; TBE gels were run at a constant 350 V.

Most staining recipes are in Murphy et al. (1990). For reactions requiring G6PDH or other coupling enzymes, agar overlays were made with five units of G6PDH, 0.5 mg PMS, 2 mg NAD, 2 mg NADP, and 1 mg MTT (15–20 ml total volume is sufficient to cover two gels). For other stains, 5 mg of NAD or NADP, 5 mg MTT, and 1 mg PMS per 100 ml of stain solution were used.

Allozymes were numbered according to increasing mobility. Subunit structures were determined by the banding patterns of heterozygotes. Monomeric enzymes showed two bands in heterozygotes, dimeric enzymes displayed three bands, and tetrameric enzymes showed five bands in heterozygotes. Putative genetic loci selected for study and the systems used to resolve them are shown in Table 2.

Analysis of Data

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 $[h_e - H_e]^2/[n(n-1)]$; the effective number of alleles n_e is $(\Sigma p_i^2)^{-1}$ (Nei 1987). Tests of homogeneity of allele frequencies in beetle samples were made using the methods of Workman and Niswander (1970). Wright's F statistics for analysis of population breeding structure were calculated. F statistics measure departures from random mating within (F_{IS}) and among (F_{ST}) populations and were estimated by the method of Weir and Cockerham (1984), whose formulae weight for variable sample sizes, number of alleles, and populations and also provide standard errors. Of particular interest was F_{ST}, defined variously as the standardized variance in allele frequencies among local populations or the correlation of random gametes within subpopulations relative to gametes within the entire population. Wright (1978) showed that, for independently assorting, selectively neutral alleles in populations at equilibrium, the relationship between F_{ST}, local population 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). Other F statistics include F_{IS}, the mean correlation between random gametes in individuals relative to those in their own subpopulation, and F_{IT}, the correlation between random gametes in individuals relative to the population as a whole. F statistics may be considered also in terms of departures from random mating at specified hierarchical levels. The null hypothesis that $F_{IS} = 0$ was tested by the statistic $\chi^2 = F_{IS}^2 N$ (Li and Horvitz 1953). That $F_{ST} = 0$ was tested by the statistic $\chi^2 = 2NF_{ST}(k-1)$, where N is the sample size and k is the number of alleles segregating at the locus (Workman and Niswander 1970). The F statistics are related as $(1 - F_{IT}) = (1 - F_{ST})(1 - F_{IS})$.

Nei's (1978) unbiased genetic distance, D, between all pairs of samples was used because it corrects for sample sizes. D is the negative natural log of the normalized genetic identity, which is the probability that a randomly chosen allele from each of two populations will be identical with

Table 2. Heterozygosity at putative allozyme loci in *H. variegata*

Enzyme	Locus	E.C. number	Buffer system	Expected heterozygosity (h_e)*
Acid phosphatase	*Acph*	E.C. 3.1.3.2	NAM	0.7229
Aconitase	*Aco*	E.C. 4.2.1.3	OD	0.6420
Aldehyde oxidase	*Aox*	E.C. 1.2.3.1.	NAM	0.4928
Adenylate kinase	*Adk*	E.C. 2.7.4.3.	NAM	0.0917
Aminopeptidase	*Amp-1*	E.C. 3.4.11.1	NAM	0
	Amp-2			0
Aspartate aminotransferase	*Aat*	E.C. 2.6.1.1.	TBE, OD	0.1884
Catalase	*Cat*	E.C. 1.11.1.6.	NAM	0.0606
Diaphorase	*Dia*	E.C. 1.8.1.4.	NAM	0.1423
Esterase	*Est-1*	E.C. 3.1.1.–	NAM	Polymorphic
	Est-2			Polymorphic
Fructose biphosphatase	*Fbp*	E.C. 3.1.3.11.	NAM	0.1125
Fumarate hydratase	*Fum*	E.C. 4.2.1.2.	OD	Polymorphic
Glucose-6-phosphate dehydrogenase	*G6pd*	E.C. 1.1.1.49	NAM	0.5831
Glucose oxidase	*Gox*	E.C. 1.1.3.4	TBE	0.7824
Glyceraldehyde-3-phosphate dehydrogenase	*G3pd*	E.C. 1.2.1.12	NAM	0
α-Glycerophosphate dehydrogenase	*α-Gpd*	E.C. 1.1.1.8.	TBE, OD	0.1355
Hexokinase-1	*Hk-1*	E.C. 2.7.1.1.	TBE	0.5717
Hexokinase-2	*HK-2*			Polymorphic
Hydroxy acid dehydrogenase	*Had*	E.C. 1.1.1.30	NAM, OD	0.7474
Isocitrate dehydrogenase-1	*Idh-1*	E.C. 1.1.1.42	NAM	0.1478
Isocitrate dehydrogenase-2	*Idh-2*		NAM	0.1666
Lactate dehydrogenase	*Ldh*	E.C. 1.1.1.27	NAM	0.2901
Malate dehydrogenase	*Mdh*	E.C. 1.1.1.37	NAM, OD	0.0496
Malic enzyme	*Me-1*	E.C. 1.1.1.40	OD	0
	Me-2		OD	0.035
Mannose-6-phosphate isomerase	*Mpi*	E.C. 5.3.1.8	NAM	Polymorphic
Phosphoglucoisomerase	*Pgi*	E.C. 5.3.1.9	OD	0.1342
Phosphoglucomutase	*Pgm*	E.C. 5.4.2.2	NAM, OD	0.3478
6-Phosphogluconate dehydrogenase	*6pgd*	E.C. 1.1.1.44	NAM	0.4290
Sorbitol dehydrogenase	*Sdh*	E.C. 1.1.1.14	NAM	Polymorphic
Superoxide dismutase	*Sod*	E.C. 1.15.1.1	OD	0.0868
Trehalase	*Tre*	E.C. 3.2.1.28	NAM	Polymorphic
Triose-phosphate isomerase	*Tpi*	E.C. 5.3.1.1	NAM	0
Xanthine dehydrogenase	*Xdh*	E.C. 1.1.1.37	TBE	0
		Mean of 22 polymorphic loci:		H_e = 0.3164
				Variance = 0.0030
		Mean of all loci (n = 28):		H_e = 0.2486
				Variance = 0.0024

* Expected proportions heterozygous when mating is random.

respect to the chance that two randomly chosen alleles will be from the same population. Thus, for $J = \Sigma p_i$ in populations x and y, $D = -\ln[\Sigma(Jxy/(2nxJx - 1)(2nyJy - 1)^{1/2})r]$ for allele frequencies p_i, sample size n, and r loci. Estimates of D were maximum because we did not include monomorphic loci in the computations.

The genetic statistics were obtained by using Biosys-1 (Swofford and Selander 1981) and Genestats (Black and Krafsur 1985b).

Results

About 29 of 35 (83%) presumptive loci were variable (Table 2). The gene diversity measures at 28 loci presenting interpretable Mendelian patterns showed that 22 were polymorphic (78.6%) at the 99% criterion. Mean heterozygosity among the 28 scorable loci was 24.86 ± 4.9% (SD). Heterozygosity was 31.6 ±5.5% among the 22 polymorphic loci.

Nine polymorphic loci were used to examine the breeding structure of diverse *H. variegata* populations (Table 3). The criterion for inclusion was that the vast majority of ladybirds in each collection stained well enough to provide unambiguous estimation of genotypes. There were 40 putative alleles segregating at these nine loci for a mean of 4.44 ± 1.13 alleles per locus. Heterozygosity averaged 25.7% among wild beetles and 22.5% among the laboratory beetles (Table 4). Five alleles segregating in laboratory populations were undetected in the wild collections (Table 4); these were, *Adk A*, detected only in Chile; *Idh-1 B*, found in Kirghizia, China, and Syria; *Mdh A* in Quebec and *Mdh D* in Kirghizia and Morocco; and α-*Gpd A*, in Quebec and Kirghizia. The frequencies of the five alleles undetected in field-collected *H. variegata* were present in low frequencies in the lab cultures, in which the total sample size was about six times greater than the field samples. All alleles detected in field-collected beetles were found also in at least one laboratory population.

Old World and New World samples did not obviously differ in their mean heterozygosities because their standard errors broadly overlapped (Table 5). Chinese beetles showed the greatest gene diversity, 29%, and Spanish beetles the least, 11% (Table 5), and these estimates seemed, on inspection, to be unrelated to the mean number of segregating alleles, the number of generations reared under artificial conditions, and the founding population sizes (Table 1).

Tests for homogeneity of allele frequencies (Workman and Niswander 1970) among wild populations showed significant differences at *Had* ($\chi^2_{[10]} = 130.6$, $P \approx 0$) and *6pgd* ($\chi^2_{[10]} = 23.7$, $P = .009$), and these two loci accounted for 83% of the total chi square ($\chi^2_{[52]} = 186.5$, $P \approx 0$). The 10 laboratory populations differed significantly at every locus, with total $\chi^2_{[279]} = 4547$, $P \approx 0$; tests of homogeneity applied to New World, western Europe, and Asian populations also showed significant differentiation at each locus. The totals were $\chi^2_{[52]} = 186.5$ for beetles cultured from the two New World collections, $\chi^2_{[28]} = 375.4$ for four cultures from Europe (including Morocco), and $\chi^2_{[104]} = 1362.2$ for cultures from Eurasia. All chi squares were highly significant ($P \approx 0$).

F statistics can measure departures from random mating within (F_{IS}) and between (F_{ST}) populations. In field-collected ladybirds, the F_{IS} statistics showed a small excess of heterozygotes at six loci and substantial deficiencies of heterozygotes at *Had* ($F_{IS} = 0.18$), *Idh-1* ($F_{IS} = 0.10$), and *6pgd* ($F_{IS} = 0.10$). The mean jackknife estimate for F_{IS} over the nine loci was 0.064 ± 0.063, not significantly different from zero.

Population differentiation of European and northeastern U.S. beetles was marginally significant ($F_{ST} = 0.107 \pm 0.0767$), but only *Had* ($F_{ST} = 0.225$, $P < .0001$) and *6pgd* ($F_{ST} = 0.04$, $P < .0001$) gave estimates that differed significantly from zero. The high interlocus errors suggest little more than larger sample sizes and more populations should be obtained.

In laboratory ladybird cultures, there was a large excess of heterozygotes only at *Idh-2*. Genetic differentiation was substantial ($F_{ST} = 0.1914 \pm 0.0208$) among laboratory populations, reflecting the diverse geographic origins of the strains and genetic drift. F_{ST} at each locus was significantly different from zero (Table 6).

Partitioning variance in gene frequencies (Wright 1978) of field-sampled ladybirds showed that 32.2% of the total variance was attributed to continents. Among

Table 3. Gene frequencies at nine polymorphic loci in *H. variegata*

	CN	RI	Que	Chile	Eur	Mor	Spain	Greece	Mold	Kirg	China	Kazak	Syria
Adk													
(*N*)	46	39	56	56	28	50	56	56	56	50	90	76	86
A	0.000	0.000	0.000	0.482	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.978	0.949	1.000	0.518	0.964	1.000	1.000	1.000	1.000	0.970	1.000	1.000	1.000
C	0.022	0.051	0.000	0.000	0.036	0.000	0.000	0.000	0.000	0.030	0.000	0.000	0.000
Dia													
(*N*)	51	33	56	56	28	50	56	56	84	50	85	84	84
A	0.000	0.000	0.000	0.000	0.018	0.000	0.000	0.000	0.089	0.070	0.000	0.000	0.000
B	0.039	0.121	0.000	0.000	0.089	0.010	0.000	0.161	0.113	0.130	0.000	0.000	0.119
C	0.961	0.879	1.000	1.000	0.875	0.980	1.000	0.839	0.798	0.800	1.000	1.000	0.881
D	0.000	0.000	0.000	0.000	0.018	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Had													
(*N*)	47	33	49	56	28	50	56	56	53	55	79	73	83
A	0.032	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.209	0.103	0.018
B	0.489	0.485	0.092	0.839	0.054	0.250	0.036	0.000	0.038	0.027	0.019	0.000	0.012
C	0.202	0.015	0.592	0.000	0.464	0.300	0.464	0.580	0.509	0.500	0.506	0.233	0.235
D	0.223	0.455	0.000	0.000	0.036	0.160	0.268	0.196	0.000	0.009	0.025	0.171	0.048
E	0.043	0.000	0.316	0.161	0.446	0.290	0.018	0.223	0.453	0.464	0.241	0.322	0.651
F	0.011	0.000	0.000	0.000	0.000	0.000	0.214	0.000	0.000	0.000	0.000	0.171	0.036
Idh-1													
(*N*)	51	32	56	56	28	50	56	56	56	50	70	62	81
A	0.020	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.040	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.143	0.000	0.056
C	0.892	0.922	0.991	1.000	0.929	1.000	1.000	0.875	0.991	0.900	0.586	1.000	0.901
D	0.059	0.047	0.009	0.000	0.018	0.000	0.000	0.125	0.009	0.000	0.157	0.000	0.043
E	0.029	0.031	0.000	0.000	0.054	0.000	0.000	0.000	0.000	0.000	0.114	0.000	0.000
Idh-2													
(*N*)	51	33	56	56	30	50	56	56	56	50	71	62	84
A	0.010	0.000	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.088	0.106	0.045	0.000	0.100	0.000	0.116	0.000	0.027	0.100	0.021	0.000	0.000
C	0.902	0.894	0.929	1.000	0.883	1.000	0.884	1.000	0.527	0.900	0.923	1.000	1.000
D	0.000	0.000	0.000	0.000	0.017	0.000	0.000	0.000	0.446	0.000	0.056	0.000	0.000
Mdh													
(*N*)	43	36	54	56	28	50	56	56	53	48	94	76	56
A	0.023	0.000	0.037	0.000	0.054	0.000	0.000	0.000	0.000	0.000	0.021	0.092	0.000
B	0.977	1.000	0.944	1.000	0.946	0.990	1.000	1.000	1.000	0.927	0.979	0.908	1.000
C	0.000	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000	0.073	0.000	0.000	0.000
Pgm													
(*N*)	51	46	54	59	28	48	56	56	54	52	55	73	84
A	0.029	0.000	0.000	0.000	0.036	0.010	0.000	0.000	0.000	0.048	0.000	0.000	0.000
B	0.118	0.163	0.046	0.042	0.143	0.458	0.027	0.420	0.074	0.173	0.091	0.151	0.161
C	0.804	0.826	0.889	0.780	0.804	0.531	0.973	0.580	0.917	0.731	0.764	0.849	0.833
D	0.020	0.011	0.056	0.178	0.018	0.000	0.000	0.000	0.009	0.048	0.145	0.000	0.006
E	0.029	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
6pgd													
(*N*)	49	32	53	56	28	49	56	57	55	50	51	73	97
A	0.020	0.016	0.028	0.089	0.000	0.000	0.018	0.000	0.000	0.000	0.039	0.000	0.314
B	0.020	0.094	0.132	0.473	0.071	0.500	0.000	0.465	0.082	0.010	0.010	0.110	0.000
C	0.092	0.109	0.000	0.000	0.000	0.000	0.000	0.000	0.009	0.000	0.275	0.000	0.021
D	0.745	0.641	0.764	0.438	0.893	0.500	0.982	0.535	0.882	0.980	0.676	0.890	0.665
E	0.112	0.141	0.057	0.000	0.000	0.000	0.000	0.000	0.027	0.000	0.000	0.000	0.000
F	0.010	0.000	0.019	0.000	0.036	0.000	0.000	0.000	0.000	0.010	0.000	0.000	0.000
α-Gpd													
(*N*)	51	32	56	56	28	49	56	56	56	50	84	73	56
A	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000	0.000	0.060	0.000	0.000	0.000
B	0.980	0.984	0.955	1.000	0.982	0.929	1.000	1.000	0.920	0.840	0.798	1.000	0.670
C	0.020	0.016	0.000	0.000	0.018	0.071	0.000	0.000	0.080	0.100	0.202	0.000	0.330

Table 4. Gene frequencies among *H. variegata* samples pooled according to origin

		Mean frequency	
Locus	Allele	Wild	USDA cultures
Adk	(*N*)	113	632
	A	0	0.0482
	B	0.9637	0.9488
	C	0.0363	0.0030
Dia	(*N*)	112	661
	A	0.0060	0.0159
	B	0.0832	0.0533
	C	0.9049	0.9298
	D	0.0059	0.0010
Had	(*N*)	108	610
	A	0.0258	0.0330
	B	0.3426	0.1313
	C	0.2272	0.3920
	D	0.2379	0.0878
	E	0.1630	0.3137
	F	0.0035	0.0422
Idh-1	(*N*)	111	593
	A	0.0065	0.0040
	B	0	0.0258
	C	0.9142	0.9244
	D	0.0412	0.0343
	E	0.0381	0.0114
Idh-2	(*N*)	114	597
	A	0.0033	0.0027
	B	0.0981	0.0308
	C	0.8931	0.9162
	D	0.0055	0.0503
Mdh	(*N*)	107	599
	A	0	0.0019
	B	0.0256	0.0150
	C	0.9744	0.9748
	D	0	0.0083
Pgm	(*N*)	125	591
	A	0.0217	0.0058
	B	0.1412	0.1643
	C	0.8112	0.7847
	D	0.0161	0.0442
	E	0.0098	0.0009
6pgd	(*N*)	109	597
	A	0.0120	0.0489
	B	0.0619	0.1781
	C	0.0671	0.0304
	D	0.7594	0.7313
	E	0.0843	0.0084
	F	0.0153	0.0029
α-Gpd	(*N*)	111	592
	A	0	0.0105
	B	0.9823	0.9111
	C	0.0177	0.0784
	Means	0.2571	0.2250
	SD[a]	0.0758	0.0721

[a] $SD = \sqrt{\Sigma (h_e - H_e)^2/[n(n-1)]}$.

the laboratory populations, only 3.4% of the variance was attributed to continents, and 96.6% was within samples (Table 7). Thus, most genetic variation in *H. variegata* exists within populations.

What do the data show with respect to the origin of New World beetles? The genetic distances (Nei 1978) of the 13 collections varied from 0.006 for Connecticut and Rhode Island to 0.177 between Chilean and Moldavian beetles (Table 8). Rhode Island and Connecticut ladybirds were each made up of several small collections and were virtually identical. Chilean ladybirds were the most distantly related, forming a separate cluster. European *H. variegata* stock was taken to South Africa in 1967 for biocontrol of aphids on grains, and stocks were then taken to Chile at some later time. Other than the Chile population, the data show no strong geographic grouping, reinforcing the view that most gene diversity is shared among samples. Visual inspection of genetic distances between the northeastern U.S. beetles and the Quebec or European samples did not suggest any particular affinity. Moreover, the genetic distances do not support a hypothesis that the feral Northeastern beetles were related to any particular USDA strain.

Discussion

Average gene diversity at 28 scorable loci was 24.9 ± 5.5% in *H. variegata*, which was greater than the 18.3 ± 3.5% observed in the ladybird *Coleomegilla maculata* (Krafsur et al. 1995b) and 16 ± 4% estimated in the ladybird *Coccinella septempunctata* (Krafsur et al. 1992). These are high levels of allozyme variation, perhaps character-

Table 5. Genetic heterozygosity at polymorphic loci in *H. variegata* populations

Population	Mean sample size	Mean no. alleles per locus	No. polymorphic loci	Heterozygosity
New world:				
CN (field)	48.9	3.6 ± 0.6	10	0.225 ± 0.073
RI (field)	35.1	2.7 ± 0.4	9	0.234 ± 0.069
Que (lab)	54.4	2.7 ± 0.4	8	0.167 ± 0.063
Chile (lab)	56.3	1.7 ± 0.3	4	0.191 ± 0.081
Europe:				
EUR (field)	28.2	3.0 ± 0.3	10	0.213 ± 0.056
Greece (lab)	56.1	1.7 ± 0.2	5	0.230 ± 0.082
Mold (lab)	58.1	2.4 ± 0.3	7	0.217 ± 0.071
Mor (lab)	49.6	2.1 ± 0.4	6	0.217 ± 0.096
Spain (lab)	56.0	1.8 ± 0.4	4	0.107 ± 0.074
Asia:				
China (lab)	75.4	2.8 ± 0.5	7	0.291 ± 0.085
Kaz (lab)	72.4	1.8 ± 0.4	4	0.156 ± 0.085
Kir (lab)	50.6	2.9 ± 0.3	10	0.245 ± 0.056
Syria (lab)	79.0	2.4 ± 0.5	6	0.234 ± 0.070

Table 6. *F* statistics for laboratory populations of *H. variegata*

Locus	F_{IS}	F_{ST}	F_{IT}
Adk	0.0039	0.4572[a]	0.4593
Dia	0.0004	0.0948[a]	0.0952
Had	0.0326	0.2037[a]	0.2296
Idh-1	−0.0015	0.1567[a]	0.1554
Idh-2	−0.3530	0.2593[a]	−0.0021
Mdh	−0.0638	0.0456[a]	−0.0153
Pgm	0.0528	0.1172[a]	0.1638
6pgd	−0.0441	0.2229[a]	0.1886
α-Gpd	0.0493	0.1426[a]	0.1849
Mean	−0.0086	0.1901	0.1832
Jackknife estimates over loci:			
Mean	−0.0036	0.1914	0.1883
SD	0.0314	0.0208	0.0276

[a] $P < .0001$.

istic of colonizing species whose populations show "boom and bust" dynamics in relation to ephemeral prey resources (Southwood et al. 1974). Other examples of colonizing species include *Drosophila* spp. (Graur 1985, Singh and Rhomberg 1987), *Aedes* mosquitoes (Black et al. 1988; Wallis et al. 1983), *Culex* mosquitoes (Raymond et al. 1991), and two *Musca* spp. (Krafsur 1993). Only 25%–33% of the amino acid substitutions in structural loci can be detected by one-dimensional electrophoresis (Nei 1987), so it would seem that we have substantially underestimated heterozygosity in *H. variegata.*

Gene diversity at nine polymorphic loci was 29% among F_5 beetles from China, the highest level of heterozygosity detected in 13 populations, and greater than the heterozygosities found in three pooled collections of feral beetles, one of which was made in Europe. The greater diversity among Chinese beetles than in the other sampled populations might argue that east Asia is the "home" of *H. variegata,* or where the largest, continuously breeding populations exist, but we should examine all resolvable loci in this strain, the field-collected beetles, and other laboratory strains to examine the question properly. Alternatively, selection may have promoted heterozygosity in the Chinese beetle culture, but, if so, why not the other cultures?

The feral ladybirds showed significant heterozygote deficiencies within populations at *Had, Idh-1,* and *6pgd,* and a substantial heterozygote excess (negative F_{IS}) at *Dia, Idh-2,* and *Pgm.* Nonrandom mating would have affected all neutral loci in the same direction, so there may have been technical problems in scoring some of the loci. Departures from random mating among populations was detected also at *Had* and *6pgd,* which might be explained by an uneven scoring of gels from the different populations but is more likely a result of geographic separation of the European and North American ladybirds. Geographic separation is supported by the analysis of variance, which showed that 32% of the variance in gene frequencies was between continents (Table 8). Technical problems could include a failure to detect heterozygotes because of inadequate resolution, but no such problems were detected in cultured beetle populations. The Wahlund effect also could explain the heterozygote deficiencies within populations because these collections were pooled from numerous small samples, but if so, would the heterozygote excesses also have been observed? Sample sizes of feral beetles were small. The jackknife means and standard deviations support the view that the *F* statistics did not differ significantly from zero. It will be interesting to examine additional samples of *H. variegata* as it enlarges its geographic range.

Would collection and laboratory propagation of independent samples from geographically diverse ladybird populations promote or diminish genetic differentiation? Cultures were begun with good numbers of beetles (Table 1), and ladybird culture is accomplished by providing aphids as prey. Mortality due to cannibalism can occur if cultures are too crowded, but in well-maintained cultures there is usually little mortality during laboratory adaptation and yields are substantial. Thus, bottlenecks in culture probably did not occur. This fairly rules out convergence of various strains to a common laboratory phenotype and, simultaneously, does not encourage a high rate of drift beyond that obtained from the original sampling of field populations. It would therefore seem that gene frequency differences among ladybird cultures may be largely representative of the differences that existed among the parental populations. Examined in this light, $F_{ST} = 0.19$ among the laboratory populations sampled from four continents would suggest a substantial amount of gene flow among ancestral populations. Average gene flow amounts to a mean 1.1 reproducing immigrants per population per generation according to Wright's island model (Wright 1978). Dropping Chilean beetles from the estimate gives $F_{ST} = 0.144 \pm 0.023$, which suggests about 1.5 reproducing immigrants per generation. These data do not suggest a significant amount of laboratory contamination among the different USDA *H. variegata* cultures. Laboratory contamination can be a serious problem in many organisms (Mulvey and Vrijenhoek 1981).

To what extent heterozygosity at structural gene loci is correlated with and enhances fitness remains controversial. The

Table 7. Analyses of variance (nested classification) in gene frequencies at nine loci in natural and laboratory *H. variegata* populations of diverse geographic origin

Source	Variance	Percentage of variance
Three field populations:		
Within continents	0.10452	67.8
Between continents	0.04970	32.2
Total	0.15422	
Ten laboratory populations:		
Within continents	0.38663	96.6
Between continents	0.01354	3.4
Total	0.40017	

Table 8. Nei's (1978) unbiased genetic distances among *H. vareigata* populations

Population	1	2	3	4	5	6	7	8	9	10	11	12	13
1 Connecticut	**												
2 Rhode Island	0.006	**											
3 Quebec	0.032	0.061	**										
4 Chile	0.075	0.078	0.118	**									
5 Europe	0.034	0.060	0.005	0.132	**								
6 Morocco	0.047	0.053	0.044	0.087	0.046	**							
7 Spain	0.027	0.044	0.019	0.143	0.021	0.072	**						
8 Greece	0.062	0.073	0.036	0.137	0.037	0.014	0.060	**					
9 Moldavia	0.069	0.097	0.031	0.177	0.023	0.089	0.047	0.077	**				
10 Kirghizia	0.049	0.079	0.014	0.161	0.002	0.060	0.031	0.049	0.030	**			
11 China	0.050	0.080	0.030	0.164	0.033	0.073	0.046	0.059	0.063	0.037	**		
12 Kazakhstan	0.029	0.044	0.016	0.121	0.010	0.042	0.015	0.044	0.046	0.021	0.041	**	
13 Syria	0.070	0.089	0.042	0.156	0.032	0.067	0.068	0.074	0.062	0.031	0.047	0.039	**

conventional wisdom in building laboratory stocks of insects for eventual pest management, be they predators, parasitoids, or insects bred for release as sterile males, is that they should incorporate as much genetic diversity as is available to the species (Hopper et al. 1993; Roush 1990). It would seem that such variation is abundant in both the natural and cultured coccinellid beetles studied to date, and that even small collections of feral beetles probably would incorporate a representative amount of diversity with which to propagate beetles for release.

Nei's (1978) unbiased genetic distances did not confirm the Old World origins of *H. variegata* resident in the New World. Chilean beetles showed the largest genetic distances, and probably reflect the longest operation of drift and selection. The genetic distance was minimal between Rhode Island and Connecticut beetles (0.006), an unsurprising result. Feral European and cultivated Quebec beetles also showed a trivial genetic distance, less than those between Quebec and the two northeastern U.S. *H. variegata* collections. Analysis of variance of gene frequencies showed a minor difference between lab cultures derived from New World and Old World populations (Table 7). While contamination could in principle account for this, the substantial fixation index for all lab cultures (F_{ST} = 0.19 ± 0.02) argues against a significant degree of contamination.

H. variegata and *C. septempunctata* are widely distributed and abundant aphid predators in Palearctic natural ecosystems and in agroecosystems (Hodek 1973; Honek 1985). They have become established fortuitously in parts of northeastern North America (Day et al. 1994; Gordon 1985, 1987; Gordon and Vandenberg 1991; Schaefer and Dysart 1988; Wheeler 1993), whereas institutional attempts to establish these species seem not to have been successful (Day et al. 1994; Flanders et al. 1991; Schaefer et al. 1987). Through all the attempts to manipulate populations of these beneficial predators, no efforts were directed toward a better understanding of their genetics and breeding structures, although genetic phenomena were discussed extensively in attempts to understand "failure" and predict "success." Recent studies on gene diversity in the aforementioned ladybird beetles suggest high levels of gene flow among populations. This gene flow may be characteristic of most species; Coll et al. (1994) and Krafsur et al. (1995b) independently investigated gene flow in a New World coccinellid beetle *Coleomegilla maculata,* again finding high levels, and similar results have been obtained in the exotic ladybird *Propylea quatuordecimpunctata* (Krafsur and Obrycki 1995) and the circumpolar ladybird *Adalia bipunctata* (Krafsur et al. 1995a). It would seem, on present evidence, that genetic phenomena have little to do with achieving the establishment of exotic ladybirds by breeding up great numbers for massive releases. After all, these are colonizing species, and they may perceive an environment mostly in terms of prey availability. Failures to achieve self-sustaining populations of released forms may be related more to the particular conditions of rearing, handling, and release with regard to diapause and reproductive physiology than they are to heritable properties of the beetles themselves.

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