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Coleomegilla maculata (Coleoptera: Coccinellidae) is a Species Complex

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Coleomegilla maculata De Geer is an abundant, widely distributed, New World polyphagous lady beetle. High levels of variation at 14 polymorphic allozyme loci were used to examine breeding structure of populations from New England, Iowa, south Texas, and Honduras. Analysis of variance of gene frequencies and *F*-statistics showed high levels of gene flow within each region and between the Texan and northern United States populations, but negligible rates of gene flow between these and the Honduran populations. Thus, gene flow was largely unrestricted in North American *C. maculata.*Honduras populations were highly differentiated genetically from the North American populations and shared with North American beetles only 41 of 70 alleles at 14 allozyme loci. Nei's genetic distances within Honduran, Texas, and Iowa-New England samples did not differ significantly from zero, but the intergroup distances were large. Reciprocal crosses within and between Texas and Iowan populations were fertile, but reciprocal crosses between Honduran and North American strains were completely sterile. No consistent morphological differences between North and Central American *C. maculata* were detected. Backcrosses of male and female hybrids of Iowa and Texas beetles to either parental strain were fertile. The results indicate two sibling species are present, one in North America and another in Honduras.

Keywords
Coleomegilla maculata, sibling species, gene flow, speciation

Comments
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Coleomegilla maculata (Coleoptera: Coccinellidae) is a Species Complex

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ABSTRACT *Coleomegilla maculata* De Geer is an abundant, widely distributed, New World polyphagous lady beetle. High levels of variation at 14 polymorphic allozyme loci were used to examine breeding structure of populations from New England, Iowa, south Texas, and Honduras. Analysis of variance of gene frequencies and *F*-statistics showed high levels of gene flow within each region and between the Texan and northern United States populations, but negligible rates of gene flow between these and the Honduran populations. Thus, gene flow was largely unrestricted in North American *C. maculata.* Honduras populations were highly differentiated genetically from the North American populations and shared with North American beetles only 41 of 70 alleles at 14 allozyme loci. Nei's genetic distances within Honduran, Texas, and Iowa–New England samples did not differ significantly from zero, but the intergroup distances were large. Reciprocal crosses within and between Texas and Iowan populations were fertile, but reciprocal crosses between Honduran and North American strains were completely sterile. No consistent morphological differences between North and Central American *C. maculata* were detected. Backcrosses of male and female hybrids of Iowa and Texas beetles to either parental strain were fertile. The results indicate two sibling species are present, one in North America and another in Honduras.

KEY WORDS *Coleomegilla maculata,* sibling species, gene flow, speciation

Coleomegilla maculata De Geer (Coleoptera: Coccinellidae) is widely distributed from southern Canada to northern South America (Gordon 1985). This polyphagous predatory species is abundant in herbaceous crops, including corn (*Zea mays* L.) (Kieckhefer and Elliot 1990), alfalfa (*Medicago sativa* L.) (Giles et al. 1994), and potato (*Solanum tuberosum* L.) (Obrycki and Tauber 1985, Groden et al. 1990; Hilbeck and Kennedy 1996). Based on adult size, color patterns, and male genitalia, three subspecies of *C. maculata* were recognized in America north of Mexico, and the ranges of the subspecies greatly overlap (Gordon 1985). Nine races or varieties of *C. maculata* were described from Mexico, Central, and South America (Timberlake 1943). These intraspecific designations were based solely on continuous variation in adult coloration and morphology. Also, geographic variation in prey suitability of *Leptinotarsa decemlineata* Say eggs for *C. maculata* larvae from North and Central America has been documented (Munyaneza and Obrycki 1998).

Allozyme loci in North American *C. maculata* are highly diverse, and the spatial components of this diversity have been reported. Coll et al. (1994) found no significant departures from random mating among six Maryland *C. maculata* populations sampled in an altitudinal transect extending from the Appalachians to the Eastern Shore. Krafsur et al. (1995) found no departures from random mating among 12 populations from Delaware, New England, and the midwestern United States. These data indicate a geographically large pool of randomly mating beetles.

Additional North American populations have now been sampled, and they too provide no evidence of significant genetic differentiation among them, thereby confirming remarkably high rates of gene flow. But an examination of allozyme genetic diversity of *C. maculata* from Honduras indicated large differences when compared with North American populations. Here we present new data on the breeding structure of geographically diverse *C. maculata* populations and provide evidence that *C. maculata* in North and Central America consists of at least two sibling species.

Materials and Methods

Biological Material. Based on geographic origins, the *C. maculata* population from Iowa represents *C. maculata lengi*, whereas the Texas samples would be *C. maculata strenua* (Gordon 1985). The Honduras samples were likely *C. maculata medialis* (Timberlake 1943). Beetles were field collected by using 35-cm sweep nets, chilled, and shipped to Ames, IA, where they were either killed by freezing at –75°C or reared for breeding studies. The frozen beetles were homogenized in 1.5-ml microfuge tubes each containing 150 μl of grinding buffer.

Collections of adult *C. maculata* were made during July and August 1995 in West Kingston, Washington County, RI; Storrs, Tolland County, CT; Watertown,

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Table 1. Allozyme variation at 14 loci in *C. maculata*

Sample	Mean sample size, *n*	Mean alleles per locus	% polymorphic loci	Mean heterozygosity	
				Observed, H_E	Expected, H_O
Iowa A	9.9	2.1 ± 0.3	71.4	0.229 ± 0.062	0.229 ± 0.066
Iowa B	14.4	2.5 ± 0.3	78.6	0.220 ± 0.063	0.239 ± 0.071
New York	11.5	2.6 ± 0.4	71.4	0.240 ± 0.068	0.264 ± 0.069
CN, RI	13.1	2.4 ± 0.3	85.7	0.242 ± 0.056	0.232 ± 0.058
TX-M	22.1	1.9 ± 0.3	50.0	0.230 ± 0.080	0.207 ± 0.063
TX-P	14.0	2.1 ± 0.3	64.3	0.209 ± 0.074	0.185 ± 0.055
TX-RG	12.3	2.0 ± 0.3	64.3	0.252 ± 0.078	0.217 ± 0.060
Honduras A	45.4	2.9 ± 0.4	78.6	0.243 ± 0.073	0.261 ± 0.076
Honduras B	22.6	2.4 ± 0.3	78.6	0.257 ± 0.063	0.270 ± 0.067
Honduras C	29.6	2.9 ± 0.4	78.6	0.292 ± 0.079	0.271 ± 0.071

Jefferson County, NY; and Ames, Story County, IA. Electromorphs form the above populations were examined with *C. maculata* collected from the Department of Francisco Morazan, Tegucigalpa, Honduras. Additional collections of *C. maculata* were made in June 1996 in Ames, Story County, IA, and in May 1996 and June 1997 from the Department of Francisco Morazan, Zamorano, Honduras. Collections were also made in Texas during June 1997 in the Rio Grande Valley, Progreso, Hidalgo County, and Moore Air Force Base, Hidalgo County. The Honduran genotypes were always compared with North American genotypes in each electrophoretic run. A sample of field collected adults was confirmed as *C. maculata* by Natalia Vandenburg, USDA, SEL, Beltsville, MD.

Voucher specimens have been placed in the Iowa State University insect collection.

Electrophoresis. Electrophoretic methods have been set forth earlier (Krafsur et al. 1995) and are only briefly outlined here. Beetles were ground individually in a pH 8.6 grinding buffer (Black and Krafsur 1985a). Vertical slab acrylamide gels consisted of 6.18% acrylamide plus 0.325% bis-acrylamide, 0.05% ammonium persulfate, and 0.15% TEMED in gel buffer.

Electrophoresis was performed in Hoefer SE600 (Amersham Pharmacia Biotech, Piscataway, NJ) gel boxes at 0–4°C. Only 1.2–2.5 μl of sample homogenate was applied to each well and homogenates from 28 beetles were run on each gel.

We examined 14 loci that were shown earlier to be polymorphic (Krafsur et al. 1995). Staining methods generally followed those of Murphy et al. (1990). Agar overlays were used to resolve coupled reactions (e.g., adenylate kinase [*Ak*], phosphoglucomutase [*Pgm*], and isocitrate dehydrogenase [*Idh*]).

Crosses Between Geographical Strains. Pairs of Iowa, Texas, and Honduras *C. maculata* were set up from field collected adults in July 1997. These individuals were reared on pea aphids, *Acrythosiphon pisum* (Harris), and green peach aphids, *Myzus persicae* (Sulzer), using standard rearing procedures (Phoofolo and Obrycki 1997). Three to five isofemale lines were established for each geographic population. Reciprocal crosses between populations were made by using F_1 laboratory reared individuals. Second laboratory generation hybrid males were backcrossed to parental populations. When feeding pairs, daily observations of mating were recorded, and egg masses were removed from the adult cages. Mating frequencies based on the foregoing observations were used to test for premating barriers. Egg masses were considered fertile if any eggs hatched.

Analysis of Data. An unbiased measure of gene diversity at a locus was measured by the statistic $h_e = n(1-\Sigma p_i^2)/(n-1)$, where p_i is each putative allele at the locus (Nei 1987). Gene diversity for n loci is $H_E = \Sigma(h_e)/n$, with variance $\Sigma(h_e - H_E)^2/[n(n-1)]$. H_E and h_e are the expected heterozygosities when mating is random and other Hardy–Weinberg assumptions apply. Gene frequency data were analyzed by using BIOSYS-1 (Swofford and Selander 1981) and GENESTATS (Black and Krafsur 1985b). Wright's (1978) methods were used to partition variance in gene frequencies into two components, within and among beetle collections. Chi-square tests of homogeneity of gene frequencies were done by using the methods of Workman and Niswander (1970). Weir and Cockerham (1984) formulae were used to calculate *F*-statistics because their methods weight for variable sample sizes, number of alleles and populations, and provide standard errors. The *F* statistics are: F_{IS} is the correlation of genes in individuals in populations; it measures the mean departure from random mating within populations and takes the expected value of $-2N^{-1}$ (Nei 1987). F_{ST} is the average correlation of genes from two randomly chosen individuals in populations relative to the total. It has the expected value of $-N^{-1}$. F_{IT} is the mean correlation of genes in individuals averaged over all populations and measures "inbreeding" from all causes. The hypothesis that $F_{ST} > 0$ at a locus of k alleles and s subpopulations was tested according to the relationship, $\chi^2 = 2N(F_{ST})(k-1)$ with $(k-1)(s-1)$ degrees of freedom.

Genetic distances were calculated by using BIOSYS-1. The measures adopted included the unbiased distance *D* of Nei (1987) and the arc distance of Cavalli-Sforza and Edwards (1967). *D* is a commonly used index and the arc distance measure is advocated by Wright (1978).

Results

Gene Diversities. Of 70 detected electrophoretic alleles, only 41 (58.6%) were shared between North American and Honduran lady beetles. There were 11

Table 2. Contingency chi-square analysis of allozyme loci in *C. maculata* populations (data are summed over 14 loci)

Locus	No. Populations	Chi-square	df	*P*
Northern U.S.	4	121.12	114	≈0.300
South Texas	3	188.52	46	<0.001
Heterogeneity[a]		309.64	92	<<0.001
North America	7	731.92	252	<0.001
Honduras	3	367.83	74	<0.001
Heterogeniety[b]		2,618.36	326	<<0.001
All populations	10	3,718.11	504	<<0.001

[a] Chi-square between northern United States and Texas.
[b] Chi-square between Honduras and North America.

"private" (unshared) alleles in the Honduran samples and 18 private alleles in the North American samples. Thus, 41.4% of the alleles represented fixed differences between the North American and Honduran samples, a large proportion. The data are set forth in *Appendix 1.*

Consistent with earlier results (Krafsur et al. 1995), there were high levels of heterozygosity in *C. maculata:* mean heterozygosity was 0.244 ± 0.068 over the 14 loci and 10 samples (Table 1). There were no significant differences among samples in mean alleles per locus ($F = 0.89$; df = 9, 379; $P \approx 0.53$). Expected sample heterozygosities differed ($F = 6.93$; df = 9, 379; $P < 0.001$). Using the Tukey multiple comparison test, nine of 45 pairwise comparisons were significant. Mean heterozygosities were 0.241 ± 0.066 for the northern U.S. samples, 0.203 ± 0.060 for the Texas samples, and 0.266 ± 0.072 for the Honduran samples. These grouped samples differed significantly, each from the other ($F = 27.8$; df = 2, 387; $P < 0.001$).

Genotypic Differentiation. Chi-square tests showed no significant differentiation among the northern populations (Table 2). South Texas populations differed significantly, however, at *Pep-2*, *6pgd*, and *Pgm.* Genotypic differentiation among the Honduras samples was significant at loci coding for *Had-1*, *Idh-1*, *Pep-2*, *6pgd*, *Pgi*, and *Sod-1.* Only *Adk*, *α-Gpd*, and *Mpi* were homogeneous over all populations, consistent with a hypothesis of selection. The large heterogeneity components (Table 2) indicate the population gene frequencies grouped by region differed greatly each from the other, and the Honduran populations differed the most.

Table 3. ANOVA in allelic frequencies for populations grouped into two (United States, Honduras) or three regions (northern United States, Texas, Honduras)

Contrast	Variance	%	*F* xy[a]	*Nm*[b]
Populations in 2 regions	0.74035	30.0	0.192	1.05
Populations in 3 regions	0.30267	12.3	0.084	2.73
Between 2 regions	1.47529	60.0	0.277	0.65
Among 3 regions	2.15569	87.7	0.373	0.42
Among all populations	2.45836	100.0	0.426	0.34

[a] Departures from random mating between contrasted groups.
[b] Equivalent number of reproducing migrants. $Nm \approx (1 - Fxy)/4Fxy$.

Table 4. *F*-statistics for 10 populations of *C. maculata*

Locus	F_{IS}[a]	F_{ST}[b]	F_{IT}[c]
Adk	−0.016	0.017	0.001
α-Gpd	−0.036	0.010	−0.026
Had	−0.006	0.424*	0.421
Idh-1	0.007	0.297*	0.301
Idh-2	−0.018	0.980*	0.979
Mdh-1	−0.048	0.958*	0.948
Mdh+2	−0.046	0.028	−0.016
Mpi	−0.026	0.002	−0.024
Pep	0.051	0.691*	0.707
6pgdh	0.097	0.374*	0.435
Pgi	−0.036	0.197*	0.168
Pgm	0.026	0.218*	0.238
Sod	0.131	0.578*	0.633
Tpi	−0.103	0.268*	0.192
Mean	0.010	0.463	0.469
Jackknife estimates over loci:			
Mean	0.010	0.465	0.471
±SD	0.019	0.089	0.090

*, $P << 0.001$.
[a] Average departure from random matings of individuals in subpopulations.
[b] Departures from random mating among subpopulations.
[c] Departures from random mating from all causes.

Analysis of variance (ANOVA) and *F*-statistics. Partitioning of gene frequencies showed populations within the three regions accounted for 12.3% of the variance, but 30% of the total variance when grouped into two regions (Table 3). The among regions variance component was 88% of the total. The contrast between North American and Honduras gene frequencies accounted for 60% of the total variance. Wright's fixation indices (each is an F_{ST} estimate) varied from 0.08 for populations within the three regions to 0.43 among 10 populations. Under Hardy–Weinberg conditions, these *F* statistics estimate the mean number of reproducing migrants per generation that would give the same gene frequencies as observed. The critical level is ≈1 (Wright 1978) above which no further drift would occur and below which drift will proceed to fixation. Populations within three regions showed high levels of gene flow (i.e., migration), but gene flow was <1 reproducing individual between regions.

F statistics complement and extend the foregoing analyses by incorporating within (F_{IS}) and among (F_{ST}) population components for each locus (Table 4). Mating was random within populations; the negative values of F_{IS} indicate slight excesses of heterozygotes because when mating is random within subpopulations, the expected value of $F_{IS} = -2N^{-1}$. Deficiencies of heterozygotes were detected only at *6pgd* and *SOD*, but these probably were caused by difficulty in observing some allele combinations. Matings among populations were clearly not random, and the mean over loci was $F_{ST} = 0.46 \pm 0.09$, a statistic that indicates about one reproducing beetle exchanged among populations every three generations. Of 14 loci, 10 were significantly greater than zero. The four loci that were not may be constrained by some kind of selection regime.

Table 5. Matrix of genetic distance coefficients

Population	1	2	3	4	5	6	7	8	9	10
1 Iowa A	—	0.011	0.010	0.011	0.037	0.143	0.131	0.626	0.612	0.514
2 Iowa B	0.200	—	0.000	0.000	0.082	0.222	0.190	0.719	0.718	0.591
3 NY	0.218	0.165	—	0.000	0.071	0.191	0.167	0.680	0.672	0.555
4 CN and RI	0.234	0.187	0.132	—	0.082	0.205	0.182	0.655	0.647	0.540
5 TX-M	0.259	0.314	0.299	0.335	—	0.088	0.086	0.575	0.559	0.522
6 TX-P	0.355	0.418	0.395	0.430	0.315	—	0.003	0.537	0.533	0.609
7 TX-RG	0.359	0.409	0.396	0.410	0.336	0.161	—	0.544	0.546	0.625
8 Honduras C	0.665	0.686	0.669	0.660	0.671	0.640	0.654	—	0.012	0.059
9 Honduras B	0.654	0.684	0.664	0.652	0.649	0.618	0.631	0.211	—	0.052
10 Honduras A	0.618	0.630	0.607	0.602	0.622	0.651	0.669	0.270	0.250	—

Above diagonal is Nei's (1978) unbiased genetic distance; below the diagonal is Cavalli-Sforza and Edwards (1967) arc distance.

Genetic Distances. Two genetic distance measures for all 45 pairwise comparisons showed close agreement (Table 5). Averaging Nei's unbiased measure D for each population group, we have for northern United States $D = 0.005 \pm 0.006$, for Texas $D = 0.059 \pm 0.048$, and for Honduras $D = 0.041 \pm 0.025$. These are small values close to zero, but much larger estimates were obtained for the intergroup distances. Thus, for the northern United States–Texas comparisons, a mean distance of $D = 0.142 \pm 0.061$ was estimated. For northern United States–Honduras comparison we obtained 0.627 ± 0.067, and for Texas–Honduras, $D = 0.561 \pm 0.035$. The foregoing relationships are set forth as a dendrogram (Fig. 1), showing the striking clustering of populations within the three groups.

Beetle Fertilities. Reciprocal crosses within and between Iowa, Texas, and Honduras *C. maculata* showed that within strain matings were fertile (9 of 11 mating pairs, 82%) as were the crosses between Texas and Iowa beetles (9 of 10 pairs) (Table 6). But Iowa and Texas *C. maculata* were bidirectionally sterile when crossed to Honduras beetles (0 of 27 mating pairs), even though matings were observed to occur. These data indicate postmating reproductive isolation.

The frequency of matings varied greatly among the crosses (Table 7). Chi-square analysis of the data are set forth in Table 8. The major component of this variation (53.4%) can be attributed to the crosses between Honduran and North American beetles (Table 8). However, in the foregoing outcrosses of Honduran beetles, only 23.9% of the chi-square variance was attributed to the strain, whereas 65.2% was attributed to the difference between Honduran males and females. Honduran females were less likely to mate than Honduran males (11.8 versus 23.5%), even in intrastrain crosses (14.3% between Honduran beetles versus 34.8% in Iowa and Texas). Thus, the strain effect is largely an artifact of the reluctance of Honduras females to mate.

The spermathecae from four F_1 Honduran females that had been crossed with F_1 Texas males were dissected and only two contained motile sperm. The spermathecae of six F_1 Texas females crossed with F_1 Honduran males were dissected and all six contained motile sperm.

Do postmating barriers to gene flow exist between south Texas and northern *C. maculata*? Testing the fertilities of hybrids of the heterogametic sex (males) provides the most sensitive test (Table 9). Male hybrids from Iowa × Texas reciprocal crosses were backcrossed to the parental strains and tested for fertility. The proportion of fertile matings was 97% when hybrids were crossed to Iowa females and 100% when crossed to Texas females. Moreover, no differences in hybrid fertility were recorded that could be attributed to the strain of the male or female parents of hybrid males. Thus, no postmating barriers to gene flow were detected.

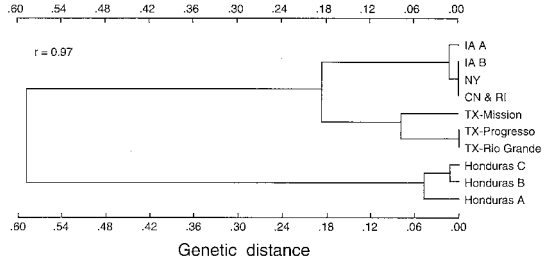

Fig. 1. Dendrogram by the unweighted pair group of Nei's unbiased genetic distance measure.

Discussion

Honduran and North American *C. maculata* populations shared only a fraction (58.6%) of their allozyme variation, whereas 41.4% was unshared. These data suggest ancient separation of gene pools. In equilib-

Table 6. Fertility of crosses within and between geographical strains of *C. maculata*

Parents		No. mating pairs	No. fertile	% fertile
Female	Male			
Iowa	Iowa	5	5	100
Texas	Texas	3	2	67
Honduras	Honduras	3	2	67
Iowa	Texas	5	4	80
Texas	Iowa	5	5	100
Honduras	Texas	5	0	0
Honduras	Iowa	8	0	0
Texas	Honduras	6	0	0
Iowa	Honduras	8	0	0

Crosses were made with F_1 beetles bred in the laboratory.

Table 7. Mating frequencies of F_1 *C. maculata* intra- and interstrain crosses

Cross Female × Male	No. observations	Matings		No. egg masses
		No.	%	
IA × IA	70	20	28.6	15
IA × TX	66	16	24.2	15
IA × Hond	108	27	25.0	34
TX × TX	42	19	45.2	5
TX × IA	55	12	21.8	23
TX × Honduras	84	22	26.2	20
Honduras × Honduras	42	6	14.3	11
Honduras × IA	112	6	5.4	20
Honduras × TX	67	14	20.9	14

rium populations, F_{ST} can be used to estimate migration. Migration rates of $Nm < 1$ per generation predict further genetic differentiation via drift, whereas values >1 prevent it (Wright 1978). The F_{ST} estimate of 0.24 between North American and Honduran lady beetles predicts much less than the critical level of one reproducing migrant per generation. This datum does not confirm speciation, but it is consistent with speciation because it connotes reproductive isolation.

Reciprocal crosses between Texas and Iowa *C. maculata* showed that the North American forms were interfertile. Honduran beetles also were fertile in intrastrain crosses. But the Central American samples were completely sterile when crossed reciprocally with North American *C. maculata*, confirming the patterns shown by gene frequencies. As genetic differentiation proceeds, postmating isolating mechanisms increase. These usually appear first in hybrids of the heterogametic sex, which can be semisterile or altogether sterile (Orr 1997). Hybrid male progeny of reciprocal Iowa × Texas intercrosses were fertile when crossed to either parental strain.

Mating frequencies were estimated to test for premating isolation. No such isolation was detected. There was much heterogeneity in the mating rates and most of this was attributed to the reluctance of Honduran females to mate even with their own males.

The genetic distances between the North American and Honduran *C. maculata* are sufficient to ask if the species represent divergence from an immediate common ancestor or convergence from different ancestors. These lady birds are aposematically colored (Brakefield 1985) and, in principle, could be Mullerian

Table 8. Chi-square analysis of mating frequencies from Table 7

Source	Avg mating rates %	df	χ^2	% variation	P
Honduras outcrosses	18.6	3	19.326	53.4	<<0.001
IA, TX		1	4.623	23.9	0.032
by sex		1	12.596	65.2	<<0.001
IA × TX	23.4	1	0.099	0.3	0.753
Intrastrain	29.2	2	9.750	26.9	0.008
IA, TX only	34.8	1	3.213	32.8	0.073
Honduras × Honduras	14.3	1	6.537	67.2	0.011
Contrast 1, 2, 3	22.0	2	7.277	20.1	0.026
All crosses	22.0	8	36.203		<<0.001

Table 9. The fertilities Texas–Iowa hybrid males backcrossed to the parental strains

Male parentage	Female	No. lines	No. tested	No. fertile	% fertile
IA ♀ × TX ♂	IA	4	14	13	93
IA ♀ × TX ♂	TX	4	14	14	100
TX ♀ × IA ♂	IA	5	15	15	100
TX ♀ × IA ♂	TX	5	14	14	100
Totals		18	57	56	98

mimics by convergence. But their gross morphological similarities were strong, so divergence from an immediate common ancestor seems a much more likely hypothesis.

The reproductive isolation we detected could, in principle, have been largely extra-chromosomal. Longstanding bidirectional cytoplasmic incompatibility caused by the rickettsia *Wolbachia* or other bacteria (Werren 1997) could account for our observations. Indeed, there is good evidence that reproductive barriers between some sympatric species and subspecies of crickets *Gryllus* and chrysomelid beetle *Diabrotica* are maintained by *Wolbachia* (Giordano et al. 1997). Different *Wolbachia* strains were detected in North American *C. maculata lengi* Timberlake and *fuscilabris* (Mulsant) (Jeyaprakash and Hoy 2000) and these beetles are reproductively incompatable (O. Perez and M. A. Hoy, personal communication). We have not sampled beetles from the southeastern United States where *C. m. fuscilabris* is found. If indeed *Wolbachia* were to have caused the separation of Honduran and North American gene pools and to maintain it, the absolute degree of incompatibility is quite remarkable. The large fraction of unshared alleles between North American and Honduran beetles indicates separation of gene pools for a very long time.

The basis for establishing subspecies in *C. maculata* appears to have been variation in adult size and color patterns among geographic populations (Timberlake 1943, Gordon 1985). For some subspecific designations, size and color variation is associated with minor morphological differences in male genitalia (Gordon 1985). Three of the races of *C. maculata* described by Timberlake (1943) were based on one or two museum specimens each from a single collection. Because color, size, and morphology are continuous variables and greatly influenced by environment, we argue that these variables cannot legitimately be used for intraspecific designations without replicated studies, coupled with breeding work, to support a measure of reproductive isolation. Because population genetic research shows that mating is essentially random among the diverse North American populations from New England, Maryland, the Midwest, and Texas (Coll et al. 1994, Krafsur et al. 1995), we question the value of some of the subspecific designations in *C. maculata.*

To conclude, our data indicate that the Honduran *C. maculata* samples represent a sibling species even though we could not find obvious morphological divergence. We base this hypothesis on the differences

in gene frequencies, lack of fertile crosses with North American populations, and differences in prey suitability (Munyaneza and Obrycki 1998).

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Appendix 1. Gene frequencies in *C. maculata* samples

Locus	Populations									
	Iowa[a]		NY	CT, RI	TX[c]	TX[d]	TX[e]	Honduras[b]		
	A	B						A	B	C
Adk										
N	10	20	12	9	23	14	12	23	30	57
A	0.000	0.000	0.042	0.056	0.000	0.000	0.000	0.065	0.017	0.009
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.000
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.017	0.018
D	1.000	0.975	0.958	0.944	1.000	1.000	0.958	0.891	0.833	0.930
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.067	0.026
F	0.000	0.025	0.000	0.000	0.000	0.000	0.042	0.043	0.050	0.018
a-Gpd										
N	10	17	15	19	23	14	15	23	27	45
A	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.019	0.000
B	0.950	0.971	0.933	0.974	1.000	1.000	1.000	0.957	0.981	0.922
C	0.000	0.029	0.033	0.000	0.000	0.000	0.000	0.043	0.000	0.078
D	0.000	0.000	0.033	0.026	0.000	0.000	0.000	0.000	0.000	0.000
Had-1										
N	10	20	3	14	23	14	15	18	27	47
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.093	0.234
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.083	0.000	0.298
C	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.694	0.574	0.351
D	0.850	0.975	1.000	1.000	0.739	0.679	0.733	0.000	0.000	0.000
E	0.100	0.025	0.000	0.000	0.000	0.000	0.000	0.222	0.148	0.117
F	0.050	0.000	0.000	0.000	0.261	0.321	0.267	0.000	0.148	0.000
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.037	0.000
Idh-1										
N	10	6	9	13	23	14	12	23	30	20
A	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.348	0.500	0.475
B	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.217	0.000	0.000
C	0.950	0.917	1.000	0.923	1.000	0.964	0.917	0.370	0.500	0.450
D	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.065	0.000	0.000
E	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.075
F	0.000	0.083	0.000	0.000	0.000	0.036	0.083	0.000	0.000	0.000
Idh-2										
N	10	6	9	13	23	14	11	23	30	28
A	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	1.000
B	1.000	1.000	0.944	0.962	1.000	1.000	1.000	0.000	0.000	0.000
C	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000	0.000
D	0.000	0.000	0.056	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mdh-1										
N	10	6	9	14	23	14	12	23	30	28
A	1.000	1.000	1.000	1.000	0.913	1.000	1.000	0.000	0.000	0.018
B	0.000	0.000	0.000	0.000	0.087	0.000	0.000	1.000	1.000	0.982
Mdh+2										
N	10	20	15	14	23	14	12	23	30	56
A	0.000	0.000	0.000	0.036	0.000	0.000	0.000	0.0.087	0.033	0.000
B	1.000	0.875	1.000	0.964	1.000	1.000	1.000	0.913	0.967	0.982
C	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018
D	0.000	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
E	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mpi										
N	9	20	14	13	14	14	12	22	30	50
A	0.000	0.000	0.000	0.000	0.000	0.000	0.042	0.023	0.083	0.040
B	0.056	0.000	0.000	0.000	0.036	0.036	0.042	0.045	0.000	0.020
C	0.000	0.025	0.071	0.000	0.036	0.036	0.000	0.000	0.000	0.000
D	0.944	0.925	0.821	0.885	0.929	0.893	0.917	0.932	0.917	0.930
E	0.000	0.050	0.107	0.115	0.000	0.036	0.000	0.000	0.000	0.010
Pep-2										
N	10	6	9	14	23	14	12	23	30	28
A	0.000	0.000	0.056	0.036	0.000	0.000	0.125	0.000	0.000	0.000
B	0.250	0.000	0.056	0.000	0.457	0.964	0.708	0.000	0.000	0.000
C	0.750	1.000	0.833	0.893	0.543	0.036	0.167	0.000	0.000	0.000
D	0.000	0.000	0.056	0.071	0.000	0.000	0.000	0.848	0.983	0.946
E	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.152	0.017	0.054
6pgd										
N	10	18	16	11	22	14	12	23	30	55
A	0.000	0.028	0.031	0.045	0.114	0.000	0.083	0.000	0.000	0.000
B	0.950	0.917	0.844	0.864	0.545	0.036	0.042	0.000	0.017	0.551
C	0.000	0.028	0.000	0.000	0.000	0.750	0.833	0.609	0.500	0.082
D	0.050	0.000	0.031	0.045	0.000	0.036	0.042	0.326	0.417	0.155
E	0.000	0.000	0.031	0.045	0.000	0.000	0.000	0.065	0.067	0.209
F	0.000	0.028	0.063	0.000	0.250	0.179	0.000	0.000	0.000	0.012
G	0.000	0.000	0.000	0.000	0.091	0.000	0.000	0.000	0.000	0.000

Locus	Populations									
	Iowa[a]		NY	CT, RI	TX[c]	TX[d]	TX[e]	Honduras[b]		
	A	B						A	B	C
Pgi										
N	10	6	9	12	23	14	12	24	30	28
A	0.150	0.083	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
B	0.250	0.167	0.167	0.125	0.000	0.000	0.000	0.021	0.017	0.018
C	0.150	0.500	0.500	0.583	0.000	0.000	0.000	0.208	0.033	0.196
D	0.450	0.250	0.278	0.250	1.000	0.821	0.833	0.521	0.417	0.375
E	0.000	0.000	0.056	0.042	0.000	0.179	0.167	0.250	0.283	0.321
F	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.250	0.089
Pgm										
N	10	17	11	12	22	14	11	23	30	54
A	0.000	0.000	0.000	0.000	0.045	0.000	0.000	0.000	0.000	0.000
B	0.000	0.000	0.000	0.000	0.045	0.036	0.273	0.000	0.000	0.000
C	0.250	0.235	0.273	0.292	0.000	0.536	0.500	0.000	0.017	0.000
D	0.550	0.382	0.318	0.500	0.614	0.393	0.227	0.370	0.467	0.231
E	0.100	0.294	0.364	0.208	0.250	0.000	0.000	0.000	0.000	0.046
F	0.100	0.088	0.045	0.000	0.045	0.036	0.000	0.609	0.500	0.667
G	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.022	0.017	0.056
Sod-1										
N	10	20	15	14	23	14	12	22	30	56
A	0.850	0.625	0.633	0.786	0.000	0.000	0.000	0.000	0.033	0.000
B	0.150	0.375	0.367	0.214	1.000	0.964	1.000	0.955	0.867	1.000
C	0.000	0.000	0.000	0.000	0.000	0.036	0.000	0.045	0.100	0.000
Tpi-2										
N	10	19	15	12	21	14	12	23	30	56
A	0.000	0.053	0.067	0.083	0.000	0.000	0.000	0.000	0.000	0.000
B	0.650	0.474	0.567	0.667	0.595	0.714	0.583	1.000	1.000	1.000
C	0.350	0.474	0.367	0.250	0.405	0.286	0.417	0.000	0.000	0.000

NY, New York; CT, RI, Connecticut and Rhode Island; TX, Texas.
[a] Locations near Ames, IA.
[b] Locations near Tegucigalpa.
[c] Moore AFB.
[d] Progreso.
[e] Rio Grande City.