Population Genetics and Gene Variation in the Predator, *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae)

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ABSTRACT An electrophoretic study of 23 allozyme loci in 15 populations of the predaceous ladybird beetle *Coleomegilla maculata* (De Geer) revealed extensive genetic polymorphism occurring as low-frequency allelic variation averaging four alleles per locus. Although all loci except *GOT-2* demonstrated variation, only 69% of the loci were polymorphic when the rare allele occurred at >5% frequency. The average observed population heterozygosity index demonstrates a north–south cline with higher allelic variation associated with both lower latitudes and corn–sorghum crop types. Nei's genetic distance values suggests high genetic identity among all populations, with some clustering based on geographic locale. We conclude this ladybird beetle is genetically very variable, and that some of this variation is maintained by selection.

KEY WORDS *Coleomagilla maculata,* population genetics, gene variation

RELATIVELY LITTLE RESEARCH has been reported for beetles of economic importance, and reports tend to concentrate on pest species. Few reports exist concerning the extent or nature of genetic variation as represented by allozyme variation in beneficial beetles. Liebherr (1986a; b) has studied genetic variation and cladistics of the Platynini, a North American carabid tribe. Most recently, Krafsur et al. (1992) have used allozyme variation to examine gene flow in the sevenspotted lady beetle, *Coccinella septempunctata* L. in European versus North American populations.

Studies of biochemical genetic variation in beneficial beetles may be used to map the arthropod genome, serve as markers to study fitness effects in differing environments, and serve to establish host race identities or at least give insight into questions of taxonomic importance. The research endeavors mentioned above could indirectly address questions associated with micro- or macroevolutionary processes.

The entomophagous ladybird beetle *Coleomegilla maculata* (De Geer) is an important predator of both the corn leaf aphid *Rhopalosiphum maidis* Fitch and the European corn borer, *Ostrinia nubilalis* (Hübner) in American monocultures of corn and sorghum (Andow & Risch 1985). It has been tested as a biological control agent on cucumbers (Gurney & Hussey 1970).

Coleomagilla maculata is known to vary somewhat in markings and coloration, but unlike *Adalia bipunctata* studied by Creed (1966), the genetics of this variation has not been investigated. We report here the first extensive study on allozyme variation in this species, and address the following three questions: (1) How genetically variable is this species? (2) Are there any patterns in genetic variation associated with geographic distribution? (3) What is the nature of population substructuring in this species? Answers to these questions give us insight into the population genetics and gene–environment interactions of this interesting and economically important predator. In general, the answers extend the evidence concerning the pervasiveness of evolutionary theory governing the behavior of genes in natural populations.

Materials and Methods

Coleomegilla maculata adults were collected by hand picking or by use of insect net sweeps at 15 localities in five states of the the Midwest in June and July from corn, sorghum, or alfalfa. One collection was made at the University of Missouri's South Farm agricultural experiment station in each year, 1985, 1986, and 1987. All other samples were collected in 1986 or 1987 (Table 1) with a specific attempt to represent populations from diverse geographic areas and different crops. Samples were frozen immediately at −85°C until electrophoretic analysis was done. Details concerning each population may be found in Table 1.

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Table 1. Locale, crop, latitude, and observed population heterozygosity (H) for 15 populations of *Coleomegilla maculata* analyzed for electromorph variation

Population	Abbreviation	State	County	Year	Crop	Latitude	Observed H
South Farm 1986	SF86	MO	Boone	1986	corn	38° 85′	0.137
J. C. Penney Farm	JCP	MO	Boone	1986	alfalfa	38° 0′	0.105
New Franklin	NF	MO	Howard	1986	clover	39° 00′	0.060
Prairie Home	PH	MO	Cooper	1986	clover	38° 80′	0.081
Westphalia	WSTPHA	MO	Osage	1986	clover	38° 30′	0.103
Fruitland	FRTLND	MO	Cape Girardeau	1986	alfalfa	37° 30′	0.061
New Madrid	NWMDRID	MO	New Madrid	1986	alfalfa	36° 20′	0.068
Braggadacio	BRGDACIO	MO	Pemiscot	1986	alfalfa	35° 70′	0.067
Urbana	URB	IL	Champaign	1986	alfalfa	40° 10′	0.074
Winchester	WNCHSTR	IL	Scott	1986	alfalfa	39° 45′	0.086
Donaldson	DNLDSN	AR	Hotsprings	1987	corn	34° 15′	0.126
Hope	HOPE	AR	Hempstead	1987	sorghum	33° 13′	0.145
Yuba	YUBA	OK	Bryan	1987	corn	33° 90′	0.139
Gober	GOBR	TX	Fannin	1987	corn	33° 25′	0.151
Honey Grove	HYNGRVE	TX	Fannin	1987	sorghum	33° 30′	0.136

Not listed in Table 1 are the laboratory populations of 1986 and 1987. These represent colonies established from field collections. Lab 86 was derived from ≈20 females collected from a field of mixed vegetables and grasses at the USDA Biological Control of Insects Research Laboratory in Columbia, MO. This colony died out in the winter of 1986–1987. Lab 87 was derived from a late-spring collection made at South Farm and was maintained in the laboratory until August 1990. Laboratory colonies were kept on eggs of *Trichoplusia ni* (Hübner). For all practical purposes, the South Farm 87 population served as our allele control for the studies reported here. The genetic analyses we report here for each laboratory colony were made about the third generation after collecting.

Electrophoretic procedures and equipment to display allozyme variation for genetic analyses were the same as described by Steiner & Joslyn (1979). We used buffer systems LiOH and CA-8 to analyze the enzymes listed in Table 2. The LIOH system has a gel made of 0.074 *M* Trizma base and 0.009 *M* citric acid, pH adjusted to 8.55. It contains 90% of gel buffer and 10% of electrode buffer. The electrode buffer is 0.036 *M* lithium hydroxide and 0.194 *M* boric acid pH adjusted to 8.40. The CA-8 electrode stock buffer consists of 1.37 *M* Trizma base and 0.314 *M* citric acid, pH adjusted to 8.15. This buffer is diluted

Table 2. Allozymes, enzyme classification codes, allele designations, and locus heterozygosity values (*h*) studied in *Coleomegilla maculata*

Allozyme	Abbreviation	Enzyme Classification Code	Alleles found[a]	*h*
Adenylate kinase-1	ADK-1	2:7:4: 3	98, 100, 102, 104, *n*	0.170
Adenylate kinase-2	ADK-2	2:7:4: 3	98, 100, 102	0.037
Adenylate kinase-3	ADK-3	2:7:4: 3	98, 100, 102	0.050
Aldehyde oxidase	ALDOX	1:2:3: 1	99, 100, 101, 102	0.132
Esterase-3	EST-3	3:1:1: 1	96, 98, 100, 102, *n*	0.157
Glutamate oxaloacetate transaminase-1	GOT-1	2:6:1: 1	97, 100, 103, 106	0.045
Glutamate oxaloacetate transaminase-2	GOT-2	2:6:1: 1	100	0.000
alpha-glycerophosphate dehydrogenase	α-GPDH	1:1:1: 8	94, 97, 100, 103	0.032
Glucose-6-phosphate dehydrogenase	G-6-PDH	1:1:1:49	97, 100, 103	0.067
Glycerophosphate-3-dehydrogenase	G-3-PDH	1:2:1:12	97, 100, 103, *n*	0.034
Hexokinase-2	HK-2	2:7:1: 1	98, 99, 100, 101, 102	0.147
Hexokinase-3	HK-3	2:7:1: 1	98, 99, 100, 101	0.147
Isocitrate dehydrogenase-1	IDH-1	1:1:1:42	96, 98, 100, 102, 104	0.091
Isocitrate dehydrogenase-2	IDH-2	1:1:1:42	98, 100, 102	0.091
Leucine aminopeptidase-1	LAP-1	3:4:1: 1	100, 101, 102	0.343
Leucine aminopeptidase-2	LAP-2	3:4:1: 1	96, 98, 100, 102, *n*	0.208
Malate dehydrogenase-1	MDH-1	1:1:1:37	97, 100, 103	0.104
Malate dehydrogenase-2	MDH-2	1:1:1:37	98, 100, 102	0.036
Malic enzyme	ME	1:1:1:40	98, 99, 100, 101	0.030
Phosphoglucose isomerase-2	PGI-2	5:3:1: 9	97, 100, 103, 106	0.174
Phosphoglucomutase-1	PGM-1	2:7:5: 1	102, *n*	0.352
Phosphoglucomutase-2	PGM-2	2:7:5: 1	96, 98, 100, 102	0.146
6-phosphogluconate dehydrogenase	6-PGDH	1:1:1:44	96, 98, 100, 102	0.088

Average number of alleles per locus, 4; total average heterozygosity, 0.110.

[a] *n*, null allele.

Table 3. Nei's genetic distances based on 10 allozyme loci for 12 populations of *C. maculata*

Population	1	2	3	4	5	6	7	8	9	10	11	12
1 SF86	0	0.014	0.032	0.023	0.031	0.004	0.007	0.013	0.048	0.007	0.013	0.014
2 SF87	—	—	0.025	0.022	0.042	0.009	0.009	0.010	0.027	0.010	0.020	0.023
3 LAB87	—	—	—	0.055	0.073	0.037	0.043	0.033	0.053	0.025	0.037	0.065
4 DNLDSN	—	—	—	—	0.012	0.013	0.015	0.030	0.048	0.027	0.039	0.028
5 HOPE	—	—	—	—	—	0.022	0.024	0.045	0.080	0.042	0.057	0.031
6 GOBR	—	—	—	—	—	—	0.004	0.011	0.035	0.008	0.018	0.014
7 HNYGRV	—	—	—	—	—	—	—	0.013	0.042	0.010	0.021	0.007
8 SF85	—	—	—	—	—	—	—	—	0.032	0.007	0.019	0.026
9 LAB86	—	—	—	—	—	—	—	—	—	0.041	0.064	0.062
10 NF	—	—	—	—	—	—	—	—	—	—	0.013	0.025
11 PH	—	—	—	—	—	—	—	—	—	—	—	0.032
12 YUBA	—	—	—	—	—	—	—	—	—	—	—	0

1:4 with distilled water for the cathode tray and 1:5 for the anode tray. The same gel buffer as for LIOH is used for CA-8. Electrostarch (Otto Hiller, Madison, WI) at 13% (wt/vol) concentration served as the gel support medium.

Individual *C. maculata* were prepared by first dissecting each individual to determine sex and if hyperparasites were present. Individuals were homogenized in 15 ml of distilled water. The homogenates were subjected to electrophoresis and stained according to the procedures outlined by Steiner & Joslyn (1979). Parasitized and unparasitized *C. maculata* were electrophoresed side-by-side to determine differences in electrophoretic profile. Parasitized specimens had extra bands for esterase and malate dehydrogenase enzymes. Voucher specimens will be deposited at the completion of a study examining genetic differences between *C. maculata* biotypes.

Gels were read using a background light source and photographed to make a permanent record. The resulting data were then subjected to genetic and statistical analyses. Gene frequencies were calculated from the observed numbers of genotypes, because allozyme systems are codominant genetic systems. The resulting gene frequencies are tabulated in the *Appendix*. Observed numbers of heterozygotes were used to calculate locus and population level heterozygosity values. Correlation coefficients and a rank sums test (Mann-Whitney) were calculated using the programs provided for the Texas Instrument-59 calculator with attached printer. The data were subjected to genetic distance analysis using Nei's (1978) method and Wright's (1965) *F* statistics to determine the extent of population genetic substructuring. Because all loci were not investigated in every population, a subset of 10 loci (*PGM-1* and *2*, *HK-3*, *MDH-1* and *2*, *GOT-1*, *IDH-1*, *alpha-GPDH*, *6-PGDH* and *ALDOX*) occurring in 12 populations (Table 3) provided the largest data subset for the Nei's estimate. The software program NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System) version 1.50 by F. James Rohlf (Exeter, New York, NY) was used to generate the dendrograms.

Here, we accept the proposition that the variants observed on starch gels are good genetic alleles from our own experience and because a multitude of studies now exist in the literature demonstrating this tenet and our own genetic crosses between specific allozyme isolines in the laboratory provide additional support. These studies also show evidence for fitness differences between some allozymes (W.W.M.S. & D. Davis, unpublished data).

We used the most commonly occurring allele at a locus in our reference population (South Farm 87) as our reference allele and assigned it the number *100*. This allele thus serves as an internal reference on each gel once it is found to occur in each population studied. Slower migrating alleles at this locus are then assigned a lower number depending on how many millimeters (mm) they occur behind (cathodal to) the commonly occurring allele. Faster alleles are assigned higher numbers depending on how much faster they migrate in the electric field beyond (anodal to) the commonly occurring allele. Thus, for example, in Table 2, alleles *96* and *98* at the *6-PGDH* locus are 4 and 2 mm slower migrating, respectively, and are nearer the cathodal end of the gel. Allele *102* is found 2 mm nearer the anodal end of the gel than the most common allele, *6-pgdh*^{100}.

Results

Table 1 lists the average observed population heterozygosity for the individual populations. Table 2 lists those allozyme loci and their alleles analyzed in this study along with locus heterozygosity. The allele frequencies of all polymorphic loci are listed in the *Appendix*. Most of the loci were segregating for two or more alleles, resulting in a high average of four alleles per locus. Only *GOT-1* was monomorphic across all populations. Locus heterozygosity was not as high as expected from the high number of observed alleles (Table 2). The average was 0.110 with a range from 0.00 to 0.352. The disparity between the two estimates suggests that most of the ob-

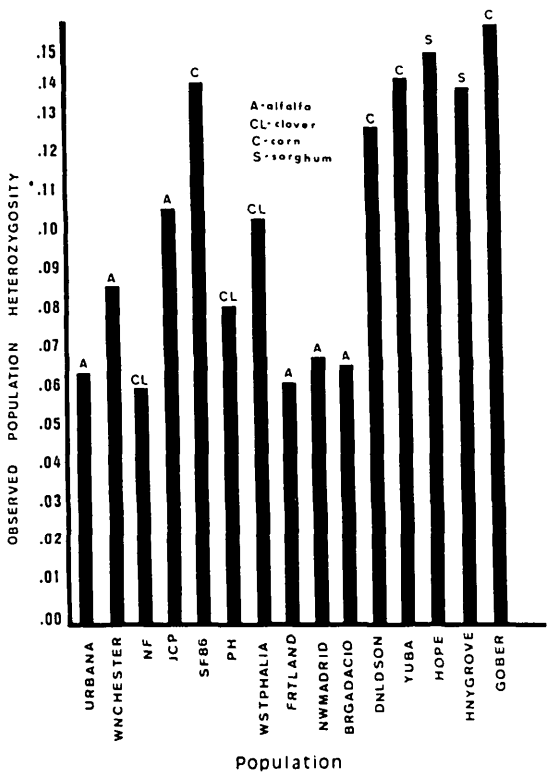

FIG. 1. Observed population heterozygosity (H) of 15 populations of the ladybird beetle predator, *Coleomegilla maculata*. Populations are distributed on the *x* axis from Urbana, Illinois in the North to Gober, Texas in the South. Heterozygosity is negatively correlated with latitude (−0.657, df = 13, P < 0.05).

served alleles occur at low frequency, an implication confirmed by examination of the allele frequencies.

Six loci demonstrated null (no activity) alleles on the starch gels, which were confirmed in the crossing studies (W.W.M.S. & D. Davis, unpublished data). These loci included *PGM-1*, *PGM-2*, *G-3-PDH-1*, *EST-3*, *ADK-1*, and *ADK-3*. Null alleles are known to occur in many insects and have been studied extensively in *Drosophila* (Bell et al. 1972, Bewley & Lucchesi 1977, David et al. 1978, Bentley et al. 1983).

The heterozygosity values demonstrated a cline correlated to both latitude and to host crop from which the sample was drawn (Fig. 1). Because crop plantings are latitudinally-related, and because latitudinal changes encompass a range of environmental parameters including temperature, hours of available daylight, humidity, and other factors, it is at best difficult to determine the basis for the correlation coefficient of −0.657 (df = 13, $P < 0.05$). The nature of the dual relationship is such that genetic variation is generally lower on crops found to the north (alfalfa and clover) and higher on crops found to the south (corn and sorghum). We do not know the extent to which the year collected confounds the heterozygosity–crop type correlation, but the correlation appears to hold across years because a 1986 northern collection on corn matches collections taken from southern populations on corn in 1987.

Nei's genetic distance estimates (Table 3) indicates that all populations are genetically very similar. The dendrogram generated from this data is found in Fig. 2. It indicates that the populations tend to cluster on geographic location. For example, the Hope and Donaldson populations are from Arkansas whereas Gober, Honeygrove, and Yuba are farther south. The Prairie Home population was collected in 1986, the same year as the Lab 86 population but 64 km to the West of Lab 86. The South Farm populations, although collected in different years clearly cluster together and include a population from New Franklin, 32 km to the north. The Texas populations which form a subcluster with South Farm

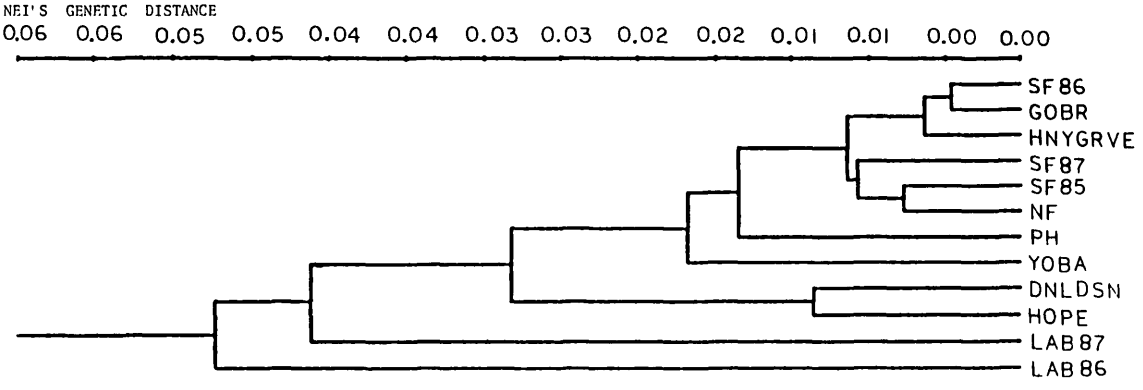

FIG. 2. Phenogram based on the unweighted pair group analysis of Nei's genetic distance data of Table 3. Software program NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System) version 1.50 by F. James Rohlf (Exeter, New York, NY) was used to generate this dendrogram. Observed clustering of populations follows their geographic distribution.

Table 4. Wright's (1965) *F* statistics by locus for populations of *C. maculata*

LOCUS	F_{IS}	F_{IT}	F_{ST}
LAP-1	0.2265	0.3222	0.1237
LAP-2	0.4049	0.4845	0.1338
HK-3	−0.0189	0.2089	0.2236
PGM-1	0.5336	0.6248	0.1955
G-3-PDH	0.4054	0.3496	0.0858
IDH-2	0.1307	0.1644	0.0388
ADK-1	0.3172	0.3886	0.1046
ADK-2	0.0995	0.2539	0.1715
ADK-3	0.1100	0.7121	0.6765
EST-3	0.1598	0.2823	0.1458
α-GPDH	−0.0416	−0.0339	0.0074
MDH-2	0.0562	0.0974	0.0437
ME	0.1225	0.2368	0.1303
PGM-2	0.6576	0.7088	0.1495
MDH-1	−0.0983	0.1539	0.2296
G-6-PDH	−0.3382	−0.2206	0.0879
HK-2	0.2129	0.2987	0.1090
IDH-1	0.0993	0.2504	0.1678
6-PGDH	0.0726	0.1162	0.0471
PGI-2	0.0259	0.1109	0.0873
ALDOX	0.0950	0.1275	0.0359

were collected on the same type of crops (corn and sorghum) that occur at South Farm.

Wright's (1965) *F* statistic values (Table 4) give a mixed picture of the mating and subdivision within the populations. The positive F_{ST} values indicate some local subdivision is present within populations, and the range of F_{IS} values suggest effective breeding sizes range from 2 to >30 individuals. Selection and random sampling effects probably influence these estimates.

Discussion

The subject of genetic variation in natural populations is widely treated in the modern scientific literature (Wagner & Selander 1974, Frankel & Soule 1981, Steiner et al. 1982, Nevo et al. 1984; see also the past 20 yrs of any major genetics journal dealing with population genetics). It is thought such variation underlies selection theory, providing a descriptive parameter or tool useful in examining gene–gene and gene–environment interactions (e.g., DeJong & Scharloo 1976, Hoorn & Scharloo 1978). Gene variation also provides genetic markers useful in gene mapping, defining genetic relationships, and predicting evolutionary age of a population (Nei 1978, Mickevich & Mitter 1981).

Is the level of variation observed for *C. maculata* typical for Coleoptera? Table 5 lists the results of other researchers on a range of beetles. It is clear that with a heterozygosity value of 11, *C. maculata* is on the low side but within the range observed for beetles. It is also clear that it has twice as many alleles per locus as the other beetles that have been studied. This could be a reflection of differences in observation or technique, but because of the congruence in heterozygosity values between studies, is more likely caused by the high number of low-frequency alleles observed for *C. maculata* (*Appendix*).

The observed cline in heterozygosity is significantly associated with latitude and host crop type (Fig. 1). Many such clines in genetic variability, using either the general heterozygosity statistic or heterozygosity at individual loci, have been reported in the literature in a wide array of species (reviewed by Steiner 1993). In many cases, the relationship is the same; that is, heterozygosity increases in southern latitudes as the equator is approached. Interestingly, evidence exists for a mirror situation south of the equator; there, Steiner et al. (1982) found heterozygosity

Table 5. Comparison of genetic variation statistics for 10 beetle species

Species	No. populations analyzed	No. loci analyzed	% loci polymorphic	Avg. no. alleles observed per locus	H[a]	Author[b]
Neaphaenops tellkampfii	8	13	47.0	1.74	0.154	1
Dendroctonus ponderosae Hopkins	6	15	20.0	1.33	NC	2
Dendroctonus pseudotsugae Hopkins	2	13	53.9	2.62	NC	3
Platynus tennicollis LeConte	5	22	86.4	1.92	0.195	4
Platynus angustatus Dejean	5	22	90.9	2.14	0.162	4
Speonomus sp.	13	13	31.0	NC	0.02–0.112	5
Hypera postica Gyllenhal	6	22	53.6	NC	0.231	6
Coccinella septempunctata L.	18	28	57.1	1.50	0.159	7
Coleomegilla maculata	15	23	69.6	4.00	0.110	8

Note that *C. maculata* carries more alleles per locus than any other species, giving it a high number of polymorphic loci. However the relatively lower heterozygosity value of 0.110 suggests that most of the alleles occur as low-frequency variation.

[a] NC, not calculated.

[b] 1 Turanchik & Kane (1979).
2 Stock & Guenther (1979); economically important pest, allele frequencies at aspartate aminotransferase show a cline.
3 Stock et al. (1979); economically important pest.
4 Liebherr (1986b); allele frequency cline correlated with altitude.
5 Crouau-Roy (1986); observed H significantly lower than expected.
6 Hsiao & Stutz (1985); economic pest, no genetic evidence for different races, but see observations of Sell et al. (1987).
7 Krafsur et al. (1992).
8 Present paper; north-south cline in heterozygosity values.

increases in *Anopheles* mosquitoes as one proceeds toward northerly latitudes approaching the equator from the south. This has been variously attributed by different authors to either higher dispersal in northern habitats versus southern ones, to migration from central populations in the southern latitudes to colonize northern latitudes, or to selection differences along some gradient. With respect to the latter case, Slatkin (1987) has found that if extinction is higher in newly colonized areas, a lower population heterozygosity would make gene flow appear higher. Endler (1973) examined several models of gene flow and differentiation along environmental gradients and concludes the two may be independent. He suggests individual cases will require unique and in-depth investigation.

Currently, we can rule out migration as the primary mechanism for maintaining genetic variability because *C. maculata* is thought to overwinter by aggregating at the base of plants or in leaf litter. Our data is consistent with the idea that selection plays an important role. The primary aphid host occurring on the hosts corn and sorghum is *Rhopalosiphum maidis* (Fitch). The plants may supply additional nutrition in the form of pollen. On alfalfa and clover, primary insect hosts may consist of different species of aphids and insect pest eggs. There is no clear evidence that pollen from these plants is used as a food source.

The magnitude and pattern of genetic variability described here are heuristic. The association between morphological variation and genetic variation in this beetle remains to be investigated. The differential effects of temperature, humidity, and host type on genetic background deserve attention. Finally, because *C. maculata* has economic importance as a biological control agent, we plan to investigate insecticide resistance and the role isozymes may play in physiological adaptation.

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Appendix

Allele frequencies for different polymorphic genes in *C. maculata* (see text for discussion and Tables 1 and 2 for abbreviations)

Allele frequencies for locus *LAP-1*.

Population	*n*	Allele frequencies						No. heterozygotes
		97	98	99	100	101	102	
SF85	10	—	—	0.222	0.778	—	—	4
SF86	22	—	—	0.065	0.522	0.391	0.022	6
SF87	124	0.044	0.157	0.112	0.566	0.067	0.054	42
LAB86	28	0.107	0.375	0.089	0.232	0.054	0.143	16
LAB87	9	—	—	0.040	0.960	—	—	1
URB	9	—	—	0.150	0.850	—	—	0
DNLDSN	20	—	—	0.125	0.700	0.075	0.100	10
HOPE	20	—	—	0.200	0.567	0.167	0.067	4
YUBA	20	—	—	0.071	0.786	0.107	0.036	9
GOBR	20	—	—	0.083	0.639	0.167	0.111	9
HYNGRVE	20	—	—	0.184	0.526	0.211	0.079	9

Allele frequencies for locus *LAP-2*.

Population	*n*	Allele frequencies				No. heterozygotes
		97	98	99	100	
SF85	34	—	0.015	0.862	0.073	4
SF86	39	.030	0.116	0.833	0.051	6
LAB86	27	—	—	0.889	0.111	4
NF	21	—	0.023	0.667	0.310	7
PH	12	—	0.080	0.500	0.417	2

Allele frequencies for the locus *IDH-2*.

Population	*n*	Allele frequencies			No. heterozygotes
		98	100	102	
SF85	57	0.009	0.991	—	1
SF86	38	0.026	0.974	—	0
LAB86	14	—	1.000	—	0
JCP	8	—	0.938	0.063	1
NF	22	0.114	0.886	—	5
WSTPHA	9	0.056	0.944	—	1

Allele frequences for the locus *G-3-PDH*.

Population	*n*	Allele frequencies				No. heterozygotes
		97	100	103	null	
SF85	71	—	0.993	0.007	—	0
SF86	178	—	0.986	0.014	—	5
SF87	60	—	0.950	—	0.050	0
LAB86	61	0.008	0.992	—	—	1
LAB87	30	—	1.00	—	—	0
JCP	20	0.025	0.925	—	0.050	1
NF	71	—	1.00	—	—	0
PH	70	0.007	0.979	0.014	—	3
WSTPHA	16	0.156	0.781	—	0.063	5
FRTLND	16	—	1.00	—	—	0
NWMDRID	7	—	1.00	—	—	0
BRGDACIO	9	—	0.944	0.056	—	1
URB	35	—	0.985	0.015	—	1
WNCHSTR	40	—	1.00	—	—	0

Allele frequences for the locus *ADK-3*.

Population	*n*	Allele frequencies				No. heterozygotes
		98	100	102	null	
SF85	60	0.092	0.767	0.058	0.083	6
SF86	137	0.004	0.012	0.972	0.012	8
LAB86	39	0.103	0.846	0.026	0.025	6
JCP	3	—	1.00	—	—	0
NF	3	—	1.00	—	—	0
PH	36	0.028	0.972	—	—	2
WSTPHA	7	—	1.00	—	—	0
FRTLND	16	—	1.00	—	—	0
NWMDRID	7	—	1.00	—	—	0
BRGDACIO	8	—	1.00	—	—	0
URB	16	—	1.00	—	—	0
WNCHSTR	39	0.017	0.966	0.017	—	2

Allele frequences for the locus *EST-3*.

Population	*n*	Allele frequencies					No. heterozygotes
		96	98	100	102	null	
SF85	57	—	—	0.833	0.149	0.018	11
SF86	70	0.015	0.150	0.762	0.027	0.046	40
LAB86	29	—	0.017	0.983	—	—	1
JCP	26	—	0.096	0.826	—	0.076	3
NF	58	—	—	1.00	—	—	0
PH	60	—	0.050	0.875	0.025	0.050	9
WSTPHA	16	0.094	0.219	0.656	0.031	—	5
FRTLND	16	—	0.031	0.969	—	—	1
NWMDRID	7	—	0.100	0.740	0.040	0.120	3
BRGDACIO	8	—	—	1.00	—	—	0
URB	24	—	0.188	0.729	—	0.083	1
WNCHSTR	47	—	0.042	0.744	0.021	0.191	6

Allele frequences for the locus *ADK-1*.

Population	*n*	Allele frequencies					No. heterozygotes
		98	100	102	104	null	
SF85	66	0.144	0.750	0.045	—	0.061	16
SF86	106	0.009	0.906	0.085	—	—	8
LAB86	34	0.103	0.662	0.059	—	0.176	5
JCP	8	0.063	0.875	0.063	—	—	2
NF	3	—	1.00	—	—	—	0
PH	67	0.075	0.791	0.134	—	—	8
WSTPHA	13	0.077	0.692	0.192	0.038	—	3
NWMDRID	3	—	1.00	—	—	—	0
BRGDACIO	8	0.062	0.938	—	—	—	1
URB	20	—	0.950	0.050	—	—	2
WNCHSTR	34	0.029	0.574	0.191	—	0.206	3

Allele frequences for the locus *ADK-2*.

Population	*n*	Allele frequencies			No. heterozygotes
		98	100	102	
SF85	42	0.023	0.818	0.114	6
SF86	152	0.010	0.980	0.010	4
LAB86	48	0.021	0.906	0.073	7
JCP	3	—	1.00	—	0
PH	46	—	0.989	0.011	1
WSTPHA	6	0.083	0.750	0.167	3
FRTLND	16	—	1.00	—	0
NWMDRID	7	—	1.00	—	0
BRGDACIO	8	—	1.00	—	0
URB	16	0.188	0.781	0.031	3
WNCHSTR	40	—	1.00	—	0

Allele frequences for the locus *HK-2*.

Population	*n*	Allele frequencies					No. heterozygotes
		98	99	100	101	102	
SF85	89	—	0.005	0.815	0.180	—	13
SF86	112	—	0.045	0.826	0.129	—	17
SF87	82	—	0.067	0.866	0.067	—	14
LAB86	31	—	—	0.667	0.333	—	10
LAB87	29	—	—	1.00	—	—	0
NF	82	—	0.006	0.994	—	—	1
PH	29	—	—	0.914	0.086	—	3
URB	29	—	—	0.690	0.310	—	6
WNCHSTR	16	—	—	1.00	—	—	0
DNLDSN	25	—	0.040	0.840	0.120	—	8
YUBA	16	—	—	0.906	0.094	—	3
GOBR	20	—	0.100	0.775	0.100	0.025	7
HNYGRVE	19	.026	0.158	0.763	0.026	—	3

Allele frequences for the locus *HK-3*.

Population	*n*	Allele frequencies				No. heterozygotes
		98	99	100	101	
SF85	42	—	—	1.00	—	0
SF86	98	0.020	0.071	0.898	0.010	15
SF87	84	—	0.018	0.964	0.018	6
LAB86	14	—	—	1.00	—	0
LAB87	20	—	—	1.00	—	0
NF	32	—	—	0.984	0.016	1
PH	23	—	0.261	0.739	—	5
URB	4	—	0.300	0.700	—	3
WNCHSTR	8	—	0.500	0.500	—	6
DNLDSN	20	—	0.075	0.875	0.050	3
HOPE	20	—	—	1.00	—	0
YUBA	20	—	—	0.969	0.031	1
GOBR	20	—	0.050	0.925	0.025	3
HNYGRVE	20	—	0.025	0.975	—	1

Allele frequencies for the locus *α-GPDH*.

Population	*n*	Allele frequencies			No. heterozygotes
		97	100	103	
SF85	94	0.005	0.995	—	1
SF86	142	0.018	0.979	0.004	8
SF87	80	0.025	0.975	—	4
LAB86	43	0.012	0.988	—	1
LAB87	30	0.017	0.983	—	1
JCP	12	—	1.00	—	0
NF	22	0.023	0.977	—	1
PH	35	—	0.989	0.011	1
WSTPHA	7	—	1.00	—	0
FRTLND	16	0.031	0.969	—	1
NWMDRID	7	—	1.00	—	0
BRGDACIO	8	—	1.00	—	0
URB	16	—	1.00	—	0
WNCHSTR	40	—	1.00	—	0
DNLDSN	20	—	1.00	—	0
HOPE	20	—	1.00	—	0
YUBA	20	—	1.00	—	0
GOBR	20	0.025	0.975	—	1
HNYGRVE	20	0.025	0.975	—	1

Allele frequencies for the locus *MDH-1*.

Population	*n*	Allele frequencies				No. heterozygotes
		97	100	103	106	
SF85	105	0.047	0.943	0.009	—	4
SF86	128	0.016	0.965	0.019	—	9
SF87	77	0.013	0.916	0.071	—	11
LAB86	24	—	0.938	0.062	—	9
LAB87	24	0.033	0.967	—	—	2
JCP	9	—	1.00	—	—	0
NF	82	—	0.982	0.018	—	3
PH	43	—	1.00	—	—	0
WSTPHA	7	—	1.00	—	—	0
URB	35	—	1.00	—	—	0
WNCHSTR	28	—	1.00	—	—	0
DNLDSN	20	0.015	0.525	0.325	—	8
HOPE	20	—	0.450	0.450	0.100	13
YUBA	20	0.025	0.900	0.025	0.050	4
GOBR	19	—	0.868	0.132	—	5
HNYGRVE	20	—	0.875	0.125	—	3

Allele frequencies for the locus *PGM-1*.

Population	*n*	Allele frequencies						No. heterozygotes
		94	96	98	100	102	null	
SF85	74	—	—	0.081	0.689	0.176	0.054	21
SF86	155	0.003	0.067	0.121	0.561	0.022	0.217	104
SF87	77	—	—	0.318	0.611	0.019	0.036	19
LAB86	14	—	—	0.500	0.464	—	0.036	2
LAB87	29	—	—	0.052	0.534	—	0.414	1
JCP	22	—	0.068	0.250	0.682	—	—	8
NF	53	—	—	0.038	0.755	—	0.208	4
PH	46	—	—	0.032	0.872	—	0.096	3
WSTPHA	16	0.094	0.219	0.625	0.063	—	—	3
FRTLND[a]	10	—	—	0.111	0.222	—	0.667	0
NWMDRID	6	—	—	0.084	0.916	—	—	1
BRGDACIO	8	—	—	—	0.250	—	0.750	0
URB	37	—	0.041	0.095	0.784	—	0.081	6
WNCHSTR	29	0.017	0.052	0.224	0.431	0.017	0.293	11
DNLDSN	20	—	—	0.325	0.525	—	0.150	3
HOPE	20	—	—	0.225	0.475	—	0.300	7
YUBA	17	—	—	0.353	0.585	—	0.059	4
GOBR	20	—	—	0.250	0.600	—	0.150	8
HNYGRVE	21	—	—	0.285	0.619	—	0.095	8

[a] Only homozygotes for each allelic type were observed.

Allele frequencies for the locus *MDH-2*.

Population	*n*	Allele frequencies			No. heterozygotes
		98	100	102	
SF85	83	—	1.00	—	0
SF86	168	0.042	0.934	0.030	11
SF87	80	—	1.00	—	0
LAB86	43	—	0.988	0.012	1
LAB87	30	—	1.00	—	0
JCP	12	0.125	0.875	—	1
NF	81	—	1.00	—	0
PH	22	—	1.00	—	0
FRTLND	16	—	1.00	—	0
NWMDRID	4	—	1.00	—	0
URB	20	—	1.00	—	0
WNCHSTR	20	0.025	0.975	—	1
DNLDSN	20	—	1.00	—	0
HOPE	20	—	1.00	—	0
YUBA	20	—	1.00	—	0
GOBR	19	0.050	0.950	—	2
HNYGRVE	20	—	1.00	—	0

Allele frequencies for the locus *ME*.

Population	*n*	Allele frequencies				No. heterozygotes
		98	99	100	101	
SF85	86	—	—	0.971	0.029	5
SF86	190	—	0.029	0.966	0.005	7
SF87	80	0.006	—	0.975	0.019	4
LAB86	24	—	—	0.938	0.062	3
LAB87	30	—	—	1.00	—	0
JCP	13	—	0.115	0.808	0.079	3
NF	2	—	—	1.00	—	0
PH	68	—	0.007	0.993	0	1
WSTPHA	16	—	—	0.813	0.188	3
FRTLND	16	—	—	0.719	0.281	1
NWMDRID	7	—	—	1.00	—	0
BRGDACIO	8	—	—	1.00	—	0
URB	35	—	—	1.00	—	0
WNCHSTR	40	—	—	1.00	—	0
DNLDSN	20	—	—	0.975	0.025	1
HOPE	20	—	—	1.00	—	0
YUBA	20	—	—	0.975	0.025	1
GOBR	20	—	—	1.00	—	0
HNYGRVE	20	—	—	0.975	0.025	1

Allele frequencies for the locus *PGI-2*.

Population	*n*	Allele frequencies				No. heterozygotes
		97	100	103	106	
SF85	36	—	0.972	0.028	—	2
SF86	55	0.145	0.709	0.145	—	12
SF87	8	—	0.938	0.062	—	1
JCP	3	—	1.00	—	—	0
NF	22	0.021	0.760	0.217	—	6
PH	20	—	0.976	0.024	—	1
FRTLND	16	—	1.00	—	—	0
NWMDRID	1	—	1.00	—	—	0
URB	9	—	0.889	0.111	—	2
WNCHSTR	8	—	1.00	—	—	0
DNLDSN	10	—	0.900	0.100	—	2
HOPE	10	—	0.900	0.050	0.050	2
YUBA	10	—	0.850	0.150	—	3
GOBR	10	—	0.800	0.200	—	2
HNYGRVE	10	—	0.900	0.100	—	2

Allele frequencies for the locus *PGM-2*.

Population	*n*	Allele frequencies					No. heterozygotes
		96	98	100	102	null	
SF85	77	—	—	0.558	0.052	0.390	6
SF86	179	0.008	0.079	0.697	0.022	0.194	20
SF87	91	0.016	0.093	0.456	0.016	0.418	14
LAB86	22	—	—	0.364	—	0.636	0
LAB87	43	0.070	0.302	0.140	0.093	0.395	9
JCP	21	—	0.071	0.500	0.048	0.381	1
NF	53	—	0.009	0.585	0.019	0.387	3
PH	64	0.008	0.195	0.547	0.023	0.203	22
WSTPHA	15	—	—	0.267	0.067	0.667	0
FRTLND	9	—	0.222	0.667	0.111	—	6
NWMDRID	6	—	0.167	0.666	0.167	—	2
BRGDACIO	8	—	0.438	0.562	—	—	3
URB	33	—	0.015	0.667	0.045	0.273	3
WNCHSTR	31	—	0.145	0.484	0.113	0.258	8
DNLDSN	20	—	0.025	0.650	0.025	0.300	2
HOPE	20	—	—	0.800	0.100	0.100	2
YUBA	20	—	—	0.925	0.025	0.050	1
GOBR	20	—	—	0.650	0.100	0.250	2
HNYGRVE	20	0.025	—	0.750	0.025	0.200	3

Allele frequencies for the locus *IDH-1*.

Population	*n*	Allele frequencies					No. heterozygotes
		96	98	100	102	104	
SF85	130	0.007	0.031	0.912	0.046	0.004	20
SF86	187	—	0.019	0.968	0.013	—	10
SF87	80	—	0.025	0.950	0.025	—	8
LAB86	41	—	—	0.671	0.329	—	27
LAB87	30	—	0.017	0.875	0.025	—	1
JCP	20	—	0.100	0.875	0.025	—	1
NF	54	0.009	0.074	0.898	0.019	—	10
PH	67	—	—	0.970	0.030	—	2
WSTPHA	9	—	—	1.00	—	—	0
FRTLND	16	—	0.031	0.969	—	—	0
NWMDRID	4	—	—	1.00	—	—	0
BRGDACIO	8	—	0.062	0.938	—	—	1
URB	24	—	—	0.958	0.042	—	2
WNCHSTR	40	—	0.012	0.988	—	—	1
DNLDSN	20	—	0.075	0.875	0.050	—	5
HOPE	20	—	—	1.00	—	—	0
YUBA	20	—	0.025	0.975	—	—	1
GOBR	20	—	0.025	0.959	0.025	—	2
HNYGRVE	20	—	0.075	0.875	0.025	—	4

Allele frequencies for the locus *G-6-PDH*.

Population	*n*	Allele frequencies			No. heterozygotes
		97	100	103	
SF87	15	0.167	0.800	0.033	4
DNLDSN	15	—	0.967	0.033	3
HOPE	15	—	0.933	0.067	2
YUBA	14	—	1.00	—	0
GOBR	15	—	1.00	—	0
HNYGRVE	20	—	0.975	0.025	1

Allele frequencies for the locus *COT-1*.

Population	*n*	Allele frequencies				No. heterozygotes
		97	100	103	106	
SF85	48	—	0.979	0.021	—	2
SF86	156	0.009	0.971	0.019	—	6
SF87	80	0.013	0.950	0.038	—	7
LAB86	15	—	0.900	0.100	—	3
LAB87	30	—	0.800	0.117	0.083	10
JCP	9	—	1.00	—	—	0
NF	81	0.006	0.982	0.012	—	3
PH	48	0.010	0.990	—	—	1
URB	19	—	1.00	—	—	0
WNCHSTR	28	—	1.00	—	—	0
DNLDSN	20	—	1.00	—	—	0
HOPE	20	0.025	0.975	—	—	1
YUBA	20	0.150	0.825	0.025	—	5
GOBR	20	—	0.975	0.025	—	1
HNYGRVE	20	0.025	0.950	0.025	—	2

Allele frequencies for the locus *ALDOX*.

Population	*n*	Allele frequencies				No. heterozygotes
		99	100	101	102	
SF85	57	0.026	0.886	0.088	—	9
SF86	225	0.022	0.969	0.009	—	14
SF87	80	0.088	0.844	0.056	0.013	25
LAB86	14	—	0.964	0.036	—	1
LAB87	30	0.016	0.883	0.100	—	6
JCP	20	0.150	0.850	—	—	0
NF	81	0.031	0.957	0.012	—	7
PH	63	0.063	0.913	0.024	—	9
WSTPHA	16	—	1.00	—	—	0
FRTLND	16	—	1.00	—	—	0
NWMDRID	7	—	1.00	—	—	0
BRGDACIO	8	—	1.00	—	—	0
URB	43	0.067	0.922	0.011	—	5
WNCHSTR	40	0.050	0.950	—	—	4
DNLDSN	20	—	0.950	0.050	—	2
HOPE	20	0.050	0.925	0.025	—	2
YUBA	20	0.100	0.875	0.025	—	4
GOBR	20	0.025	0.975	—	—	1
HNYGRVE	20	0.075	0.875	0.050	—	4

Allele frequencies for the locus *6-PGDH*.

Population	*n*	Allele frequencies				No. heterozygotes
		96	98	100	102	
SF85	94	0.043	0.144	0.814	—	19
SF86	191	0.003	0.019	0.955	0.021	22
SF87	70	0.006	0.019	0.969	0.006	5
LAB86	46	0.065	0.228	0.696	0.011	7
LAB87	30	—	—	1.00	—	0
JCP	12	—	0.042	0.958	—	1
NF	82	—	0.006	0.982	0.012	3
PH	70	—	0.021	0.964	0.014	4
WSTPHA	7	—	0.071	0.929	—	1
FRTLND	16	—	0.031	0.906	0.063	2
NWMDRID	7	—	0.071	0.929	—	1
BRGDACIO	8	—	—	1.00	—	0
URB	35	—	—	0.957	0.043	1
WNCHSTR	40	—	0.025	0.950	0.025	4
DNLDSN	20	—	—	0.975	0.025	1
HOPE	20	0.025	0.050	0.900	0.025	4
YUBA	20	0.025	0.025	0.950	—	2
GOBR	20	—	0.075	0.900	0.025	3
HNYGRVE	20	—	0.025	0.975	—	1