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GENETIC VARIABILITY OF SPINED SOLDIER BUGS (HEMIPTERA: PENTATOMIDAE) SAMPLED FROM DISTINCT FIELD SITES AND LABORATORY COLONIES IN THE UNITED STATES

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ABSTRACT

The spined soldier bug, *Podisus maculiventris* (Say), is an important biological control agent of agricultural and forest pests; and it preys on eggs and larvae of lepidopteran and coleopteran species. Genetic variability among field samples collected from Michigan, Mississippi, Missouri, Florida and established laboratory colonies was examined using Amplified Fragment Length Polymorphism-Polymerase Chain Reaction (AFLP-PCR). Four AFLP primer pairs generated a total of 340 molecular markers for evaluation. Results from Analysis of Molecular Variance showed that the majority of the genetic variation occurred within populations (individuals from each sample site). Nei's method indicated reduced genetic diversity in laboratory populations compared to field populations. No major differences or deficiencies were apparent among the field samples from different areas or among the laboratory reared samples. We conclude that field populations are panmictic and laboratory reared spined soldier bug could be useful as biological control agents in the field.

Key Words: *Podisus maculiventris*, population genetics, mass rearing, AFLP

RESUMEN

La chinche espinosa soldado, *Podisus maculiventris* (Say), es un agente de control biológico importante para plagas agrícolas y forestales; ésta depreda en huevos y larvas de especies de lepidópteros y coleópteros. La variabilidad genética entre muestras de campo colectadas de Michigan, Mississippi, Missouri y Florida y de colonias establecidas en el laboratorio fue examinada usando Longitud del Fragmento de Polimorfismo Amplificado-Reacción de la Cadena de Polimerasa (AFLP-PCR). Cuatro pares de primers para AFLP generaron marcadores moleculares para la evaluación. Los análisis de varianza molecular mostraron que la mayoría de la variación genética se presentó dentro de las poblaciones (individuos de cada sitio de muestreo). El método de Nei indicó que existe diversidad genética reducida en las poblaciones de laboratorio en comparación con las poblaciones colectadas en campo. No se encontraron diferencias mayores o deficiencias entre las muestras de campo de diferentes áreas o entre las muestras criadas en el laboratorio. Concluimos que las poblaciones de campo son panmícticas y que las chinches espinosas soldado criadas en el laboratorio pudieran ser agentes de control biológico útiles en el campo.

Palabras Clave: *Podisus maculiventris*, genética de poblaciones, cría masiva, AFLP

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The spined soldier bug, *Podisus maculiventris,* is a generalist predator native to North America (Warren & Willis 1971; De Clercq 2000). With the exception of the sap-feeding first nymphal stage (Landis 1937; De Clercq & Degheele 1992), immatures and adults prey on larvae and adults of soft bodied coleopterans and lepidopterans (Gallopin & Kitching 1972; Richman & Whitcomb 1978; McPherson 1980). Spined soldier bugs are associated with many crop plant species including alfalfa (*Medicago sativa* L.), celery *(Apium graveolens* L.), apple (*Malus domestica*), cotton (*Gossypium* Spp.), soybean [*Glycine max* (L.) Merr.], onion (*Allium cepa* L.), and tomato (*Solanum lycopersicum* L.) (Deitz et al. 1976). This insect has substantial potential for field and greenhouse biological control programs.

Developing environmentally friendly and low cost integrated pest management (IPM) technologies to boost crop production is essential to sustainable agriculture (Zalucki et al. 2009). Biological control, through the use of natural enemies, can be an effective component of IPM. *P. maculiventris* has significant impacts on populations of more than 100 insect pest species (De Clercq et al. 1998b; Herrick & Reitz 2004), including the Colorado potato beetle *Leptinotarsa decemlineata* (Say) and the cabbage looper *Trichoplusia ni* (Hübner) (De Clercq et al. 1998a; Aldrich & Cantelo 1999).

Enhancing the efficacy of natural *P. maculiventris* populations by augmentation may benefit from a better understanding of the genetic diversity of the predator and the relationship of that diversity to its geographic range. There is currently no knowledge of potential genetic differences among wild populations, nor possible inbreeding within established laboratory mass-reared colonies (Herrick & Reitz 2004). A molecular genetic comparison of field populations with selected generations of established colonies will help assess genetic differences. This information may contribute to improving the population structures within mass-rearing programs. Characterization of genetic variability among geographically separated colonies may help interpret results of biological studies concerning spined soldier bug susceptibility to insecticides, GMOs (Bell et al. 2003; Alvarez-Alfageme et al. 2007; Torres & Ruberson 2007) and preferred prey species among field populations.

Amplified Fragment Length Polymorphism (AFLP) has been used to assess the genetic variability of a number of insect species, including geographically separated populations of fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Belay et al. 2012) and the dung beetle, *Digitonthophagus gazella* (F.) (Whipple et al. 2012). Recently, AFLP was used to assess the genetic variation of spined soldier bug taken from one laboratory colony and from 4 field locations; 2 field locations were near the collection site of the founding individuals used to establish the laboratory colony and 2 were more distant (Kneeland et al. 2012). In this study, samples from 7 field locations and 3 laboratory colonies (from which were made 7 sample collections) were used to test 3 hypotheses. First, that spined soldier bugs from the geographically distant field locations have greater genetic variation compared to laboratory colonies; second, that individuals from different field samples have a high degree of genetic variation and gene flow between locations; and, third, that laboratory reared colonies will retain genetic diversity despite restricted gene flow.

Materials and Methods

Sample Collection

The 14 *P. maculiventris* sample sets reported on in this paper and collection dates are listed in Table 1. All colonies originated from the collection of 15 to 30 adults at the designated field sites using a Whalon modified Tedder's trap (Great Lakes IPM, Inc., Vestaburg, Michigan, USA) fitted with a WHY trap (Sterling International, Inc., Spokane, Washington, USA) containing septa impregnated with male pheromone (trans-2-hexenal, a terpineol, benzyl alcohol, linalool, terpinen-4-ol and S-1-isopropyl-4-methyl-3-cyclohexen-1-ol (Sigma-Aldrich, St. Louis, MO) (Aldrich 1988). Species confirmation was performed for field collected specimens (Slater & Baranowski 1978; McPherson 1982). All colonies were maintained in walk-in growth chambers held at 26 °C with 70% relative humidity (Coudron et al. 2002), and continuously fed live and coddled 4th instar *Trichoplusia ni* (Hübner) larvae and provided water via a dental wick (Kneeland et al. 2012).

The Missouri laboratory colony originated from adults collected in the spring of 2004 from an alfalfa field near Columbia, Missouri and has been continuously maintained in the laboratory for over 50 generations before use in these experiments. The 2011 Missouri field colony from which the 1st, 2nd and 10th generations were formed, originated from the same collection site as the 2004 Missouri laboratory colony. The high and low fecundity selection pressure for each subsequent generation consisted of using eggs from 3-5 mated pairs with the highest number of hatched eggs and lowest number above a minimum of 10 hatched eggs laid during the first 10 days after mating, respectively. Each subsequent generation of the control colony consisted of using eggs from all mated pairs. The adults that contributed progeny for the next generation were used for these genetic analyses.

All sample individuals were provided with only water for 3 days prior to analysis to clear the digestive tract and then placed into 1.5 ml Ep-

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TABLE 1. SAMPLE SITES FOR SPINED SOLDIER BUGS ANALYZED IN THIS STUDY.

Sample site number	Origin	Collection Site Lat: Long:	Collection year	No. individuals	♂♀
1	Michigan field site	42.673872 -84.485044	2011	18	14:4
2	Maryland field site	39.028169 -76.895993	2011	6	3:3
3	Florida field site	30.154986 -84.083004	2011	8	4:4
4	Mississippi field site	33.434306 -90.905342	2010	57	43:14
5	Missouri Powell Garden site	38.873661 -94.04151	2010	8	Not sexed
6	Missouri alfalfa site	38.891267 -92.206364	2010	12	6:6
7	Missouri dairy pasture site	38.99067 -92.472267	2010	20	10:10
8	1st generation lab colony from Maryland field	Same as #2	2011	22	14:8
9	Established Missouri laboratory colony	38.893897 -92.20336	2004	20	10:10
10	1st generation Missouri field colony under selection for high and low fecundity	Same as #6	2011	6	3:3
11	2nd generation Missouri field colony under selection for high and low fecundity	Same as #6	2011	15	8:7
12	10th generation Missouri field colony under selection for low fecundity	Same as #6	2011	8	4:4
13	10th generation Missouri field colony under selection for high fecundity	Same as #6	2011	5	3:2
14	10th generation Missouri field colony under no selection (control)	Same as #6	2011	6	3:3

pendorf tubes. Each adult specimen was washed 3 times in 1.5 ml 95% ethanol. Each wash lasted 24 h and afterward the samples were preserved in 95% ethanol at 4 °C (Kneeland et al. 2012).

DNA Extraction and Quantification

DNA was extracted from the thorax of each individual following the CTAB protocol modified from Doyle & Doyle (1987). The procedures for extraction (which included homogenization of the thorax, addition and incubation of Proteinase K and RNaseA, and centrifugation followed by supernatant transfer and cold storage) and quantification of DNA from individuals for each sample location using a spectrophotometer) were the same as previously reported (Kneeland et al. 2012).

Amplified Fragment Length Polymorphism-Polymerase Chain Reaction (AFLP-PCR) Genotyping

All steps of AFLP-PCR were completed following the same procedure as in Kneeland et al. (2012) and included: 1) restriction of the DNA with *MseI* and *EcoRI* restriction enzymes; 2) adapter ligation with *MseI* and *EcoRI* adapters; 3) preamplification; and 4) selective amplification with 4 combinations of *MseI* and *EcoRI* primers (Table 2). Afterwards, 1.5µL of each sample with specific primer combinations was electrophoresed on KB^{plus} 6.5% polyacrylamide gel in the GeneReadIR 4200 DNA analyzer (LI-COR, Lincoln, Nebraska) for 2 h to separate the DNA markers. The gel image was saved for scoring and further analysis.

Statistical Analysis

The molecular markers obtained on gels were scored using SAGA^{MX} Generation 2 software version 3.2 (LI-COR, Lincoln, Nebraska); this automated gel scoring system enhances the consistency of gel scoring compared to visual gel scoring and the data of 1's (band present) and 0's (band absent) are automatically generated and electronically stored for later analyses. Bootstrap analysis, with 1000 resamplings, was carried

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TABLE 2. NUCLEOTIDE SEQUENCES FOR ADAPTERS AND PRIMERS USED IN THE AFLP PROCESS, DESCRIBED BY VOS ET AL. (1995).

Oligonucleotides	Purpose	Sequence (5'-3')
*Eco*R1-forward adapter	Adapter ligation	CTCGTAGACTGCGTACC
*Eco*R1-reverse adapter	Adapter ligation	AATTGGTACGCAGTCTAC
*Mse*1-forward adapter	Adapter ligation	GACGATGAGTCCTGAG
*Mse*1-reverse adapter	Adapter ligation	TACTCAGGACTCAT
*Eco*R1 primer	Pre-amplification	GACTGCGTACCAATTC
*Mse*1 primer	Pre-amplification	GATGAGTCCTGAGTAA
E-ACA	Selective amplification	GACTGCGTACCAATTC + ACA
E-ACT	Selective amplification	GACTGCGTACCAATTC + ACT
M-CTA	Selective amplification	GATGAGTCCTGAGTAA + CTA
M-CTG	Selective amplification	GATGAGTCCTGAGTAA + CTG

out using the DBOOT software (Departmento de Biologia Geral ICB, UFG, C.P. Goiania, Goiás, Brazil) (Coelho 2001) to estimate the coefficient of variation. The data were analyzed for Nei's genetic diversity (G_{ST}) with POPGENE v. 1.32 (Yeh et al. 1997). The potential that the sample sites exhibited population structure was investigated using the program STRUCTURE (Falush et al. 2007). The analysis of molecular variance (AMOVA) and Φ_{PT} (an analogue of F_{ST}) were obtained using GenAlEx 6.5 (Peakall & Smouse 2006, 2012); sample sites (14) were divided into 2 and 3 subpopulations based on output from STRUCTURE, 2 sub-populations representing field and laboratory collections (Table 3) and, finally, we also used AMOVA to investigate, separately, only the field sites and then only the laboratory sites. A dendrogram was constructed using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in PHYLIP v. 3.6 (Felsenstein 1989).

RESULTS

Each primer pair generated an average of 85 polymorphic loci. A total of 340 polymorphic loci were used to estimate the genetic variability among all sample sites (Table 3). Bootstrap analysis (Coelho 2001) indicated that, for this number of samples, the amount of marker data was statistically sufficient and accounted for approximately 94% of the genetic variation in the *P. maculiventris* samples.

Results from STRUCTURE indicated most strongly that the 14 sites divided, through genetic differentiation, into 2 groups; the 2011 Missouri field colony sites (5 collections) under selection for high and low fecundity formed one group and the remaining 9 sites formed the other (Fig. 1a; Table 3). However there was also indication of the existence of 3 groups generally characterized as: 1) the 4 eastern field and colony sites; 2)

TABLE 3. POPGENE STATISTICS OF THE *P. MACULIVENTRIS* FROM EACH SAMPLE SITE SHOWING GENETIC DIVERSITY AND % POLYMORPHIC LOCI.

Sample Site; Structure indicated Group[a]	Nei's genetic diversity (H)	No. of usable loci	% Polymorphic loci
Michigan Field; 1, 1	0.2574	338	74.12
Maryland Field; 1, 1	0.1992	338	55.0
Florida Field; 1, 1	0.2000	338	57.94
Mississippi Field; 1, 2	0.3029	340	87.65
MO Field Powell garden;1, 2	0.2530	325	65.59
MO Field Alfalfa; 1,2	0.2336	325	65.59
MO Field dairy pasture; 1, 2	0.2350	325	75.00
Maryland Lab. G1; 1, 1	0.2225	340	68.82
MO Laboratory G50+; 1, 2	0.2455	337	76.47
MO G1 High; 2, 3	0.0878	335	30.00
MO G2 High; 2, 3	0.1949	340	64.41
MO G10 Low; 2, 3	0.1008	340	30.00
MO G10 High; 2, 3	0.0844	336	22.35
MO G10 Control; 2, 3	0.0919	337	26.76

[a]First number after the semicolon is for indicating membership in 1 of 2 groups (related clusters), second number is for 3 groups.

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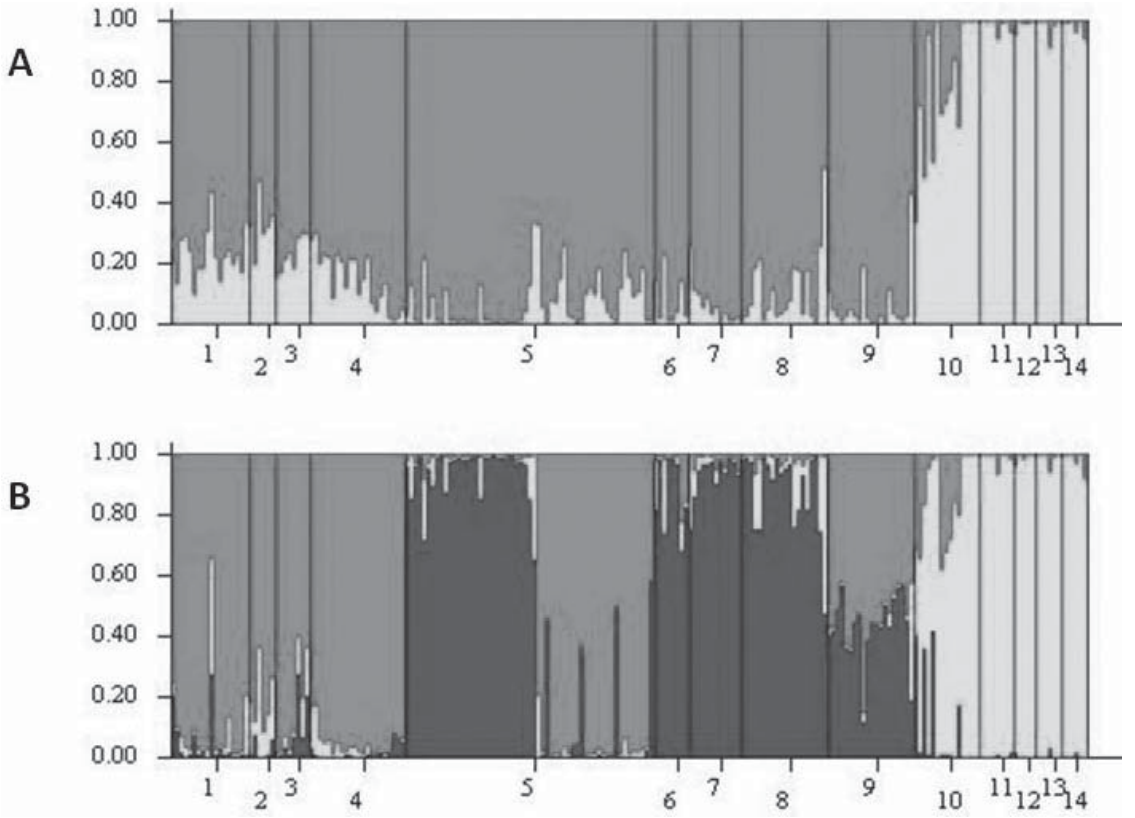

Fig. 1: Results from analysis using STRUCTURE indicating support that samples of *Podisus maculiventris* from 14[a] sites (7 field and 7 laboratory) are structured into 2 (A) or 3 (B) 'sub-populations'.

[a] Key for the identification of sites (numbers across bottom of each graph): 1 = Michigan field site; 2 = Maryland field site; 3 = Florida field site; 4 = Maryland colony; 5 = Mississippi field site; 6 = Missouri Powell Gardens field site; 7= Missouri alfalfa field site; 8 = Missouri dairy pasture field site; 9 = Missouri laboratory site (> 50 generations); 10 = Missouri laboratory site (2nd generation of high selection); 11 = Missouri laboratory site (10^{th} generation of high selection); 12 = Missouri laboratory site (10th generation of low selection); 13 = Missouri laboratory site (10th generation of no selection); 14 = Missouri laboratory site (1^{st} generation of high selection).

the Mississippi and Missouri field sites (4 sites) and established Missouri colony (> 50 generations); and 3) the 5 Missouri colony sites under selection for high/low fecundity (Fig. 1; Table 3). Under the 3 group scenario, the Mississippi field and established Missouri colony sites (unlike the other sites) were not strongly affiliated into one group but rather were nearly equally contained in groups 1 and 2 as defined.

AMOVA results were consistent, using all sample sites and regardless of grouping, with 54-65% of genetic variability within sample sites (the program uses the term populations; here it equates to our sample sites), ≈25-30% of genetic variability among populations (within groups), and ≈5-21% of genetic variability among groups (Table 4). The same trend of the highest genetic variation being within populations was recorded when separately analyzing field samples (72%) and laboratory colonies (54%); genetic variation among groups for field samples and laboratory colonies was 5.44% and 18.15%, respectively (data not shown). When all samples were analyzed by groups, the fixation index (Φ_{PT}) was between ≈0.30-0.35 (Table 4); when analyzed separately, Φ_{PT} values of 0.27 for the 7 field samples and 0.45 for the 7 laboratory colony samples resulted (data not shown).

Nei's genetic diversity (G_{ST}) results were consistent with the AMOVA results (Table 5). The G_{ST} value (0.395) for the combined analysis (field and laboratory) showed differentiation within populations. The Nm value (0.767), representing gene flow among all sample sites, was low (<1.0). Results were similar when laboratory colonies were analyzed separately, with a G_{ST} of 0.392 and Nm of 0.775. However, when the field samples were analyzed separately the lower G_{ST} (0.180)

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TABLE 4. AMOVA OF ALL 14 *P. MACULIVENTRIS* SUB-POPULATIONS, WITH 1000 BOOTSTRAPS, AND GROUPINGS OF: GROUP 1 = 7 FIELD SITES, ESTABLISHED (>50 GENERATIONS) MISSOURI LABORATORY COLONY, MARYLAND LABORATORY COLONY AND GROUP 2 = 5 MISSOURI LABORATORY COLONIES UNDER SELECTION (A); GROUP 1 = FIELD SAMPLES AND GROUP 2 = LABORATORY SAMPLES (B); GROUP 1 = MARYLAND FIELD SITE AND LABORATORY COLONY, MICHIGAN AND FLORIDA FIELD SITES, GROUP 2 = 3 MISSOURI AND 1 MISSISSIPPI FIELD SITES AND ESTABLISHED MISSOURI LABORATORY COLONY, AND GROUP 3 = 5 MISSOURI LABORATORY COLONIES UNDER SELECTION (C).

A

Source of Variation	Degree of Freedom	Source of Variation	Variance Components	Percentage of Variance
Among groups	1	1360.38	16.31	21.11
Among pops within groups	12	3805.29	19.32	25.01
Within pops	197	8197.37	41.61	53.88

Φ_{PT} = 0.317

B

Source of Variation	Degree of Freedom	Source of Variation	Variance Components	Percentage of Variance
Among groups	1	697.648	2.804	4.96
Among pops within groups 12	3197.735	16.980	30.010	
Within pops	197	7248.086	36.792	65.03

Φ_{PT} = 0.350

C

Source of Variation	Degree of Freedom	Source of Variation	Variance Components	Percentage of Variance
Among groups	2	2129.22	11.30	16.08
Among pops within groups 11	3036.44	17.41	24.75	
Within pops	197	8197.36	41.61	59.17

Φ_{PT} = 0.295

indicated moderate differentiation and the higher Nm (2.276) indicated sufficient migration to support genetic variation (Table 5).

The maximum percentage of polymorphic loci was found in the Mississippi samples, and the minimum in the 10th generation of the Missouri field colony selected for high fecundity. A similar trend of Nei's genetic diversity (H) was observed among all sample sites and grouped sites (Tables 3 and 5).

TABLE 5. MULTI-POPULATION DESCRIPTIVE STATISTICS OF SUBDIVIDED POPULATIONS: TOTAL DIVERSITY (H_T), DIVERSITY WITHIN POPULATIONS (H_S), DIVERSITY AMONG POPULATIONS (G_{ST}) AND GENE FLOW (NM) OF ALL POPULATIONS OF *P. MACULIVENTRIS* (A), FIELD POPULATIONS ALONE (B) AND LAB POPULATIONS ALONE (C).

	H_T	H_S	G_{ST}	Nm (G_{ST})
A	0.3117	0.1887	0.3946	0.7671
B	0.2860	0.2351	0.1801	2.2761
C	0.2404	0.1461	0.3923	0.7747

The UPGMA dendrogram showed that *P. maculiventris* from the same geographic locations were clustered; supporting the AMOVA and G_{ST} results of some genetic differentiation. The Missouri laboratory collections under selection for high/low fecundity formed the first clade and showed high bootstrap support. The second clade consisted of the field samples, the established Missouri colony (> 50 generations) and the Maryland colony; the 3 Missouri locations (Powell garden, alfalfa field and dairy pasture) formed one sub-clade with high bootstrap support, the other field sites formed a 2nd sub-clade also with strong bootstrap support, and the Maryland and established Missouri (> 50 generations) formed a 3rd sub-clade with, again, strong bootstrap support (Fig. 2). The dendrogram most strongly supports the result from STRUCTURE indicating that the samples form 2 genetically related groups.

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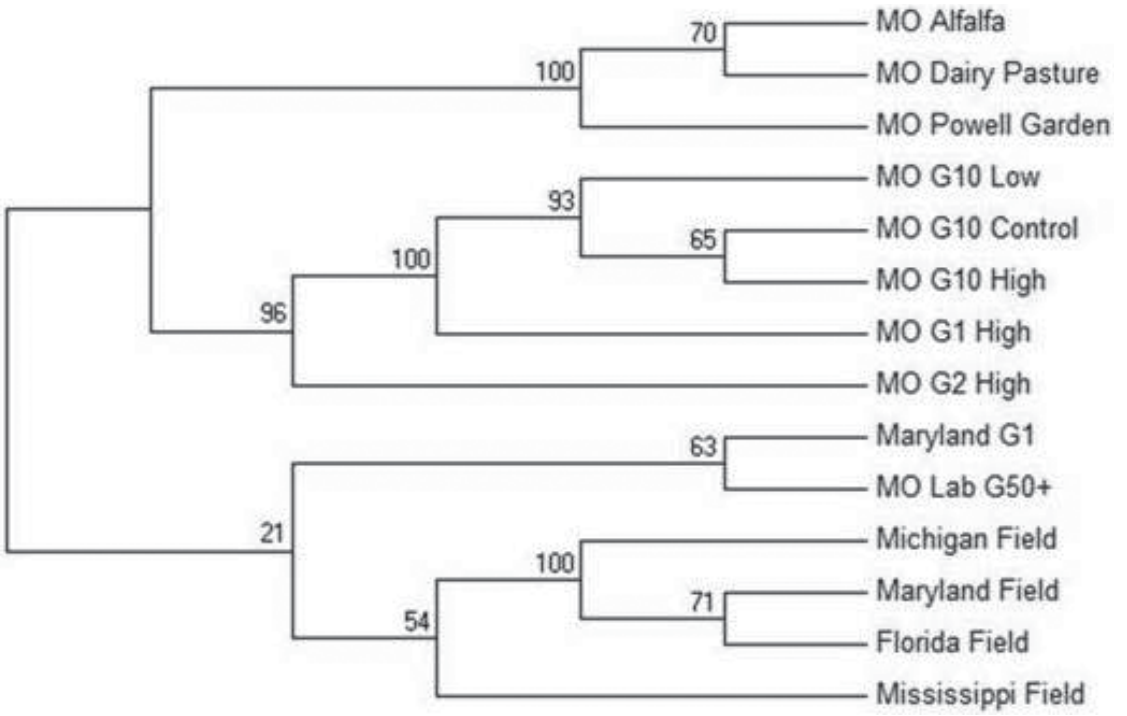

Fig. 2: Dendrogram (unrooted) presenting the relationship of various *P. maculiventris* sample locations and their genetic differences calculated by using pairwise genetic distances with Phylip.

Discussion

The data reported in this paper support our 3 hypotheses posed in the introduction. Our first hypothesis holds that spined soldier bugs from geographically distant field locations have greater genetic variation compared to established laboratory colonies. Two points support the argument. First, with over 300 polymorphic loci, there was sufficient data (even with a modest number of sampled individuals) to statistically account for 94% of the genetic variation in the spined soldier bug populations. Second, Nei's genetic diversity, (H), indicates greater diversity in most field locations than in the established laboratory colonies. Because the distinct sample sites vary in habitat characteristics, including plant populations, prey species, water availability, temperature and seasonal variation, substantial genetic diversity among field samples is not surprising. Our second hypothesis addresses the possibility of gene flow between distant locations. The concept of gene flow between individuals from distant locations and its role as a force in maintaining species integrity has been controversial for decades (Larson et al. 1984). While the controversy is not germane to our hypotheses, use of the gene flow parameter, Nm, has become solidly substantiated as a reliable indicator of gene flow (Clark et al. 2007; Krumm et al. 2008). Our calculated Nm values, indicating substantial gene flow among distant field locations and relatively little among laboratory samples, strongly bolster our second hypothesis.

The third hypothesis, that established laboratory colonies retain genetic diversity despite restricted gene flow, may bring to mind a post-hoc appreciation of the data. The underlying logic, however, is otherwise. Laboratory colonies are typically established from relatively few individuals, 15 to 30 insects in this study. The laboratory populations established from these few adults were spatially separated, maintained under similar conditions of temperature, photoperiod and food stream, and were not genetically refreshed by serial injections of field individuals. Gene flow among these populations is extremely low. Maintaining sexually-reproducing, small populations of virtually any life form under such conditions is necessarily attended by concerns about genetic bottlenecks within populations and about genetic movement toward stationary populations. These may certainly become issues in long-term production facilities. Our data show the lowest percentage of polymorphic loci occurred in the 10th generation of the Missouri field population that had been selected for high fecundity. For laboratory populations maintained for research purposes,

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particularly research designed to improve the efficacy of insect predators in biological control programs, there is very little assurance that the test populations represent the species as it occurs in nature. Our data indicate lower total genetic diversity and lower diversity within laboratory populations, compared to all sampled locations and, separately, to field locations. As may be expected from in-laboratory rearing conditions, we recorded gene flow among laboratory populations, albeit at considerably lower rates than recorded among the sampled field locations. These data confirm the fundamental assumption of our third hypothesis, that is, restricted gene flow among laboratory populations and its attendant long-term ramifications. Despite the imposed gene flow restrictions, our data support the third hypothesis that genetic diversity is present within and among established spined soldier bug colonies.

Overall, most genetic variation occurred within sample locations and among sample locations within groups. Variation among groups was low, generally < 20%. Similar findings have been recorded for other insect species, for example, the widely distributed fall armyworm (Lewter et al. 2006; Belay et al. 2012). Our data show genetic diversity in the laboratory populations declined, but it was not eliminated over many generations of living under constant conditions. Unlike the low genetic diversity, the reproductive capacity of multi-generation laboratory colonies has remained stable in several research programs (De Clercq et al. 1998b). This pattern recurs in other beneficial insect rearing programs, such as the egg parasitoids, *Trichogramma* spp. and the pupal parasitoids *Muscidifurax* spp. (Hopper et al. 1993; Sorati et al. 1996).

Substantial differences between the samples from Mississippi and Missouri locations have been reported (Kneeland et al. 2012). Here, the dendrogram, as well as the results from analysis of genetic structure, shows that the Mississippi field population is most closely related to the sample locations other than those from Missouri. Perhaps more interesting from the current results is the separation of the laboratory samples that were under selection for high/low fecundity from the rest of the samples, including the established Missouri colony and the Maryland colony. This may have resulted from the fact that selection induced another genetic bottleneck or the fact that this colony resulted from newly collected field samples.

The Missouri field samples form a group, as do the remaining field samples collected in Florida, Maryland and Michigan. The lack of bootstrap support for the branch separating the field samples is consistent with the results of other analyses on genetic parameters discussed above. The most curios, and with high bootstrap support, branch of the dendrogram is that containing the samples from the established (> 50 generations) Missouri colony and the Maryland colony. The Maryland colony was quite 'young', 1st generation, and thus the relationship to the field samples is understandable. The established Missouri colony, apparently, has not experienced a significant bottleneck or genetic drift as compared to the more recently established Missouri colony that was selected for high/low fecundity.

The functional significance of our analyses of the genetic diversity within and among *P. maculiventris* populations lies in the context of improving the mass production and efficacy of beneficial insect species. Decades of research into insect nutrition have effectively reduced production costs of some species of predators and parasitoids. We assert that future improvements in these areas depend on directed breeding programs designed to select for traits of value in mass production programs. In our exploration of this concept, we produced high- and low-fecundity spined soldier bug lines. Our data show no genetic separation between the high- and low-fecundity lines. More to the point, we recorded no statistically valid differences in overall fecundity following group-selection protocols (data not shown). Taken with the low genetic diversity in established laboratory populations reported here, we infer that a higher level of selection pressure will be necessary to produce spined soldier bug lines with stable high-fecundity traits.

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