

6-2007

# Genetic Diversity in Laboratory Colonies of Western Corn Rootworm (Coleoptera: Chrysomelidae), Including a Nondiapause Colony

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## Abstract

Laboratory-reared western corn rootworms, *Diabrotica virgifera virgifera*, from colonies maintained at the North Central Agricultural Research Laboratory (NCARL) in Brookings, SD, are used extensively by many researchers in studies of the biology, ecology, behavior, and genetics of this major insect pest. A nondiapause colony developed through artificial selection in the early 1970s is particularly attractive for many studies because its generation time is much shorter than that of typical diapause colonies. However, the nondiapause colony has been in culture for  $\approx 190$  generations without out-crossing. We compared variation at six microsatellite loci among individuals from the NCARL nondiapause colony ( $\approx 190$  generations), main diapause colony ( $\approx 22$  generations), four regional diapause colonies (3–8 generations), and four wild populations. Genetic diversity was very similar among the diapause laboratory colonies and wild populations. However, the nondiapause colony showed  $\approx 15$ – $39\%$  loss of diversity depending on the measure. Pairwise estimates of  $F_{ST}$  were very low, revealing little genetic differentiation among laboratory colonies and natural populations. The nondiapause colony showed the greatest genetic differentiation with an average pairwise  $F_{ST}$  of 0.153. There was little evidence that the laboratory colonies had undergone genetic bottlenecks except for the nondiapause colony. The nondiapause colony has suffered a moderate loss in genetic diversity and is somewhat differentiated from wild populations. This was not unexpected given its history of artificial selection for the nondiapause trait, and the large number of generations in culture. In contrast, the results indicate that the diapause colonies maintained at NCARL are genetically similar to wild populations.

## Keywords

*Diabrotica virgifera virgifera*, Western corn rootworm, genetic diversity, laboratory colonies, selection

## Disciplines

Entomology | Genetics | Genomics | Laboratory and Basic Science Research | Molecular Genetics

## Comments

This article is from *Environmental Entomology* 36 (2007): 637, doi:[10.1603/0046-225X\(2007\)36\[637:GDILCO\]2.0.CO;2](https://doi.org/10.1603/0046-225X(2007)36[637:GDILCO]2.0.CO;2).

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# Genetic Diversity in Laboratory Colonies of Western Corn Rootworm (Coleoptera: Chrysomelidae), Including a Nondiapause Colony

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Environ. Entomol. 36(3): 637–645 (2007)

**ABSTRACT** Laboratory-reared western corn rootworms, *Diabrotica virgifera virgifera*, from colonies maintained at the North Central Agricultural Research Laboratory (NCARL) in Brookings, SD, are used extensively by many researchers in studies of the biology, ecology, behavior, and genetics of this major insect pest. A nondiapause colony developed through artificial selection in the early 1970s is particularly attractive for many studies because its generation time is much shorter than that of typical diapause colonies. However, the nondiapause colony has been in culture for  $\approx 190$  generations without out-crossing. We compared variation at six microsatellite loci among individuals from the NCARL nondiapause colony ( $\approx 190$  generations), main diapause colony ( $\approx 22$  generations), four regional diapause colonies (3–8 generations), and four wild populations. Genetic diversity was very similar among the diapause laboratory colonies and wild populations. However, the nondiapause colony showed  $\approx 15$ –39% loss of diversity depending on the measure. Pairwise estimates of  $F_{ST}$  were very low, revealing little genetic differentiation among laboratory colonies and natural populations. The nondiapause colony showed the greatest genetic differentiation with an average pairwise  $F_{ST}$  of 0.153. There was little evidence that the laboratory colonies had undergone genetic bottlenecks except for the nondiapause colony. The nondiapause colony has suffered a moderate loss in genetic diversity and is somewhat differentiated from wild populations. This was not unexpected given its history of artificial selection for the nondiapause trait, and the large number of generations in culture. In contrast, the results indicate that the diapause colonies maintained at NCARL are genetically similar to wild populations.

**KEY WORDS** Western corn rootworm, *Diabrotica virgifera virgifera*, genetic diversity, laboratory colonies, selection

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, is the main pest of field corn, *Zea mays* L., in North America (Levine and Oloumi-Sadeghi 1991, Rice 2004) and is now a major invasive pest in Europe (Hemerik et al. 2004, Miller et al. 2005). This insect is increasingly difficult to manage (Ostlie 2001, Sappington et al. 2006) because of its propensity to evolve resistance to both chemical (Wright et al. 2000, Zhu et al. 2001) and cultural (Levine et al. 2002) control. Transgenic *Bt* corn targeting rootworms was recently commercialized (Rice 2004). There is concern that the western corn rootworm may also evolve resistance to expressed *Bt* toxins (Ostlie 2001, Storer 2003, Siegfried et al. 2005); thus, there is renewed impetus for developing effective insect resistance management (IRM) strategies. Because of the enormous and growing impact of this insect on agriculture, there is a great deal of ongoing research focused on the

western corn rootworm in the areas of genetics, ecology, behavior, physiology, toxicology, development, general biology, pest management and biocontrol methods, and modeling.

Many research projects on the western corn rootworm rely on the use of laboratory-reared insects. For example, field plots can be infested with known numbers of rootworm eggs or larvae in plant screening trials, control efficacy trials, and behavior studies (Riedell 1989, Sutter et al. 1991, Moellenbeck et al. 1994, Journey and Ostlie 2000, Urías-López et al. 2000, Hibbard et al. 2004). Greenhouse experiments can involve infestation of plants with neonate larvae to examine treatment effects on larval biology and development or host range (Clark and Hibbard 2004, Chege et al. 2005). Laboratory bioassays are used to explore larval behavior, survivorship, development, physiology, and plant response to damage (Riedell and Reese 1999, Davis et al. 2000, Pleau et al. 2002, Zhou et al. 2003, Clark et al. 2006).

The western corn rootworm is univoltine, undergoing an obligate diapause in the egg stage (Krysan 1972, Krysan and Branson 1977). This diapause makes rearing and timely availability of laboratory-reared

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**Table 1.** Description of *D. v. virgifera* laboratory colonies maintained at the NCARL in Brookings, SD, and wild populations, from which *n* specimens were genotyped at six microsatellite loci

Type	Origin	Text designation	Table and figure abbreviation	<i>n</i>	Year <sup>a</sup>	Approximate generations in laboratory
Laboratory nondiapause	Brookings Co., SD	Nondiapause colony	LSDnd	53	1966	190
Laboratory diapause	Moody Co., SD	Main diapause colony	LSDd	54	1987	22
	Potter Co., SD	South Dakota colony	LSD	53	1995	8
	Tippecanoe Co., IN	Indiana colony	LIN	55	1997	6
	Centre Co., PA	Pennsylvania colony	LPA	51	2000	3
	Finney Co., KS	Kansas colony	LKS	52	2000	3
Wild samples	Polk Co., IA	Iowa wild population	WIA	61	2003	0
	Centre Co., PA	Pennsylvania wild population	WPA	62	2003	0
	Ford Co., KS	Western Kansas wild population	WFKS	59	2003	0
	Cloud Co., KS	Eastern Kansas wild population	WCKS	59	2003	0

<sup>a</sup> Year of initiation of laboratory colonies or year of collection of beetles from wild populations.

insects a challenge because of long generation times. This hindrance was overcome by the successful laboratory selection of a nondiapause strain in the early 1970s at the USDA-ARS Northern Grain Insects Research Laboratory (NGIRL) in Brookings, SD (Branson 1976). Starting with a diapause population collected in South Dakota in the mid-1960s and reared without selection for six generations, recurrent selection for nondiapause was applied for nine generations before no further response was achieved (Branson 1976). NGIRL (recently renamed the North Central Agricultural Research Laboratory [NCARL]) has maintained this nondiapause colony continuously since then without introduction of new genetic material. By 1980, Branson et al. (1981) estimated the colony had been maintained for >60 generations, and Hibbard et al. (1999) conservatively estimated that this colony had been in culture for >100 generations when they tested it for virulence against corn beginning in 1995. Approximately six generations per year are normal (Branson et al. 1981), so the actual number of generations by the time of the Hibbard et al. (1999) study may have been close to 140. Other laboratories have established their own nondiapause colonies (e.g., Bernklau and Bjostad 2005, Chege et al. 2005), and nondiapause insects can be purchased from commercial suppliers as well (e.g., Pleau et al. 2002, Clark et al. 2006). Although independent diapause colonies of western corn rootworm are sometimes initiated from wild material (e.g., Hoback et al. 2002, Moeser and Vidal 2004), to our knowledge all existing nondiapause colonies were started with material from the original nondiapause colony of NCARL and are either maintained without outcrossing or they are the result of the nondiapause genes being introgressed into a diapause colony. NCARL also maintains a main diapause colony that has not been outcrossed since its establishment in 1987 (Hibbard et al. 1999) and several other diapause colonies founded from regional collections of adults in 1995–2000.

The numbers of adults used to mate and produce the next generation for each of the colonies at NCARL are quite large ( $\approx 600$  males and 1,000 females), with the intent of reducing the amount of inbreeding and effects of inadvertent laboratory selection as much as

possible (Jackson 1986, Branson et al. 1988). Because these colonies are used so extensively in studies of western corn rootworm biology, ecology, behavior, and genetics (e.g., Dunn and Frommelt 1998, Riedell and Reese 1999, Wilson and Hibbard 2004, Ellsbury et al. 2005, Nowatzki et al. 2006, Oyediran et al. 2005, Siegfried et al. 2005), it is important to know how much genetic variation may have been lost during the purposeful selection for nondiapause (Branson 1976), from unintended selection for laboratory performance, or from bottlenecks and inbreeding associated with rearing. Inadvertent loss of genetic variation affecting traits of interest could lead to experimental results that are not reflective of wild-type populations (Mukhopadhyay et al. 1997, Arias et al. 2005). In addition, with the concern over potential development of resistance to *Bt* corn, scientists seeking to conduct laboratory selection experiments will find the nondiapause trait logistically attractive, because generation times are only  $\approx 60$  d instead of  $\approx 260$  d for diapause colonies (Hibbard et al. 1999). However, response of this colony to selection will depend on how much genetic variation at resistance loci is still present.

Analyses of several microsatellite markers showed that genetic variation is uniformly high in western corn rootworm populations across the U.S. Corn Belt and that there is little differentiation between populations (Kim and Sappington 2005b). In this study, we compare variation at six microsatellite loci among the NCARL nondiapause, main diapause, four regional diapause colonies, and four natural populations. The results indicate the relative magnitude of loss of neutral genetic variation in the laboratory colonies and will help researchers design and interpret experiments conducted with insects from these colonies.

## Materials and Methods

### *Diabrotica virgifera virgifera* Colonies and DNA Isolation

Details of laboratory-reared *D. v. virgifera* diapause and nondiapause colonies are described in Table 1. The nondiapause colony was established from wild individuals collected near Brookings, SD, in the 1960s

and subjected to artificial selection for the nondiapaue trait (Branson 1976). The main diapaue colony was initiated from wild insects collected near Brookings in 1987, and the remaining regional diapaue colonies of *D. v. virgifera* were established from wild beetles captured from various locations across the Corn Belt from 1995 to 2000 (Table 1). None of the laboratory colonies have been outcrossed since establishment. Wild populations of adult *D. v. virgifera* from four widely separated geographic locations were sampled in 2003 (Table 1; Kim and Sappington 2005b). Adults of mixed sexes were collected and frozen at  $-20^{\circ}\text{C}$ . Genomic DNA was extracted from individual rootworms using the Aqua Pure isolation kit (Bio-Rad, Hercules, CA) according to the manufacturer's protocol.

### Microsatellites

Four polymorphic dinucleotide repeat (*DVV-D2*, *DVV-D4*, *DVV-D8*, *DVV-D11*) and two trinucleotide repeat (*DVV-T2*, *DVV-ET1*) microsatellite markers were used in this study. *DVV-ET1* was derived by database mining of *D. v. virgifera* expressed sequence tags (Miller et al. 2005; Kim and Sappington, unpublished data). The other five were developed for *D. v. virgifera* in a previous study (Kim and Sappington 2005a). All of these markers segregated in Mendelian fashion in an analysis of 10 families (25 offspring per family), and the DNA was amplified successfully by polymerase chain reaction (PCR) in all *D. v. virgifera* individuals examined (data not shown). Thus, these markers are not expected to have null alleles. Linkage analyses of controlled families have shown that these markers are not linked with one another (unpublished data). The loci were amplified from 51 to 62 individuals per laboratory colony or wild population in multiplexed PCR reactions, and individuals were genotyped using a Beckman-Coulter CEQ 8000 Genetic Analysis System (Beckman Coulter, Fullerton, CA) following the methods described in Kim and Sappington (2005a). In the case of the wild populations, genotypes at all microsatellite loci, except *DVV-ET1*, were determined in a previous study by Kim and Sappington (2005b).

### Data Analysis

**Levels of Genetic Variation.** Genetic diversity was assessed by examining four parameters for each population: (1) allelic diversity (AD), the mean number of alleles per locus; (2) observed heterozygosity ( $H_O$ ); (3) unbiased estimates of expected heterozygosity ( $H_E$ ) under Hardy-Weinberg assumptions; and (4) allelic richness (AR), a measure of the number of alleles independent of sample size. AD,  $H_O$ , and  $H_E$  were estimated using the Microsatellite Toolkit (Park 2001). AR was calculated using the program FSTAT v. 2.9.3 (Goudet 2001). Significant differences in allele numbers among *D. v. virgifera* populations were assessed across all loci with the nonparametric Kruskal-Wallis test ( $\alpha = 0.05$ ) corrected for experimentwise

error rate (Daniel 1990) using Statistix software (Analytical Software 2000). Evidence of genotypic linkage disequilibrium between all pairs of loci in each population was assessed using the genotype disequilibrium option implemented in FSTAT v. 2.9.3 (Goudet 2001).

**Partitioning of Genetic Variation.** *F*-statistics and pairwise  $F_{STs}$  (Weir and Cockerham 1984) were calculated with FSTAT v. 2.9.3 (Goudet 2001), and the sequential Bonferroni correction was applied to derive significance levels for multiple comparisons (Rice 1989). The probability of a population being in Hardy-Weinberg equilibrium (HWE) was determined using the exact probability test approach (Guo and Thompson 1992) implemented by the program GENEPOP (Raymond and Rousset 1995). For the hierarchical analysis of variance (ANOVA) of gene frequencies, an analysis of molecular variance (AMOVA) (Excoffier et al. 1992) was performed using the program ARLEQUIN (Excoffier et al. 2005). Three groups were formed and tested for group structure: (1) the nondiapaue colony, (2) the main diapaue and four regional diapaue colonies, and (3) the four wild populations.

**Population Bottleneck Tests.** The program BOTTLENECK 1.2 (Cornuet and Luikart 1996) was used to scan for evidence of recent population bottlenecks, under both a strict stepwise mutation model (SMM) (Kimura and Ohta 1978) and a two-phase model (TPM) (Di Rienzo et al. 1994) with 1,000 iterations. A generalized stepwise mutation model (GSM) was assumed for the TPM, in which the proportion of SMM was set to 0 with a variance in mutation lengths of 0.36 (Estoup et al. 2001). Deviations of observed heterozygosity relative to that expected at drift-mutation equilibrium were evaluated with the Wilcoxon sign-rank test (Luikart et al. 1998a). We examined allele frequency distributions for a mode-shift, which can serve as a qualitative indicator of a population bottleneck (Luikart et al. 1998b). Last, the *M* value of Garza and Williamson (2001) and its variance across loci were calculated using the program AGARST (Harley 2001). *M* is the mean ratio of the number of alleles to the range of allele size and can be used to detect reductions in population size, both recent and historical (Garza and Williamson 2001, Spear et al. 2006).

## Results

### Allele Frequencies and Genetic Diversity

A total of 70 alleles were detected across the six *D. v. virgifera* microsatellite loci, and 558 individuals were analyzed. The number of alleles per locus ranged from 3 for *DVV-T2* to 21 for *DVV-D8*, with an average of 11.8. Nine of 70 alleles were unique, or private, to a single population, but they occurred at very low frequency (average frequency of private alleles = 0.017). The highest frequency of a private allele, 0.057, was observed in the nondiapaue colony at the *DVV-D4* locus. The frequency of most private alleles was  $<0.01$ .

**Table 2.** AD, AR,  $H_O$ , and  $H_E$  at HWE,  $F_{IS}$ , and the probability ( $P$ ) of being in HWE for *D. v. virgifera* populations

Population	AD	AR	$H_E$	$H_O$	$F_{IS}$	$P^a$
LSDnd	5.33	5.29	0.588	0.594	-0.011 <sup>NS</sup>	0.983
LSDd	9.00	8.92	0.727	0.762	-0.049 <sup>NS</sup>	0.396
LSD	8.50	8.48	0.724	0.708	0.023 <sup>NS</sup>	0.080
LPA	7.33	7.33	0.694	0.693	0.002 <sup>NS</sup>	0.214
LKS	8.83	8.81	0.737	0.724	0.017 <sup>NS</sup>	0.823
LIN	8.17	8.08	0.710	0.700	0.014 <sup>NS</sup>	0.453
WIA	9.17	8.94	0.720	0.711	0.013 <sup>NS</sup>	0.346
WPA	8.67	8.43	0.708	0.704	0.005 <sup>NS</sup>	0.912
WFKS	8.50	8.34	0.713	0.678	0.049 <sup>NS</sup>	0.018
WCKS	8.67	8.52	0.721	0.689	0.045 <sup>NS</sup>	0.127
Mean	8.22	8.11	0.704	0.696	0.012 <sup>NS</sup>	0.172

Population abbreviations as in Table 1.

<sup>a</sup> Probability values using Fisher's method implemented by the program GENEPOP.

NS, not significant.

Different measures of genetic diversity—AD, AR,  $H_O$ , and  $H_E$ —were calculated over six loci for each *D. v. virgifera* population (Table 2). AD and AR ranged from 5.3 (nondiapauses colony) to 9.2 (Iowa wild population) and 5.3 (nondiapauses colony) to 8.9 (Iowa wild population), respectively.  $H_O$  values were very similar to  $H_E$  values, the latter ranging from 0.588 (nondiapauses colony) to 0.737 (Kansas colony), with an average of 0.704. Thus, the four related measures of genetic diversity were uniformly high in all populations, although values for the nondiapauses colony were consistently lower than those of the other laboratory colonies and wild populations. Nevertheless, genetic diversity remains high in the nondiapauses colony, as indicated by Kruskal-Wallis tests ( $\alpha = 0.05$ ), which revealed no significant pairwise differences in the medians of AD or AR among populations (AD: KW statistic = 3.38;  $P = 0.947$ ; AR: KW statistic = 4.25;  $P = 0.894$ ).

$F_{IS}$  estimates for populations across all loci ranged from -0.049 to 0.049, with no significant evidence of inbreeding (Table 2). None of the diapauses or nondiapauses colonies showed a significant departure from HWE over all loci, indicating that the *D. v. virgifera*

laboratory colonies are maintained as essentially random mating populations.

Tests of association between genotypes at all pairwise combinations of loci in each population showed no significant evidence of linkage disequilibrium for most locus pairs. From 150 comparisons over all 10 populations and over all 15 locus pairs, only *DVV-D4* and *DVV-ET1* showed significant linkage disequilibrium in one population, the South Dakota colony, at the adjusted nominal level (5%) for multiple comparisons.

### Genetic Differentiation Among Populations and Partitioning of Genetic Variation

The multilocus  $F_{ST}$  estimate over all populations (0.036) was low but significant. However, when the nondiapauses colony was excluded, the multilocus  $F_{ST}$  over all other populations was only 0.006. This indicates that the overall genetic differentiation can be attributed to differentiation of the nondiapauses colony from the others and that there is very little genetic differentiation between the laboratory-reared diapauses colonies and wild populations.

Pairwise  $F_{ST}$  estimates calculated by the method of Weir and Cockerham (1984) ranged from -0.003 (main diapauses colony versus Iowa wild population) to 0.172 (Pennsylvania colony versus nondiapauses colony; Table 3). Apart from nondiapauses colony versus other populations, most paired comparisons exhibited a very low  $F_{ST}$ , revealing little genetic differentiation among populations, irrespective of whether they are laboratory colonies or natural populations. The nondiapauses colony showed the greatest genetic differentiation with respect to all other populations, with an average pairwise  $F_{ST}$  of 0.153. The average pairwise  $F_{ST}$  for other populations ranged from 0.018 (Iowa wild population) to 0.031 (Pennsylvania colony), but when the nondiapauses colony was excluded, average pairwise  $F_{ST}$  estimates of other populations ranged from 0.002 (Iowa wild population) to 0.013 (Pennsylvania colony). The results from hierarchical AMOVAs of *D. v. virgifera* sorted into three groups—one nondia-

**Table 3.** Pairwise estimates of  $F_{ST}$  (below diagonal) and  $D_A$  genetic distance (above diagonal) between populations

	LSDnd	LSDd	LSD	LPA	LKS	LIN	WIA	WPA	WFKS	WCKS
LSDnd	—	0.274	0.248	0.312	0.269	0.275	0.260	0.244	0.241	0.280
LSDd	0.157 <sup>b</sup>	—	0.039	0.060	0.033	0.032	0.022	0.038	0.032	0.040
LSD	0.132 <sup>b</sup>	0.005 <sup>NS</sup>	—	0.077	0.049	0.058	0.046	0.055	0.049	0.056
LPA	0.172 <sup>b</sup>	0.011 <sup>b</sup>	0.016 <sup>b</sup>	—	0.071	0.062	0.054	0.057	0.069	0.061
LKS	0.139 <sup>b</sup>	0.001 <sup>NS</sup>	0.006 <sup>NS</sup>	0.021 <sup>b</sup>	—	0.048	0.032	0.043	0.037	0.034
LIN	0.163 <sup>b</sup>	0.003 <sup>NS</sup>	0.012 <sup>b</sup>	0.014 <sup>b</sup>	0.010 <sup>b</sup>	—	0.036	0.037	0.039	0.047
WIA	0.149 <sup>b</sup>	-0.003 <sup>NS</sup>	0.006 <sup>b</sup>	0.009 <sup>b</sup>	-0.001 <sup>NS</sup>	0.006 <sup>NS</sup>	—	0.021	0.023	0.020
WPA	0.159 <sup>b</sup>	0.001 <sup>NS</sup>	0.009 <sup>b</sup>	0.007 <sup>b</sup>	0.010 <sup>NS</sup>	0.007 <sup>NS</sup>	0.002 <sup>NS</sup>	—	0.031	0.032
WFKS	0.144 <sup>b</sup>	0.001 <sup>NS</sup>	0.004 <sup>NS</sup>	0.016 <sup>b</sup>	0.001 <sup>NS</sup>	0.004 <sup>NS</sup>	0.000 <sup>NS</sup>	0.003 <sup>NS</sup>	—	0.026
WCKS	0.162 <sup>b</sup>	0.001 <sup>NS</sup>	0.008 <sup>b</sup>	0.013 <sup>b</sup>	0.004 <sup>NS</sup>	0.012 <sup>b</sup>	-0.003 <sup>NS</sup>	0.004 <sup>NS</sup>	-0.000 <sup>NS</sup>	—
Mean <sup>a</sup>	0.153	0.020	0.022	0.031	0.021	0.026	0.018	0.022	0.019	0.022

Population abbreviations as in Table 1.

Probability of  $F_{ST}$  being significantly different than zero after corrections for multiple comparisons.

<sup>a</sup> The average value of pairwise  $F_{ST}$  estimates of a population with respect to all others.

<sup>b</sup>  $P < 0.05$ .

NS, not significant.

**Table 4. Results of hierarchical AMOVA for three groupings of *D. v. virgifera* populations: nondiapause colony, diapause colonies, and wild populations**

Source of variation	df	Sum of squares	Variance components	Percentage of variation	<i>P</i> <sup>a</sup>
Among groups	2	72.54	0.101	4.53	0.056
Among populations within groups	7	24.98	0.013	0.57	<0.001
Among individuals within populations	548	1,172.53	0.025	1.12	0.123
Within individuals	558	1,166.00	2.090	93.77	<0.001
Total	1,115	2,436.04	2.223	—	—

<sup>a</sup> Probability based on 5,040 permutations using a nonparametric permutation approach described in Excoffier et al. (1992).

pause colony, five diapause colonies, and four wild populations—revealed that most of the genetic variation was partitioned within individuals, accounting for 93.8% of the total variation (Table 4). Only 4.53% of the variation partitioned among groups, 0.57% of the variation partitioned among populations within groups, and 1.12% of the variation partitioned among individuals within populations.

**Genetic Relationships Among Populations**

The *D<sub>A</sub>* genetic distances of Nei et al. (1983) were calculated to infer genetic relationships among populations (Table 3) and ranged from 0.020 (Iowa wild population versus eastern Kansas wild population) to 0.312 (nondiapause colony versus Pennsylvania colony). The nondiapause colony showed consistently high levels of genetic divergence with respect to other populations (average *D<sub>A</sub>* = 0.267). The neighbor-joining (NJ) tree based on the *D<sub>A</sub>* genetic distances of Nei et al. (1983) revealed that apart from the South Dakota colony and nondiapause colony, which were segregated from the others, most populations were grouped into a single large clade with moderate bootstrap support of 57% (Fig. 1).

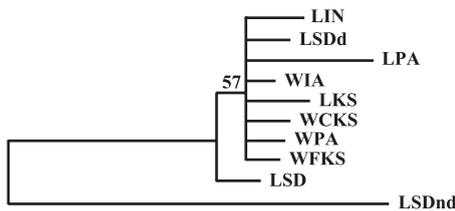
A principal component (PC) analysis showed that the first two PC axes together account for 80% of the total variance in the covariance matrix. The largest portion of the variance (72%) was accounted for by PC axis 1. A scattergram of mean factor scores revealed that the nondiapause colony is clearly divergent from the other populations along PC axis 1, whereas all

other populations are closely clustered along that axis (Fig. 2). Although populations are divergent along PC axis 2, that axis accounts for only 8% of the total variance.

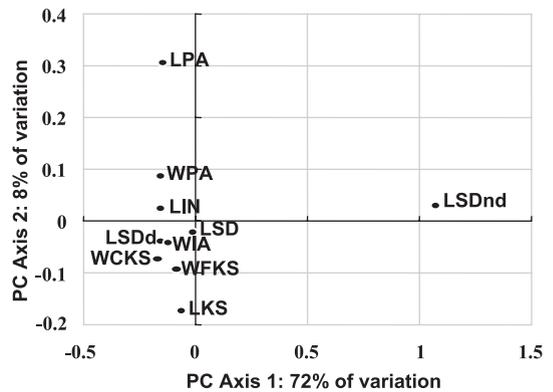
**Population Bottlenecks**

Results of tests for recent genetic bottleneck events differed depending on which of the three approaches was used (Table 5). Wilcoxon sign-rank tests revealed no significant excess of observed heterozygosity in any population of *D. v. virgifera* tested under either the strict stepwise mutation model or generalized stepwise mutation model, and thus no evidence of bottlenecks. Likewise, the mode shift test detected no deviations from the normal L-shaped allele frequency distribution expected for a large, stable, nonbottlenecked population.

In contrast, *M* values (Garza and Williamson 2001), ranging from 0.558 (in nondiapause colony) to 0.752 (in Iowa wild population and eastern Kansas wild population; Table 5), provided evidence for bottleneck events in all populations. *M* values of all populations are below those expected from historically stable populations (0.82) and also are below the equilibrium value of *M* (0.814) at the recommended parameterizations of 90% for *p<sub>s</sub>* (percent one-step



**Fig. 1.** Neighbor-joining dendrogram based on the *D<sub>A</sub>* genetic distance (Nei et al. 1983) showing genetic relationships among 10 *D. v. virgifera* populations. The numbers at the nodes are the percentage bootstrap values from 1,000 replications of resampled loci. Only nodes with at least 50% bootstrap support are shown. Population abbreviations as in Table 1.



**Fig. 2.** Scatter diagram of factor scores derived from PC analysis of a covariance matrix of allele frequencies to show genetic relationships among 10 *D. v. virgifera* populations. Population abbreviations as in Table 1.

**Table 5.** Results of various tests to detect a recent population bottleneck event within each *D. v. virgifera* population

Population	Wilcoxon sign-rank tests <sup>a</sup>		Mode shift	M <sup>c</sup>
	SMM	TPM <sup>b</sup>		
LSDnd	0.781	0.578	Normal	0.558 (0.029)
LSDD	0.977	0.781	Normal	0.740 (0.026)
LSD	0.922	0.219	Normal	0.685 (0.040)
LPA	0.961	0.656	Normal	0.685 (0.037)
LKS	0.945	0.422	Normal	0.736 (0.027)
LIN	0.922	0.922	Normal	0.715 (0.031)
WIA	0.977	0.656	Normal	0.752 (0.039)
WPA	0.977	0.719	Normal	0.713 (0.027)
WDKS	0.922	0.922	Normal	0.713 (0.028)
WCKS	0.977	0.656	Normal	0.752 (0.024)

Population abbreviations as in Table 1.

<sup>a</sup> One-tailed probability for observed heterozygosity excess relative to the expected equilibrium heterozygosity, which is computed from the observed no. of alleles under drift-mutation equilibrium. SMM, stepwise mutation model; TPM, two-phased model of mutation.

<sup>b</sup> The test was conducted assuming a generalized stepwise mutation model (GSM) with a variance of 0.36 in geometric distribution of mutation lengths (Estoup et al. 2001).

<sup>c</sup> The M value of Garza and Williamson (2001) and its variance (in parentheses). M, the mean ratio of the no. of alleles to the range of allele size.

mutations) and of 3.5 for  $\Delta_g$  (mean size of non-one-step mutations) (Garza and Williamson 2001).

## Discussion

Loss in genetic diversity is predicted over time for populations of fixed size (Crow and Kimura 1970), and changes in the genetics of laboratory insect populations can be substantial, even when maintained in high numbers in the absence of artificial selection (Mackauer 1976, Briscoe et al. 1992, Miyatake and Yamagishi 1999). Such losses in diversity can occur through founder effects at the time of colony initiation (Bartlett 1985, van Lenteren 2003, Arias et al. 2005), inadvertent selection for laboratory performance (Mackauer 1976, Norris et al. 2001, Fry 2003), which can lead to inbreeding (van Lenteren 2003), genetic drift during periods of low effective population size (i.e., population bottlenecks) (Matthews and Craig 1987, Miyatake and Yamagishi 1999), or during purposeful artificial selection, which is often accompanied by inbreeding (Fry 2003). Although major losses in genetic diversity in laboratory colonies are common, they are not inevitable. Lanzaro et al. (1998) found little genetic divergence between a long-established laboratory colony and three wild populations of the South American sand fly *Lutzomyia longipalpis* (Lutz and Neiva) based on isozyme markers.

In this study, we used microsatellite markers to provide an index of how much neutral genetic variation may have been lost in laboratory populations of *D. v. virgifera* maintained at NCARL. The results clearly indicate that the nondiapause colony harbors less genetic variation than the other diapause colonies or wild populations tested. Depending on the parameter measured, there has been a loss of 15–39% genetic diversity in the nondiapause colony compared with

contemporary wild populations. Genetic divergence of this colony from wild populations is evidenced by moderate and significant pairwise  $F_{ST}$  values and relatively high pairwise values of the  $D_A$  genetic distance of Nei et al. (1983). In stark contrast, there is no evidence for loss of genetic variation or of genetic divergence among any of the diapause laboratory colonies relative to wild populations.

The loss of genetic diversity in the nondiapause colony was not unexpected given its history, but the precise reason for the loss is not known. The most obvious differences between the nondiapause colony and the various diapause colonies is that the former had been in culture for  $\approx 190$  generations versus  $\leq 22$  generations for the latter at the time we collected specimens, and it underwent several generations of artificial selection for the nondiapause trait, which could have increased inbreeding (Fry 2003). Uniformly low  $F_{IS}$  values indicate that inbreeding is currently not a problem in any of the colonies. There was no apparent founder effect at the initiation of any of the diapause populations, wherein a reduced sample of alleles from the wild population is caused by a limited number of founding individuals. This is evidenced by low frequencies of private alleles and by values of allelic diversity and heterozygosity similar to those of wild populations. It is possible a founder effect may have occurred during the initial establishment of the nondiapause colony based on it having the highest frequency of a private allele (for *DVV-D4*), although the frequency of that private allele was low (0.057). The South Dakota, Pennsylvania, Indiana, and Kansas colonies each were initiated with 400–500 individuals. However, the number of individuals founding the nondiapause and main diapause colonies are not known, and it is possible that a small initial starting population in the former may have contributed to the lower diversity now observed for this colony.

Based on allele frequency mode-shift and heterozygosity tests (Cornuet and Luikart 1996, Luikart et al. 1998b), none of the populations, including the nondiapause colony, have experienced a recent bottleneck. Beginning in the mid-20th century, *D. v. virgifera* underwent a rapid range expansion across the Corn Belt out of a small area of the central Great Plains (Chiang 1973, Metcalf 1983). However, Kim and Sappington (2005b) found no evidence for bottlenecks in 10 wild populations of *D. v. virgifera* from Kansas to New York (including the 4 tested in this study), but the M statistic was not calculated in that study. The M statistic has the advantage that it can detect bottlenecks after  $>100$  generations, long after the signatures of a bottleneck sought by other tests are no longer detectable (Garza and Williamson 2001, Spear et al. 2006). Based on a survey of microsatellite data for 17 taxa (13 mammals, 2 fish, 2 insects) of known population histories, Garza and Williamson (2001) found that stable populations all had M values  $\geq 0.82$ , whereas populations known to have undergone a demographic reduction all had M values  $\leq 0.69$ . These values are generally used to evaluate estimates of M for other species.

Some of the  $M$  values for *D. v. virgifera* observed in this study are difficult to interpret. The nondiapause colony, South Dakota colony, and Pennsylvania colony all have values below the 0.69 threshold, indicating that they likely underwent a bottleneck sometime in the past. However, the main diapause colony, Kansas colony, and all of the wild populations have intermediate  $M$  values (0.71–0.75). In studies of salamanders, Spear et al. (2006) reported  $M$  values of 0.697 and 0.741 for two presumably stable populations in Arizona that had no history of demographic reductions. These values were higher than those of populations in Yellowstone National Park where demographic reductions were known or suspected (all  $\leq 0.67$ ). Although the  $M$  values of the Arizona populations were below the critical value of Garza and Williamson (2001) of 0.82 for stable populations, they could serve as reference populations for the Yellowstone populations because of their known history of relative stability (Spear et al. 2006). Similarly, the wild populations of *D. v. virgifera* can serve as reference populations for the laboratory colony populations, even though the former may be recovering from a bottleneck many generations ago related to the range expansion out of the central Great Plains. Although the main diapause and Kansas laboratory colonies have  $M$  values less than the critical value of 0.82, they fall within the range of  $M$  values of the wild reference populations, indicating that they have not undergone a bottleneck while in culture. However, the nondiapause, South Dakota, and Pennsylvania colonies all have values below the range of the wild reference populations, strongly implicating a past bottleneck in these colonies.

One of the reasons we conducted this study was to directly assess changes in genetic diversity in laboratory colonies of *D. v. virgifera* maintained at NCARL as an indicator of how similar they are to wild populations. Hibbard et al. (1999) found that infestations of corn by the main diapause colony caused damage similar to that of wild populations in field trials in Missouri, whereas infestations by the nondiapause colony caused significantly greater damage. At the time of their studies, the main diapause colony had been in culture for  $\approx 11$  generations and the nondiapause colony for  $\approx 140$  generations. In contrast, Branson et al. (1981) observed less damage by the nondiapause colony than by wild rootworms and suggested that eggs from the nondiapause colony may have been less viable. The different outcomes were probably related to soil temperatures (Hibbard et al. 1999). Our results indicate that little genetic variability has been lost in any of the diapause colonies at NCARL, although the nondiapause colony has experienced some loss. Investigators can expect a response to artificial selection for insecticide resistance or other traits of interest in laboratory colonies to be similar to that expected of wild populations, possibly even in the nondiapause colony. However, instead of working directly with the nondiapause colony in selection experiments, it might be advisable to introgress the nondiapause trait into other colonies to maximize variability in the genome before initiating selection experiments. We emphasize

that our results are an index of genetic variation in general. Loci under inadvertent selection for performance under mass-rearing conditions can be affected directly (Mackauer 1976, Fry 2003), and thus undergo genetic changes different than the neutral markers examined in this study.

### Acknowledgments

We thank C. Nielson and J. Gibson for expert technical assistance in rearing the colonies and processing samples for DNA extraction, respectively.

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Received for publication 21 September 2006; accepted 9 February 2007.