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Frequency and Distribution of Extended Diapause in Nebraska Populations of *Diabrotica barberi* (Coleoptera: Chrysomelidae)

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Frequency and Distribution of Extended Diapause in Nebraska Populations of *Diabrotica barberi* (Coleoptera: Chrysomelidae)

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Abstract

The frequency of extended diapause in populations of the northern corn rootworm, *Diabrotica barberi* Smith & Lawrence (Coleoptera: Chrysomelidae), was measured in eastern Nebraska. Adult collections were made during late summer in 2008 and 2009 from eight sites each year (seven sites were consistent over years). Eggs were obtained from 12 to 20 females per site and were held on moist soil under appropriate temperature profiles to facilitate egg survival, diapause development, and diapause termination. Percentage egg hatch was recorded after the first and second year for the 2008 and 2009 collections. Additional extended diapause expression was estimated for the 2008 and 2009 collections by counting remaining live eggs after the second-year egg hatch was completed. These data also were used to estimate each site's maximum egg viability. Results collectively indicate that the extended diapause trait was present in all eastern Nebraska populations; however, significant variation in the frequency of extended diapause was observed within and among *D. barberi* populations (first-year mean percentage egg hatch range: 2008 collections: 4.5–38.6%; 2009 collections: 10.7–42.5%). Geographically, the highest incidence of extended diapause was found along a north-south transect in eastern counties of Nebraska that parallels the Missouri River. In general, the frequency of extended diapause was lower in sites found west of the north-south transect. This study contributes to the knowledge base needed to develop appropriate *D. barberi* management strategies in Nebraska. These data can be used to pinpoint areas of Nebraska that may have the greatest risk of economic injury in first-year corn (*Zea mays* L.) when *D. barberi* densities are high.

Keywords: northern corn rootworm, population variation, overwintering

The northern corn rootworm, *Diabrotica barberi* Smith & Lawrence (Coleoptera: Chrysomelidae), is an important insect pest of corn, *Zea mays* L., in the western Corn Belt (Krysan 1986). *D. barberi* larvae feed on the roots of corn, which can inhibit the plant's capacity to uptake water and nutrients from the soil, lead to plant instability (lodging), and cause yield loss (Krysan et al. 1983, Woodson and Jackson 1996). *D. barberi* is univoltine with initial egg hatch occurring in late May to early June in the U.S. Corn Belt and adult emergence often in July and August. Female *D. barberi* oviposit primarily in the soil of cornfields (Chiang 1973, Boetel et al. 1992, Krysan 1999). *D. barberi* eggs enter obligatory diapause, which allows them to survive the winter (Krysan 1999).

Traditionally, crop rotation from a host to nonhost crop (i.e., annual corn-soybean rotation) has been an effective way to manage *D. barberi* in corn, as larvae cannot survive on nonhost roots. However, rootworm injury has periodically been reported in Minnesota, Iowa, South Dakota, and Illinois in first-year corn after annual rotation (Bigger 1932, Ostlie 1987). The most likely cause of this phenomenon is the expression of an extended egg diapause in *D. barberi* populations (Chiang 1965; Krysan et al. 1984, 1986; Ostlie 1987; Levine et al. 1992). Extended diapause is a process in which *D. barberi* remain in the egg stage for more than one winter (Ostlie 1987). Extended egg diapause in *D. barberi* was first characterized by Chiang (1965) in Minnesota, but the frequency was only 0.3% of the population. Over time, the frequency of extended diapause has increased to 40–50% in some areas (Krysan et al. 1984, Levine et al. 1992). Selection pressure on *D. barberi* created by crop rotation has been reported as a possible mechanism that increased the frequency of extended diapause in some populations (Ostlie 1987). In a corn-soybean rotation, eggs that exhibit extended diapause could hatch after two winters when corn is present and therefore pass on the trait, whereas eggs that do not exhibit extended diapause would die when they hatch after 1 yr near an unsuitable host (Ostlie 1987).

Over the past decade, northern corn rootworm densities and reports of rootworm injury in first-year corn have increased in eastern Nebraska (Hunt et al. 2007). The reason is unclear, but a series of warm winters that may have increased overwintering survival, possible increases in frequency of *D. barberi* extended diapause, or both, may be contributing factors. A 2006–2007 survey of *D. barberi* emergence patterns in first-year cornfields revealed that population densities are highly variable in eastern Nebraska, but, in general, are much higher in the northern two-thirds of the state than locations farther south (Hunt et al. 2007, L.J.M., unpublished data). The presence of *D. barberi* extended diapause has been confirmed and characterized in a population from Saunders County, Nebraska (Campbell 2009), but the frequency of extended diapause has not been evaluated in other *D. barberi* populations around the state. Therefore, as part of a larger study to more clearly understand the biology and pest potential of *D. barberi* in Nebraska, a study was conducted from 2008 to 2010 to measure the frequency and distribution of extended diapause in populations of *D. barberi* from eastern Nebraska.

Materials and Methods

D. barberi females were collected from seven counties in eastern Nebraska: Sherman, Nance, Dixon, Saunders, Lancaster, Jefferson, Nemaha, and one location in Brookings

County, South Dakota, during August–September 2008 (Fig. 1). Additional collections were made during August–September 2009 from the same seven Nebraska sites plus a site in Webster County, NE (Fig. 1). Most collection sites were ≈ 80–160 km (50–100 miles) apart. Beetles were collected from blooming plants (e.g., *Cirsium* spp., *Helianthus* spp.) that were found in roadside or fence-row habitat alongside cornfields. After corn has pollinated, many *D. barberi* beetles will move to alternative pollen sources to feed (Campbell and Meinke 2006). The intent was to use the alternative feeding sites in an area as a trap crop, which enabled a composite sample of beetles from the local neighborhood to be obtained. Beetles were collected using lung-powered aspirators or funnel-equipped bags (Tollefson 1986). During the August–September period each year, most *D. barberi* collected were visibly gravid females.
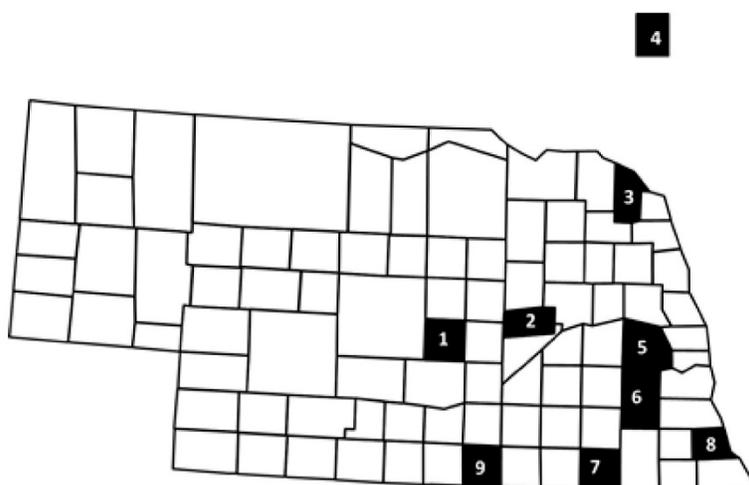
Figure 1. Nebraska counties where *Diabrotica barberi* females were collected. 1, Sherman; 2, Nance; 3, Dixon; 4, Brookings, South Dakota; 5, Saunders; 6, Lancaster; 7, Jefferson; 8, Nemaha; 9, Webster.

Individual gravid females were placed in food and ovipositional boxes, as described by Campbell and Meinke (2010). The polystyrene oviposition boxes were 5.9 cm long by 5.9 cm wide by 7.8 cm high, which included a lid 0.64 cm deep (ShowMan box, Althor Products, Wilton, Connecticut). A food "shelf" made of a rectangular piece of plastic (4.5 cm long by 2.5 cm wide by 1.5 cm high) was attached to the lid with Velcro (Velcro USA, Manchester, New Hampshire). Boxes contained moist autoclaved silty clay loam field soil (≈ 30% moisture by volume) that was presifted through a 60-mesh sieve. The beetles were fed a diet consisting of field and sweet corn ear tissue that was changed every 3–5 d. The beetles were maintained in the ovipositional boxes until they died.

The eggs were separated from the soil by washing soil through a 60-mesh sieve. Eggs recovered on the sieve were placed on a milk filter (KenAG Animal Care Group, product D110, Ashland, Ohio), and then counted under a stereomicroscope. After counting, eggs

again were placed on autoclaved moist soil in petri dishes and partially covered with a layer of 60-mesh soil.

Eggs from each female were held separately. Eggs for the 2008 collections were obtained from 13 to 22 females per site. Eggs for the 2009 collections were obtained from 13 to 15 females per site. Only boxes with a minimum of 50 eggs per female were used in the study. The overall mean number of eggs recovered per female was 176.76 ± 10.31 in 2008 and 153.09 ± 6.32 in 2009. Mean eggs obtained per female are presented in Tables 1 and 2, respectively, for each collection site and year.

To facilitate prediapause development, diapause termination (if egg is not in extended diapause), and postdiapause development, eggs were maintained at 22°C for 1–2 mo after oviposition, 10°C for ≈ 30 d, 5°C for ≈ 6 mo, and 22°C until eclosion of neonate larvae. Research has shown that *D. barberi* eggs require a specific chill period to complete diapause and obtain maximum postdiapause egg hatch (Krysan 1982, 1999; Krysan et al. 1984). Extended diapause eggs need to experience more than one chill period (i.e., multiple winters) to terminate diapause (Levine et al. 1992). Once egg hatch began, the petri plates were checked daily to count and remove any neonate larvae present. Unhatched eggs were maintained at 22°C for 3–4 mo and then subjected to the aforementioned temperature profile for a second year.

After the second-year egg hatch totals from the 2008 and 2009 collections had been counted, the number of remaining eggs that appeared normal (milky white, whole eggs) were counted. The eggs were separated from the soil in the petri dishes by using the same method described in the previous section to recover eggs from ovipositional boxes.

Statistical Analysis

The percentage of eggs that hatched after 1 yr (2008, 2009 collections analyzed separately) were compared among sites with analysis of variance (ANOVA) using the PROC MIXED procedure in SAS (version 9.2, SAS Institute 2009). Mean maximum estimated egg viability after 2 yr ([hatched eggs + remaining normal unhatched eggs]/initial egg sample size) and mean proportion of remaining normal unhatched eggs after 2 yr (remaining normal unhatched eggs/initial egg sample size) from 2008 and 2009 collections also were analyzed with ANOVA using PROC MIXED (version 9.2, SAS Institute 2009). Based on evaluations carried out in PROCUNIVARIATE and PROCGPLOT (version 9.2, SAS Institute 2009), the distributions of the percentage hatch data and the proportion of remaining unhatched egg data were well approximated by the normal distribution (Steel et al 1997, p. 246). Because of this, percentage and proportion data were not transformed before analysis. Within populations, chi-square analyses using PROCFREQ in SAS (version 9.2, SAS Institute 2009) were performed to determine if there were significant differences between the frequencies of hatched to unhatched eggs among egg cohorts. A significance level of $P < 0.05$ was used in all analyses. For ANOVA, means were separated using Fisher protected least significant difference test. Means and standard errors are reported as the least-squares means obtained from analyses.

Results

The first-year average percentage hatch for the 2008 collection ranged from 4.5 to 38.6% (Table 1). Sherman County had a significantly larger first-year average percentage hatch (38.6 ± 5.13) than all other populations collected that year. The second highest average percentage hatch came from Jefferson County, which was also significantly different from all other counties (Table 1). Egg hatch percentages recorded from Brookings, Nance, Saunders, Dixon, and Nemaha counties were not significantly different from each other (Table 1). Lancaster County had the lowest average percentage hatch (4.5 ± 1.48) but was not significantly different from Nance, Saunders, Dixon, and Nemaha counties (Table 1). Second-year egg hatch occurred in every population from the 2008 collections, and ranged from 5.3 to 15.9% of the original egg cohort sizes (Table 1).

Table 1. 2008 collections. Mean northern corn rootworm egg cohort size, percentage egg hatch, and estimated maximum egg viability per site.

Collection site	N	Mean eggs per (♀) ± SE	Mean first-year percentage egg hatch ± SE[a]	Mean second-year percentage egg hatch ± SE[b]	Post year 2 hatch mean estimated egg max viability ± SE[c]	Post year 2 hatch mean proportion normal eggs remaining ± SE[d]
Lancaster	22	103.55 ± 21.92	4.46 ± 2.32A	5.33 ± 1.43A	0.67 ± 0.04C	0.57 ± 0.05D
Nemaha	17	113.94 ± 24.94	5.56 ± 2.63AB	7.89 ± 1.63AB	0.62 ± 0.05BC	0.49 ± 0.05D
Dixon	14	156.00 ± 27.48	6.29 ± 2.90AB	6.31 ± 1.79A	0.35 ± 0.05A	0.22 ± 0.05BC
Saunders	13	262.08 ± 28.52	8.69 ± 3.01AB	6.84 ± 1.86A	0.64 ± 0.05BC	0.49 ± 0.06D
Nance	15	228.73 ± 26.55	10.37 ± 2.80AB	8.63 ± 1.73ABC	0.53 ± 0.05B	0.34 ± 0.05CD
Brookings	16	261.75 ± 25.70	12.69 ± 2.72B	15.86 ± 1.68D	0.54 ± 0.05B	0.26 ± 0.05BC
Jefferson	15	170.33 ± 26.55	23.09 ± 2.80C	12.43 ± 1.73BC	0.54 ± 0.05B	0.18 ± 0.05AB
Sherman	15	164.53 ± 26.55	38.56 ± 2.80D	13.02 ± 1.73CD	0.56 ± 0.05BC	0.04 ± 0.05A
P			< 0.0001	< 0.0001	0.0003	< 0.0001
F			17.98	5.19	4.23	13.12
df			7, 119	7, 119	7, 119	7, 119

Means presented are least-squares means (LSMEANS); within columns, means followed by the same uppercase letter are not significantly different ($P > 0.05$). N represents the number of females per site that laid > 50 eggs; egg cohorts then were used in the diapause study.

a. Mean first-year percentage egg hatch per site = (total eggs that hatched after exposure to one chill period/total eggs collected) × 100.
b. Mean second-year percentage egg hatch per site = (total eggs that hatched after exposure to second chill period/total eggs collected) × 100.
c. Post year 2 hatch mean estimated max egg viability = ([hatched eggs + remaining normal unhatched eggs]/initial egg sample size); normal eggs = milky white, whole eggs.
d. Post year 2 hatch mean proportion normal eggs remaining = (remaining normal unhatched eggs/initial egg sample size).

The first-year percentage egg hatch for the 2009 collection ranged from 10.7 to 42.5% (Table 2). First-year average percentage egg hatch data from the 2009 collections followed a pattern that was similar to that observed from 2008 collections. The Sherman County site

again had the highest mean percentage hatch (42.4 ± 3.4), but in this case, it was not significantly different than the Jefferson County site (38.6 ± 3.4). Mean percentage first-year egg hatch values from both Sherman and Jefferson counties were significantly greater than the mean percentage egg hatch from other collection sites. The mean from Webster County was significantly lower than means from Sherman and Jefferson counties but significantly greater than means from Dixon, Nemaha, and Lancaster counties (Table 2). Lancaster, Nemaha, Dixon, Saunders, and Nance counties exhibited the lowest mean percentage hatch values and were not significantly different from each other (Table 2). Second-year egg hatch occurred in every population from the 2009 collections, which ranged from 4.97 to 21.04% of the original egg cohort sizes (Table 2).

Table 2. 2009 collections. Mean northern corn rootworm egg cohort size, percentage egg hatch, and estimated maximum egg viability per site.

Collection site	N	Mean eggs per (♀) ± SE	Mean first-year percentage egg hatch ± SE[a]	Mean second-year percentage egg hatch ± SE[b]	Post year 2 hatch mean estimated egg max viability ± SE[c]	Post year 2 hatch mean proportion normal eggs remaining ± SE[d]
Lancaster	13	101.08 ± 16.75	12.18 ± 3.69A	15.50 ± 3.41BC	0.45 ± 0.05A	0.17 ± 0.04B
Nemaha	15	127.67 ± 15.59	10.67 ± 3.44A	18.06 ± 3.19BC	0.64 ± 0.05B	0.35 ± 0.04C
Dixon	15	149.27 ± 15.59	11.61 ± 3.44A	17.91 ± 3.17BC	0.44 ± 0.05A	0.14 ± 0.04AB
Saunders	15	114.07 ± 15.59	16.25 ± 3.44AB	15.37 ± 3.28BC	0.49 ± 0.05A	0.17 ± 0.04B
Nance	15	211.80 ± 15.59	16.65 ± 3.44AB	12.79 ± 2.90BC	0.51 ± 0.05A	0.21 ± 0.04AB
Webster	15	172.87 ± 15.59	25.24 ± 3.44B	21.04 ± 4.20C	0.57 ± 0.05AB	0.10 ± 0.04AB
Jefferson	15	148.07 ± 15.59	38.60 ± 3.44C	11.86 ± 2.54AB	0.55 ± 0.05AB	0.05 ± 0.04A
Sherman	15	193.00 ± 15.59	42.45 ± 3.44C	4.97 ± 1.73A	0.51 ± 0.05A	0.03 ± 0.04A
P			< 0.0001	0.0188	< 0.0001	< 0.0001
F			13.13	2.53	1.99	6.44
df			7, 110	7, 110	7, 110	7, 110

Means presented are least-squares means (LSMEANS); within columns, means followed by the same uppercase letter are not significantly different (*P* > 0.05). *N* represents the number of females per site that laid > 50 eggs; egg cohorts then were used in the diapause study.

a. Mean first-year percentage egg hatch per site = (total eggs that hatched after exposure to one chill period/ total eggs collected) × 100.

b. Mean second-year percentage egg hatch per site = (total eggs that hatched after exposure to second chill period/total eggs collected) × 100.

c. Post year 2 hatch mean estimated max egg viability = ([hatched eggs + remaining normal unhatched eggs]/initial egg sample size); normal eggs = milky white, whole eggs.

d. Post year 2 hatch mean proportion normal eggs remaining = (remaining normal unhatched eggs/initial egg sample size).

The Lancaster, Nemaha, Saunders, and Nance 2008 collection sites had the highest mean proportion of remaining normal unhatched eggs after 2 yr and were not significantly different from each other. The Sherman (0.04 ± 0.05) County site had the lowest mean proportion of remaining normal unhatched eggs, which was significantly different from all other populations, except Jefferson County (Table 1).

The 2009 Nemaha population had the highest mean proportion of remaining normal unhatched eggs after 2 yr (0.35 ± 0.04) and was significantly different from all other populations (Table 2). The numerically lowest mean proportions of remaining normal unhatched eggs were found among the Sherman (0.03 ± 0.04) and Jefferson (0.05 ± 0.04) populations (Table 2).

The estimated maximum egg viability, after completion of second-year hatch, was fairly consistent among the 2008 populations (Table 1). Most estimates of mean maximum egg viability ranged from 0.53 to 0.67. Only the Dixon population mean fell below this range and was significantly different from the other populations (Table 1). The Lancaster population had the greatest mean maximum egg viability (0.67 ± 0.04) but was not significantly different than the means from Sherman, Saunders, and Nemaha populations (Table 1). The maximum estimated egg viabilities of the Brookings, Nance, Jefferson, Saunders, Nemaha, and Sherman populations were not significantly different from each other (Table 1).

Similar results for the estimated maximum egg viability occurred for the 2009 collection data. Estimates of mean maximum egg viability were mostly consistent among the 2009 populations, ranging from 0.44 to 0.64 (Table 2). The Nemaha population had the greatest mean maximum egg viability (0.64 ± 0.05) and was significantly different from all other populations, except for the Jefferson (0.55 ± 0.05) and Webster (0.57 ± 0.05) populations (Table 2). All other populations were not significantly different from one another (Table 2).

All 2008 and 2009 populations had significant within-population variation with regard to first-year percentage egg hatch (Tables 3 and 4). Figures 2 and 3 graphically present examples of the within-population variation in first-year egg hatch from populations with relatively high (Sherman) and relatively low (Saunders, Nemaha) mean first-year percentage egg hatch.
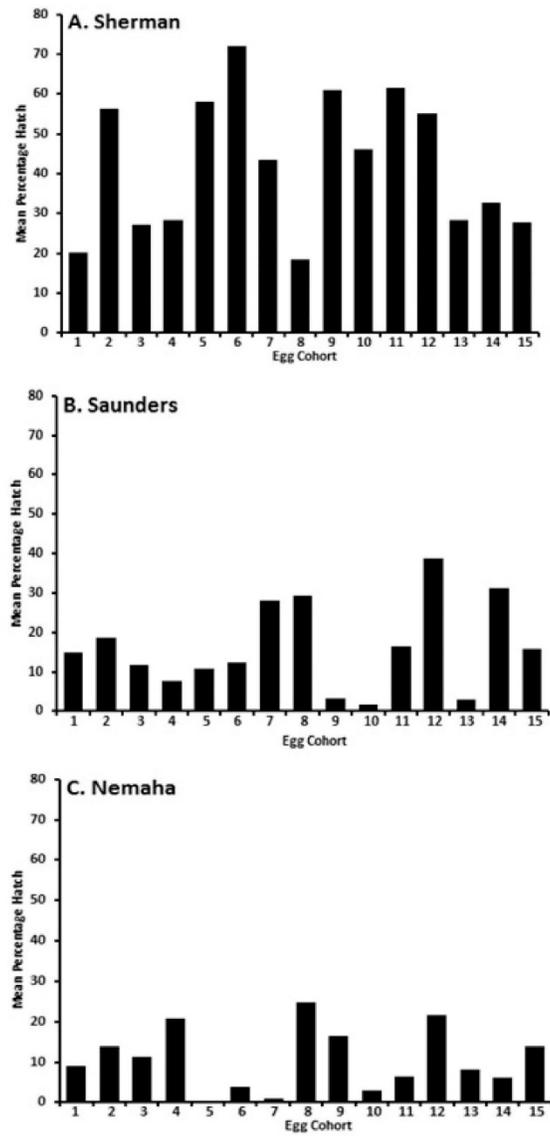
Table 3. Results of χ^2 analysis of within population variation of first-year egg hatch data, 2008 collections

Collection site	N	df	χ^2 value	χ^2 probability
Lancaster	22	21	230.81	< 0.0001
Nemaha	17	16	129.54	< 0.0001
Dixon	14	13	139.33	< 0.0001
Saunders	13	12	321.11	< 0.0001
Nance	15	14	199.66	< 0.0001
Brookings	16	15	336.43	< 0.0001
Jefferson	15	14	260.91	< 0.0001
Sherman	15	14	388.71	< 0.0001

N represents the number of females per site that laid > 50 eggs; egg cohorts were then used in the diapause study.
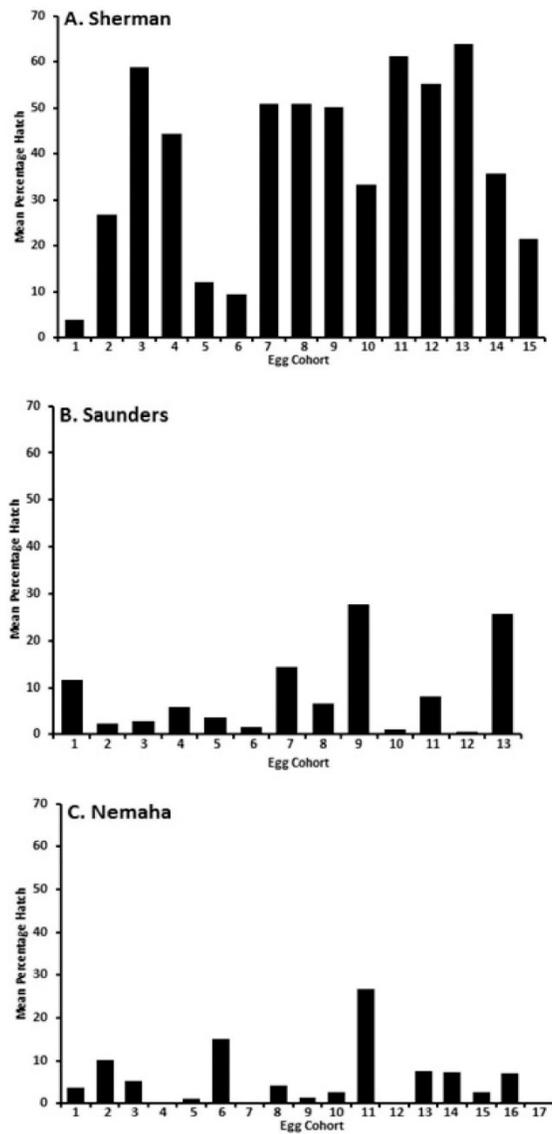
Figure 2. 2008 northern corn rootworm collection: percentage first-year egg hatch from egg cohorts obtained from individual females; (A) Sherman site, (B) Saunders site, and (C) Nemaha site.

Figure 3. 2009 northern corn rootworm collection: percentage first-year egg hatch from egg cohorts obtained from individual females; (A) Sherman site, (B) Saunders site, and (C) Nemaha site.

Table 4. Results of χ^2 analysis of within population variation of first-year egg hatch data, 2009 collections

Collection site	N	df	χ^2 value	χ^2 probability
Lancaster	13	13	117.36	< 0.0001
Nemaha	15	15	106.34	< 0.0001
Dixon	15	15	361.63	< 0.0001
Saunders	15	15	162.47	< 0.0001
Nance	15	15	376.93	< 0.0001
Webster	15	15	291.63	< 0.0001
Jefferson	15	15	147.17	< 0.0001
Sherman	15	15	341.13	< 0.0001

N represents the number of females per site that laid > 50 eggs; egg cohorts were then used in the diapause study.

Discussion

The data from this study collectively indicate that the extended diapause trait is expressed in all *D. barberi* populations included in this study. It is also clear that significant differences in the frequency of extended diapause occur among populations of *D. barberi* in eastern Nebraska. The consistent significant differences in percentage first-year egg hatch among populations from 2008 and 2009 collections show that results were repeatable within sites (Tables 1 and 2). Within collection years, mean estimated maximum egg viability was fairly consistent among populations (Tables 1 and 2). The Sherman population, which exhibited elevated mean percentage first-year egg hatch, was not significantly different in estimated maximum egg viability from six of the seven populations included in 2008 or 2009 collections. This strongly supports the premise that first-year egg hatch variation among sites was because of differences in extended diapause expression and not variation in egg viability. Finally, the mean proportion of normal eggs remaining after the second-year hatch was completed strongly mirrored the first-year hatch data. A significantly lower proportion of normal eggs remained from the Sherman site than many of the other populations (Tables 1 and 2). This study was terminated after 2 yr, but previous studies have shown that *D. barberi* is capable of remaining in diapause up to 4 yr (Levine et al. 1992, Campbell 2009). Populations such as Nemaha, Lancaster, and Saunders appeared to have many viable eggs that could still hatch in year 3, whereas potential egg hatch from the Sherman site was nearly completed.

In general, the highest frequency of extended diapause appeared to occur in a north–south transect from Brookings County, South Dakota, to Nemaha County, Nebraska, that parallels the Missouri River. *D. barberi* populations collected farther west from the north–south transect exhibited lower frequencies of extended diapause. It is unclear why the current geographic patterns of extended diapause exist, but factors such as historical cropping practices and gene flow may have contributed to the trends observed at some locations. Growers in central Nebraska historically have grown more continuous corn in contrast to growers in very eastern Nebraska, who have predominately followed a corn-soybean annual rotation (L.J.M., unpublished data). The latter scenario favors selection for an existing

extended diapause trait in local *D. barberi* populations (Krysan 1993). Composite agriculture census data from 2002 and 2007 (USDA NASS 2009) indicate that the mean ratio of harvested hectares of soybean-corn was 0.92 for eastern counties Dixon, Saunders, Lancaster, and Nemaha (high mean frequency of extended diapause), but only 0.36 for the central Nebraska counties Sherman and Webster (lower mean frequency of extended diapause). Interestingly, this positive relationship between cropping history and observed frequency of *D. barberi* extended diapause was not observed at the Nance and Jefferson County locations (i.e., Nance: soybean-corn ratio: 0.54, high mean frequency extended diapause; Jefferson: soybean-corn ratio: 0.96, lower mean frequency extended diapause). Each is geographically intermediate between the far-eastern and central Nebraska counties mentioned. If the extended diapause trait followed a diffusion pattern from the north (i.e., southeast South Dakota), where the trait has been present at high frequency for some time (Krysan et al. 1986), the prevailing cropping pattern of annual corn-soybean rotation in very eastern Nebraska would have facilitated survival of immigrant individuals exhibiting the trait (Hill and Mayo 1980). Establishment and increase in frequency of extended diapause could have been a gradual process that fits the limited dispersal capability of this insect (Naranjo 1990), which would have gone unnoticed until densities increased to a level capable of causing spotty economic injury in first-year corn. Central Nebraska *D. barberi* populations are at the western edge of the geographical range of the species (Krysan and Smith 1987), where populations are spotty and densities are often low, which could isolate populations from the main north-south transect. It would be informative to sample *D. barberi* populations at varying distances from the Missouri River east into Iowa where annual corn-soybean rotation has been a common practice to help clarify if the broad agricultural flood plain associated with the river may have facilitated gene flow from the north. Because a complex interaction of factors impacts *D. barberi* population density and distribution over time (French et al. 2004), a modeling approach may be useful to identify key relationships that drive frequency of extended diapause in Nebraska.

This study provides another example of genetic variation that can occur both within and among *D. barberi* populations. Previous research has shown that *D. barberi* can exhibit relatively high levels of genetic diversity and variation of traits across the United States. Several studies have documented that significant genetic differences can be maintained among *D. barberi* populations that are in close geographic proximity, such as aldrin susceptibility (Ball and Weekman 1963, Krysan and Sutter 1986), amplified fragment length polymorphism variation (Campbell et al. 2011), and polymorphic allozyme loci (Krafsur et al. 1993). It has been suggested that small-scale variation could be due in part to limited flight activity of *D. barberi*, which may lead to partially isolated breeding populations (McDonald et al. 1985, Krafsur et al. 1993). *D. barberi* typically moves outside of cornfields to find new sources of food (Cinereski and Chiang 1968, Naranjo and Sawyer 1988); however, tethered flight studies have shown that *D. barberi* typically only takes short trivial flights and does not undertake long-range migratory flights (Naranjo 1990).

French et al. (2012) recently reported the *D. barberi* extended diapause trait to be highly heritable with genetic dominance of the trait strongly influenced by female genotype. In their study, extended diapause females laid significantly more extended diapause eggs than females with a 1-yr diapause trait, regardless of male genotype. However, genetic

variability was maintained in all laboratory crosses. A *D. barberi* colony selected for a normal 1-yr diapause over 10 yr still produced a low proportion of extended diapause eggs. The authors then suggested that the genetic variability in diapause maintained in natural *D. barberi* populations is selectively advantageous because of year-to-year environmental (and cropping pattern) uncertainty.

Results from this study support this premise, as within-population variation in first-year egg hatch percentages of field populations was highly significant (Figs. 2 and 3). For example, the Sherman population, which exhibited the lowest mean frequency of extended diapause, included both females that produced a high proportion of extended diapause eggs and females that produced primarily 1-yr diapause eggs. Results from this study contribute to the knowledge base needed to develop appropriate *D. barberi* management recommendations for different areas of eastern Nebraska. Because the extended diapause trait appears to occur throughout eastern Nebraska, growers using crop rotation to manage rootworms in these areas should expect to see some *D. barberi* in their first-year corn. A better understanding of the extent of extended diapause in an area can also help to determine the effectiveness of crop rotation as a rootworm management tactic. In areas with a high frequency of extended diapause expression, crop rotation alone may not always be adequate to keep *D. barberi* populations from causing economic damage in first-year corn, especially when *D. barberi* densities are high. Growers in areas west of the north-south transect should be able to control *D. barberi* populations by using crop rotation because of a lower frequency of extended diapause and relatively low *D. barberi* densities.

From a resistance management perspective, the occurrence of extended diapause in *D. barberi* populations may appear to be a positive attribute. If conventional insecticides or plant-incorporated protectants (i.e., cry proteins expressed in corn hybrids) are used as part of rootworm management programs, *D. barberi* populations emerging from eggs laid 2, 3, or 4 yr earlier would provide a natural temporal refuge. Populations of *D. barberi* adults would be emerging in the same geographic area over time with less previous exposure to the pesticides or specific transgenic events being used locally. These beetles with lower resistance allele frequencies could potentially mate with individuals that are offspring of more highly selected individuals, effectively lowering the potential of resistance evolution in an area. This benefit would be in addition to any structured refuge that may be in place. This hypothesis has been supported by modeling efforts to measure the effect of extended diapause on the evolution of *D. barberi* resistance to Bt corn, but only under certain scenarios (Mitchell and Onstad 2005). Model results indicated that other operational factors (e.g., toxin dose, soil insecticide use, Bt adoption level, and crop rotation pattern in the area) strongly interacted with extended diapause frequency over years to either reduce or increase the rate of resistance evolution. Because of the diversity of extended diapause frequency and variable operational factors present across eastern Nebraska, complex interactions of these variables may lead to different rates of resistance evolution at the local landscape level.

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