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Laura A. Campbell

University of Nebraska-Lincoln, lacampbell2@gmail.com

Lance J. Meinke

University of Nebraska-Lincoln, lmeinke1@unl.edu

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Fitness of *Diabrotica barberi*, *Diabrotica longicornis*, and Their Hybrids (Coleoptera: Chrysomelidae)

LAURA A. CAMPBELL¹ AND LANCE J. MEINKE

Department of Entomology, University of Nebraska-Lincoln, 202 Entomology Hall, Lincoln, NE 68583-0816

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ABSTRACT *Diabrotica barberi* Smith & Lawrence, the northern corn rootworm, and *Diabrotica longicornis* (Say) (Coleoptera: Chrysomelidae), are currently recognized as closely related chrysomelid species. Hybridization has been proposed to occur between them, although the viability of hybrids has never been tested. The objective of this study was to assess life-history parameters of *D. barberi*, *D. longicornis*, and their hybrids under laboratory conditions to examine the potential for field hybridization. *D. barberi* and *D. longicornis* were collected in allopatry and were used to create lab colonies. Parental species were crossed to obtain F₁ hybrids, F₂ hybrids, and backcrosses to either parental species. Various life-history traits, which may contribute to overall fitness, were measured, and population growth rates were calculated for all crosses. *D. barberi* had greater reproductive potential than *D. longicornis*, but *D. longicornis* individuals lived longer than *D. barberi* individuals. In other traits, the two parental species were similar. The fitness of hybrids of a *D. longicornis* female and *D. barberi* male, as estimated by reproduction, survival, developmental time, longevity, and head capsule width, was similar to that of the parental species. Hybrids of a *D. barberi* female and *D. longicornis* male demonstrated consistently poor egg viability, low survival, and shortened adult life span. The cause of the hybrid unidirectionality is unknown, but these data collectively suggest that hybrids of a *D. longicornis* female and *D. barberi* male and F₂ backcrosses with parentals could potentially be as viable as either parental species and contribute to population growth under field conditions.

KEY WORDS northern corn rootworm, life-history traits, population growth rates, introgression

Diabrotica barberi Smith & Lawrence, the northern corn rootworm, and *Diabrotica longicornis* (Say) (Coleoptera: Chrysomelidae) are closely related galerucine chrysomelid beetles. Morphological (Krysan et al. 1983), allozyme (McDonald et al. 1982, Krysan et al. 1989), and molecular data (Clark et al. 2001a) strongly support the idea that *D. barberi* and *D. longicornis* are sister taxa. The range of *D. barberi* extends north and eastward from Nebraska and Kansas to the east coast and north to Ontario and Quebec in Canada (Krysan et al. 1983, Krysan and Smith 1987). *D. longicornis* can be found from eastern Nebraska and Kansas west and south to Arizona and Chihuahua, Mexico, but its range seems to be limited to habitats associated with river drainages (Krysan et al. 1983, Krysan and Smith 1987). *D. barberi* and *D. longicornis* are sympatric in an area that includes the eastern half of Nebraska and Kansas, plus northeastern Oklahoma (Krysan et al. 1983, Golden 1990).

D. longicornis was first collected on wild cucurbits in what is now Colorado (Say 1824). In the 1870s, a rootworm species with a similar description was documented feeding on field corn, *Zea mays* L., in Illinois

and Missouri and also was called *D. longicornis* (Webster 1913, Krysan et al. 1983). Based on range, color differences of certain morphological structures, and economic impact, Smith and Lawrence (1967) named two subspecies, *D. l. longicornis*, the nominate subspecies, and *D. longicornis barberi*, the pest in Midwest corn monocultures (Krysan et al. 1983). In 1983, Krysan et al. elevated *D. l. barberi* to species rank, i.e., *D. barberi*, basing the decision on differences in color, habitat preferences, morphological development in embryogenesis at diapause, mating behavior, and pheromone response. *D. barberi* and *D. longicornis* use different stereoisomers of the sex pheromone 8-methyl-2-decyl propanoate (Guss et al. 1985, Krysan et al. 1986b).

The two species seem to have distinct habitat preferences. Adult *D. barberi* are commonly found in or near corn, whereas adult *D. longicornis* are often collected on buffalo gourd, *Cucurbita foetidissima* HBK, a wild cucurbit, and are rarely found in corn (Krysan et al. 1983, Krysan and Smith 1987). *D. barberi*, as a corn pest, uses corn most often as a larval host and infrequently lays eggs near plants other than corn (Naranjo and Sawyer 1988, Boetel et al. 1992). However, *D. barberi* may complete some or all develop-

¹ Corresponding author, e-mail: lacampbell2@gmail.com.

ment on other weedy and native grass species (Branson and Ortman 1967, Branson and Ortman 1971, Oyediran et al. 2008). Larval host range for *D. longicornis* is unknown but most likely includes native prairie grasses (Krysan and Smith 1987); *D. longicornis* can be reared to adults on corn under laboratory conditions (Golden and Meinke 1991).

Morphologically, *D. barberi* and *D. longicornis* are very similar (Krysan et al. 1983). *D. barberi* and *D. longicornis* cannot be distinguished by male genitalia, female spermathecae, and egg chorion sculpturing, characters which can be used to successfully distinguish most *Diabrotica* species (Krysan and Smith 1987). In addition, both species exhibit an extended egg diapause, in which a proportion of the population can survive for two or more winters in the soil (Krysan et al. 1986a, Golden and Meinke 1991, Levine et al. 1992).

D. barberi and *D. longicornis* can successfully hybridize in lab situations, but unidirectional incompatibility occurs (Krysan et al. 1983, Golden 1990). Although interbreeding between *D. barberi* and *D. longicornis* has not been directly observed in the field, the results of several studies suggest that hybridization occurs in the area of sympatry. Allopatric populations of *D. barberi* and *D. longicornis* have distinct cuticular hydrocarbon profiles (Golden et al. 1992). When cuticular hydrocarbon profiles are used as a diagnostic tool, individuals from some sympatric populations in Nebraska exhibit cuticular hydrocarbon scores that are intermediate to the parental species, similar to lab-created hybrids (Golden 1990). Enzyme data from *D. barberi* populations in Nebraska and Kansas were most different from other *D. barberi* populations, and the authors attributed this difference to the range overlap with *D. longicornis* (McDonald et al. 1985).

Although the pest species *D. barberi* has been well-studied, many questions remain about its basic biology, and little is known about the biology of *D. longicornis*. In addition, no studies have been conducted to evaluate the fitness of hybrids from these taxa. Gaining further understanding of hybrid fitness in the *D. barberi* and *D. longicornis* system will help to determine what effect, if any, hybridization could be having on populations of either taxa under field conditions. Often, hybrids formed between two species are sterile or have very low fitness, contributing to reinforcement between the two species (Mayr 1963, Coyne and Orr 2004). However, genetic variability from introgression can sometimes be beneficial and maintained by natural selection (Stebbins 1959, Mayr 1963), and hybrids may have many phenotypes and varying levels of reproductive success (Stebbins 1959, Barton and Hewitt 1985). In the *D. barberi* and *D. longicornis* system, introgression could potentially affect the behavior (e.g., habitat and ovipositional preference) and subsequent pest status of the taxa in the sympatric area. Therefore, as an initial step of a larger project to understand the potential evolutionary role of hybrids of *D. barberi* and *D. longicornis*, the objective of this study was to assess various life-history parameters of *D. barberi*, *D. longicornis*, F₁ hybrids of

Table 1. Abbreviations for *D. barberi*, *D. longicornis*, and hybrid crosses

| Classification | Cross (♀ × ♂) ^a |
|-----------------------------------|---|
| Parental species | BARxBAR |
| <i>D. barberi</i> backcrosses | BARxF ₁ F ₁ xBAR |
| F ₁ hybrids | BARxLON LONxBAR |
| F ₂ hybrids | F ₁ xF ₁ |
| <i>D. longicornis</i> backcrosses | F ₁ xLON LONxF ₁ |
| Parental species | LONxLON |

^a BAR is *D. barberi*, LON is *D. longicornis*, and the F₁ hybrid used for the F₂ hybrids and backcrosses is the LONxBAR hybrid cross.

the two species, F₂ hybrids, and backcrosses to evaluate the relative fitness of hybrids to the parental species.

Materials and Methods

***D. barberi*, *D. longicornis*, and Hybrid Colonies.** *D. barberi* (BAR) colonies originated from 2004 collections from first-year cornfields near Pipestone (Pipestone Co.), MN. *D. longicornis* (LON) colonies originated from 2004 collections from buffalo gourd near Lewellen (Garden Co.) and near Rock Creek State Recreation Area (Dundy Co.), NE. The collection sites for this study were located in geographic areas where these species are allopatric (Krysan et al. 1983, Krysan and Smith 1987) to reduce the potential of collecting from introgressed populations, but near enough to the area of sympatry to provide a realistic picture of potential hybrid fitness within the sympatric zone.

For all crosses, the female of the pair is listed first (Table 1). *D. barberi* colonies originated from 26 females separated into seven oviposition boxes (see below for description of oviposition boxes). *D. longicornis* colonies originated from six females. Eggs from these females were obtained and in 2005, their offspring were mated to form LONxBAR hybrids, BARxLON hybrids, and parental species lines. In 2006, offspring from the 2005 crosses were mated to form nine types of crosses: both types of hybrid crosses, both parental species, backcrosses to each parental species (using LONxBAR hybrids as the male or female member of the pair: four crosses), and hybrid-hybrid crosses (all LONxBAR). With the exception of the BARxLON hybrid cross, 12 randomly selected crosses of each cross type were subsampled to 35 eggs in 2007, and larvae from these eggs were reared to the adult stage. Because of low numbers of viable eggs from the BARxLON crosses, all available eggs were used for rearing. Fitness parameters were measured for individuals obtained in 2006 and 2007 (see below).

Rearing, Mating, and Oviposition. Individual females were placed in oviposition boxes with soil using a modification of the box design by Boetel and Fuller (1997). The soil was moistened ($\approx 30\%$ moisture by volume), autoclaved silty clay loam soil presifted through a 60-mesh sieve. 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, CT). 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; beetles were provided with a fresh slice of sweet corn, *Z. mays*, ear every 4–5 d. Eggs were collected by washing the oviposition soil through a 60-mesh sieve, then placed on milk filters (KenAG Animal Care Group, Ashland, OH) and counted. Eggs were then placed in petri dishes and were partially covered with a light layer of 60-mesh soil. To facilitate diapause development and termination, 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.

In 2005–2007, in a method modified from Golden and Meinke (1991), larvae were provided with corn seedlings and were reared individually in 5.9-cm³ plastic cups with lids (SYSCO, Houston, TX, and Sweetheart (Solo), Highland Park, IL) to ensure production of virgin adults. Over the course of its development, each larva received three fresh corn seedlings; corn had previously been shown to be a suitable larval host for both species (Golden and Meinke 1991). Pioneer Brand 31G66 (Johnston, IA) corn, treated with fungicides Fludioxonil and Mefenoxam, was used for rearing. Peat moss was used as the soil medium to maintain corn growth and to provide an environment for pupae. The peat moss was moistened with distilled water in large batches, with 30% moisture by volume, before planting. The peat moss retained sufficient moisture for corn seedling growth for 2–3 wk, particularly in the covered rearing cups; therefore, additional water was generally not necessary. Larvae were reared at room temperature, ≈22°C. Each individual was left undisturbed during the prepupal or pupal stages to avoid causing damage to the developing pupa. Adult emergence was determined when the beetle was found on the surface of the soil. Adult gender was identified using the method of White (1977).

Beetles were maintained individually in fresh plastic cups on a diet of sweet corn ears and lettuce, *Lactuca sativa* L., until mating. Randomly chosen male-female pairs were held in the plastic cups for 7–10 d, and then the pairs were transferred to the oviposition boxes as described above. Unmated beetles were maintained individually in the plastic cups as described above until death.

Fitness Parameters. Various life-history parameters were measured as an indication of the fitness of individuals from each type of cross. The selected parameters were chosen because of their potential effects on beetle development and population growth. Lifetime oviposition was determined for BARxBAR, LONxLON, BARxLON, and LONxBAR in 2005 (eggs hatched 2006) and for the BARxBAR, LONxLON, BARxLON, LONxBAR, F₁xBAR, BARxF₁, F₁xLON, LONxF₁, and F₁xF₁ crosses in 2006 (eggs hatched 2007). For each individual cross in both years, percentage larval eclosion, percentage adult emergence, and percentage male were calculated. For in-

dividuals of each cross type, developmental time (larval eclosion to adult emergence, in days) and adult longevity (adult emergence to death, in days) also were recorded.

Adult head capsule widths were measured for males and females of each cross in 2006 and 2007. Head capsule widths have been previously used as an indication of larval fitness (Branson et al. 1988), because larval conditions, i.e., diet (Ellsbury et al. 2005), temperature (Woodson and Jackson 1996), or crowding (Branson and Sutter 1985, Naranjo 1991), can influence adult head capsule width. In addition, adult head capsule width generally correlates with overall beetle size, and, unlike other structures, such as the abdomen, the head width does not vary with beetle age. For this study, adult head capsule widths were used as an additional measure of species differences. Head capsules were measured at the widest point of the head, from the outside edge of each eye, with a dissecting microscope (Wild Heerbrugg) with an ocular lens of 20× and an objective lens at 12×. The eyepiece on the scope was calibrated using a 2-mm micrometer (Ward's Natural Science Establishment, Inc., Rochester, NY).

Population Growth. To examine the relative differences between the two species and among the various crosses, the net reproductive rate (R_0) and intrinsic rate of population increase (r_m) were calculated (Birch 1948). This method has been used as a way to compare fitness costs of resistance to *Bacillus thuringiensis* toxins in coleopteran and lepidopteran insect populations (Alyokhin and Ferro 1999, Sayyed and Wright 2001). In this study, calculation of the population growth parameters allowed an assessment of the possibility that hybrids of *D. barberi* and *D. longicornis* and backcrosses could successfully contribute to population growth.

R_0 is the lifetime number of female offspring produced by each female of a cohort (Birch 1948). The net reproductive rate is calculated as follows: $R_0 = \sum (l(x) m(x))$, where $l(x)$ is the survivorship at age class x and $m(x)$ is the number of adult females produced by females at age class x (Birch 1948). If $R_0 > 1$, the population will increase exponentially; if $R_0 < 1$, the population will decline to extinction. If R_0 is equal to 1, then the population size will be maintained, but it will not increase (Gotelli 2001). r_m is calculated using iterations of the Euler equation: $\sum ((e^{-rx}) l(x) m(x)) = 1$. The intrinsic rate of population increase is expressed in terms of the number of females produced per female per unit of time (Birch 1948, Gotelli 2001). Population growth rates were calculated using the SAS program designed by Maia et al. (2000).

Parameters for the population growth analysis (female age, fertility, gender ratio, and percentage survival) were obtained only from the 2006–2007 life-history data. Percentage survival was treated in two different ways: as the survival to adult emergence and as the survival to 10 d postemergence. This second value was chosen to account for the preovipositional period that occurs in many *Diabrotica* species (Hill

1975, Sherwood and Levine 1993). The preovipositional period in females has not been documented for *D. barberi* or *D. longicornis*, but it was assumed to be similar.

Data Analyses. Life-history parameters were analyzed using analysis of variance (ANOVA) by the PROC MIXED procedure in SAS version 9.1 (SAS Institute 2003). Cross was used as the treatment effect for fecundity, percentage larval eclosion, percentage adult emergence, and percentage male. Developmental time, longevity, and head capsule widths were analyzed as a factorial of cross and gender. Percentage values (percentage hatch, percentage survival to adult, and percentage male) were not transformed because arcsine square-root transformation did not improve normality of the data. No other life-history parameters were transformed.

Means were separated using Fisher protected least significant difference (LSD) test. If interactions were significant at the $P < 0.10$ level, treatment combination (cell) means were used to summarize the results. If interactions were not significant at the 0.10 level, factor level (marginal) means were used to summarize the results. In either case, a significance level of $P < 0.05$ was used to separate means in all analyses. Means and standard errors for all parameters, excluding the population growth parameters, were obtained from the LSMEANS statement in the PROC MIXED procedure (Littell et al. 2006).

Population growth parameter estimates and their variances were obtained using the SAS program LIFETABLE by Maia et al. (2000), which incorporates a jackknife method for computing variances (Meyer et al. 1986). In addition, LIFETABLE allows comparison of population growth parameters through one-sided and two-sided *t*-tests and their associated *P* values (Maia et al. 2000). The two-sided *t*-tests were used to separate means for the population growth parameters obtained here.

Results

Life-History Parameters. The mean number of eggs in both years from BARxBAR pairs averaged higher than the mean obtained from LONxLON pairs, although this difference was only significant in 2005 (Fig. 1A). The mean number of eggs resulting from the LONxBAR crosses was not significantly different than means from either parental species in either year (Figs. 1A and 2A). In 2005, the number of eggs from the BARxLON crosses was very low, although not significantly different than the LONxBAR or LONxLON crosses, given the sample size. In 2006, there were significantly fewer eggs laid from the BARxLON crosses than any other cross except BARxF₁ (Fig. 2A). Mean oviposition per female from any cross with a LONxBAR parent (BARxF₁, F₁xBAR, F₁xF₁, F₁xLON, and LONxF₁), was not significantly different than the means from either parental species (Figs. 1A and 2A).

Mean percentage eclosion ranged from 0 to 42% in 2006. The LONxBAR cross had the highest percentage larval eclosion, although it was not significantly dif-

ferent from the BARxBAR cross (Fig. 2A). There was no larval eclosion from the BARxLON cross, but this was not significantly different from either parental species (Fig. 2A). Mean percentage eclosion ranged between 5 and 57% in 2007. All crosses with a LONxBAR parent had a mean percentage eclosion similar to the parental species (Fig. 2B). Mean percentage eclosion for BARxLON was significantly less than all other crosses (Fig. 2B), and viable eggs were only obtained from two BARxLON pairs.

In both years, survival to the adult stage was relatively high (58–79%) for most crosses (Figs. 1C and 2C). In 2007, survival to the adult stage for the BARxLON cross was low and significantly different than the other crosses ($22.2 \pm 11.6\%$; Fig. 2C), and all adults resulted from one BARxLON pair.

In both years, the percentage of adults that were male varied among crosses. In 2006, there were no significant differences among crosses (Fig. 1D). In 2007, the BARxLON cross was 75.7% male, and all other crosses ranged between 36.8% (F₁xF₁) and 59.9% (BARxBAR) males (Fig. 2D).

The interaction of gender and cross significantly affected developmental time (neonate larva to adult emergence) in 2006 ($F = 2.98$; $df = 2, 1,410$; $P = 0.05$). In general, female BARxBAR had a longer mean developmental time than LONxBAR or LONxLON females (Table 2). Male BARxBAR also had the longest mean developmental time among males, whereas LONxBAR males had the shortest mean developmental time (Table 2). LONxLON females developed significantly faster than LONxLON males, but there were no other differences by gender (Table 2). In 2007, only cross had a significant effect on developmental time ($F = 3.84$; $df = 8, 1,219$; $P = 0.0002$); the effect of gender was not significant ($F = 0.32$; $df = 8, 1,219$; $P = 0.57$). BARxLON individuals had a significantly longer mean developmental time than individuals of all other crosses (Table 3).

Individual beetle longevity was significantly affected by mating status (mated or virgin) in 2006, as well as gender and cross in both years (Tables 4 and 5). In 2006, the interaction of mating status, gender, and cross was significant ($F = 4.44$; $df = 2, 1,202$; $P = 0.01$). Males that mated lived significantly longer (54.1 ± 2.0 d) than males that did not mate (46.9 ± 1.9 d), whereas females that did not mate lived significantly longer (69.9 ± 1.8 d) than females that mated (49.1 ± 2.0 d); this resulted in a significant interaction of gender by mating status, $F = 52.5$; $df = 1, 1,202$; $P < 0.0001$). Results from mated and virgin individuals are presented separately in Tables 4 and 5. For individuals that mated in 2006, BARxBAR individuals lived a significantly shorter time than LONxBAR or LONxLON individuals (Table 4). Mated males also lived significantly longer than mated females (Table 4). For virgin individuals, the interaction of cross and gender was significant in both years (2006: $F = 9.48$; $df = 2, 819$; $P < 0.0001$; 2007: $F = 1.78$; $df = 8, 992$; $P = 0.08$). In 2006, males always had shorter life spans than females, but the strength of the effect varied among crosses, with BARxBAR significantly lower

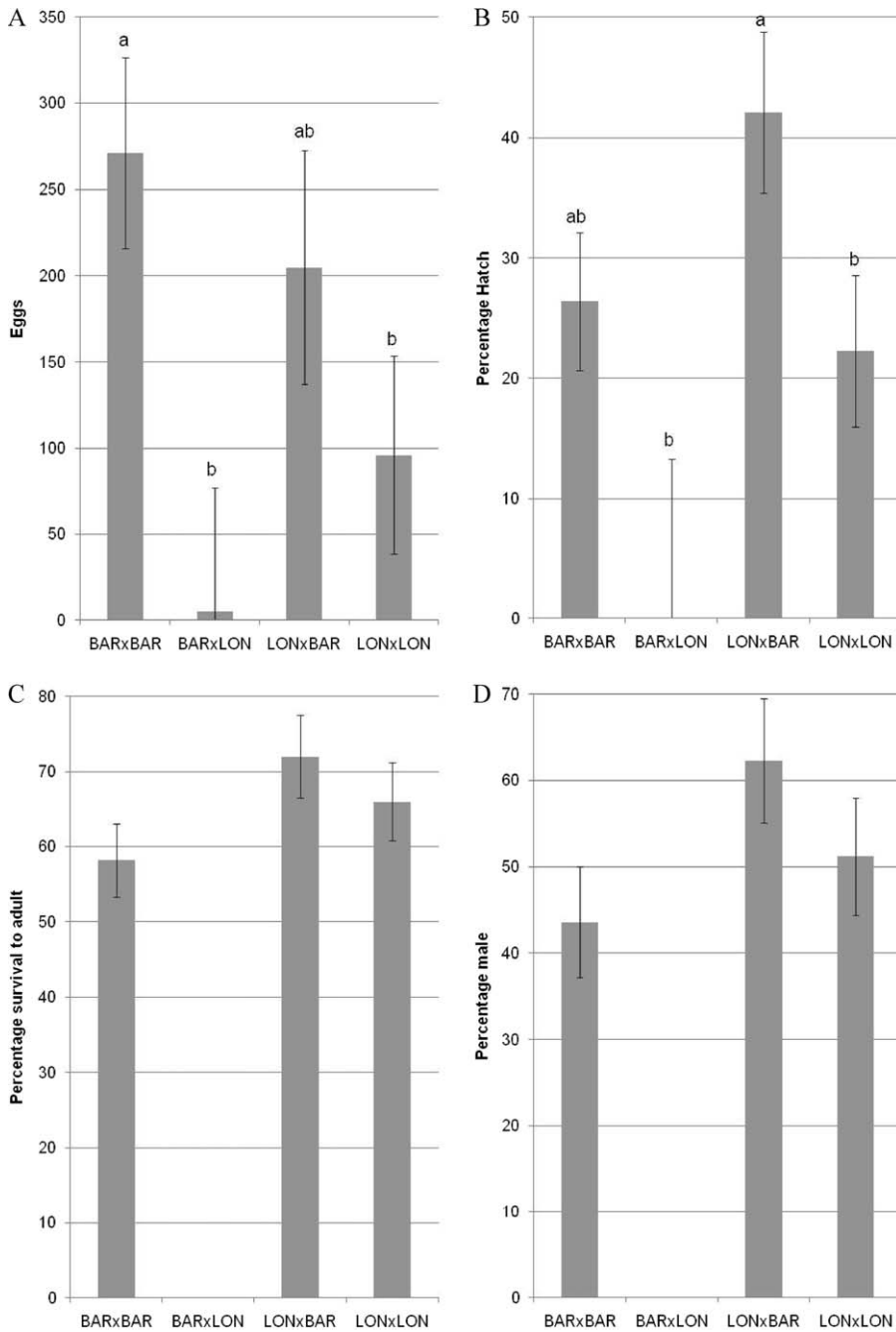


Fig. 1. Mean eggs (A), percentage eclosion (hatch; B), percentage survival to the adult stage (C), and percentage male (D) for all crosses in 2005–2006. Number of male–female pairs used to obtain mean egg values was as follows: BARxBAR, 12; BARxLON, 7; LONxBAR, 8; and LONxLON, 11. Percentage eclosion, percentage survival to adult, and percentage male data were obtained from the following number of pairs: BARxBAR, 11; BARxLON, 2; LONxBAR, 8; and LONxLON, 9. Effect of cross on eggs was significant ($F = 3.43$; $df = 3, 34$; $P = 0.03$), and effect of cross on percentage hatch was significant ($F = 3.28$; $df = 3, 26$; $P = 0.04$). Effect of cross was not significant for percentage survival to adult ($F = 1.80$; $df = 2, 21$; $P = 0.19$) or percentage male ($F = 1.88$; $df = 2, 21$; $P = 0.18$).

than the other two crosses (Table 5). In 2007, males generally had shorter life spans than females again, except the differences for BARxBAR and BARxLON

crosses were not significant (Table 5). LONxLON and LONxBAR, and their offspring, had the overall longest mean longevity in 2006 and 2007 (Table 5). In general,

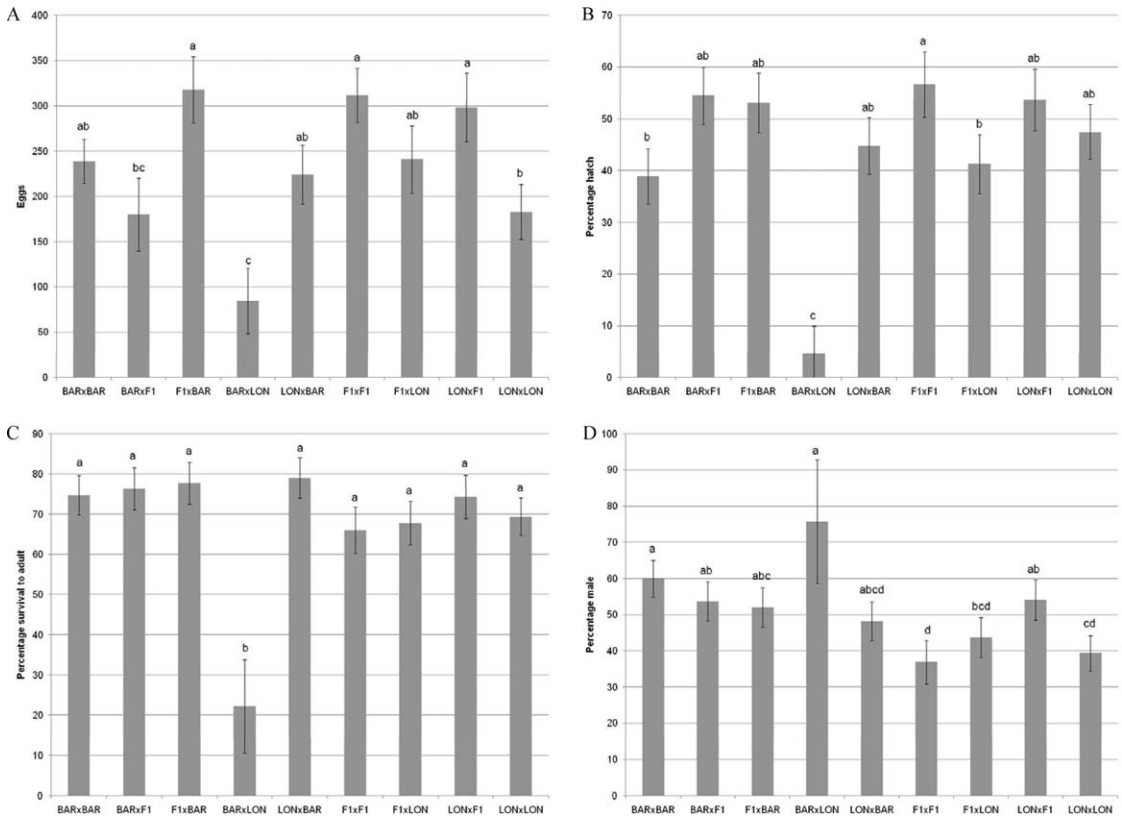


Fig. 2. Mean eggs (A), percentage eclosion (hatch; B), percentage survival to the adult stage (C), and percentage male (D) for all crosses in 2005–2006. Eggs from 12 pairs were subsampled to 35 eggs for each cross. Exceptions were as follows: BARxF₁, 13 pairs, with a mean of 34.4 eggs each; LONxBAR, 12 pairs, with a mean of 34.8 eggs each; and BARxLON, 13 pairs, with a mean of 40.5 eggs each. All BARxLON eggs were used because of low viability observed in 2005–2006. Effect of cross was significant for all parameters (eggs: $F = 4.62$; $df = 8, 225$; $P < 0.0001$; percentage eclosion: $F = 8.88$; $df = 8, 99$; $P < 0.0001$; percentage survival to adult: $F = 3.30$; $df = 8, 85$; $P = 0.003$; and percentage male: $F = 2.56$; $df = 8, 84$; $P = 0.02$).

virgin females lived significantly longer than virgin males, which is the opposite trend seen among mated beetles (Tables 4 and 5). These results suggest that mating and reproductive processes significantly affected adult physiology in males and females of each species in different ways.

Both cross and gender had significant effects on beetle head capsule widths (Tables 6 and 7). In 2006, the interaction of cross and gender was significant

($F = 2.38$; $df = 1, 294$; $P = 0.09$). Mean female head capsule width was not significantly different among crosses in 2006; however, the mean head capsule widths of females were significantly smaller than the respective male head capsule widths for LONxLON

Table 2. Mean developmental time, in days, for males and females in 2006

| Cross (♀ × ♂) | Female | | Male | |
|---------------|--------|--------------------|------|--------------------|
| | N | Developmental time | N | Developmental time |
| BARxBAR | 321 | 35.4 ± 0.1aA | 279 | 35.2 ± 0.1aA |
| LONxBAR | 261 | 33.7 ± 0.2bA | 303 | 33.9 ± 0.1cA |
| LONxLON | 134 | 34.0 ± 0.2bB | 118 | 34.6 ± 0.2bA |

Means presented are least-squares means (LSMEANS). Interaction between cross and gender was significant ($F = 2.98$; $df = 2, 1,410$; $P = 0.05$). Within columns, means followed by the same lowercase letter are not significantly different. Within rows, means followed by the same uppercase letter are not significantly different ($P > 0.05$).

Table 3. Mean developmental time, in days, for individuals in 2007

| Cross (♀ × ♂) | N | Developmental time |
|--------------------------------|-----|--------------------|
| BARxBAR | 110 | 35.7 ± 0.2b |
| BARxF ₁ | 179 | 35.6 ± 0.2b |
| F ₁ xBAR | 183 | 35.1 ± 0.2a |
| BARxLON | 13 | 38.6 ± 0.7c |
| LONxBAR | 154 | 35.5 ± 0.2ab |
| F ₁ xF ₁ | 169 | 35.4 ± 0.2ab |
| F ₁ xLON | 126 | 35.4 ± 0.2ab |
| LONxF ₁ | 174 | 35.1 ± 0.2a |
| LONxLON | 141 | 35.5 ± 0.2ab |

Means presented are least-squares means (LSMEANS). Effect of cross was significant ($F = 3.84$; $df = 8, 1,219$; $P = 0.0002$). The effect of gender was not significant ($F = 0.32$; $df = 8, 1,219$; $P = 0.57$). Within columns, means followed by the same lowercase letter are not significantly different ($P > 0.05$).

Table 4. Mean longevity in days, by cross and gender, for mated individuals in 2006

| Cross (♀ × ♂) | Gender | N | Days ± SE |
|---------------|--------|-----|-------------|
| BARxBAR | | 136 | 42.5 ± 1.5c |
| LONxBAR | | 123 | 52.1 ± 1.5b |
| LONxLON | | 130 | 60.2 ± 1.5a |
| | ♂ | 191 | 54.1 ± 1.2a |
| | ♀ | 198 | 49.1 ± 1.2b |

Means presented are least-squares means (LSMEANS). Effect of cross was significant ($F = 35.47$; $df = 2, 383$; $P < 0.0001$). Effect of gender was also significant ($F = 8.26$; $df = 1, 383$; $P = 0.004$), but the interaction of gender and cross was not significant ($F = 1.16$; $df = 2, 383$; $P = 0.31$). Within cross and within gender comparisons, means followed by the same lowercase letter are not significantly different ($P > 0.05$).

and LONxBAR crosses (Table 6). Mean male head capsule widths were generally larger than mean female head capsule widths, regardless of cross (Tables 6 and 7). BARxLON individuals had significantly smaller mean head capsule widths compared with all other crosses; in contrast, LONxBAR had the largest mean head capsule widths, although this difference was not always significant (Table 7).

Population Growth. The net reproductive rate (R_0) and the intrinsic rate of population increase (r_m) were highly variable among crosses (Table 8). The BARxLON cross had the lowest net reproductive rates and intrinsic rates of population increase based on both survival to emergence and survival to 10 d post-emergence (Table 8). All other crosses had net reproductive rates and intrinsic rates of population increase that were comparable to, or greater than, those of the parental species (Table 8).

Discussion

This study adds to our knowledge about the basic biology of *D. barberi* and *D. longicornis* and has pro-

Table 5. Mean longevity in days, by cross and gender, for virgin individuals in 2006 and 2007

| Cross (♀ × ♂) | N | ♀ Days ± SE | N | ♂ Days ± SE |
|--------------------------------|-----|---------------|-----|----------------|
| 2006 | | | | |
| BARxBAR | 218 | 51.4 ± 2.2bA | 174 | 37.0 ± 2.4bB |
| LONxBAR | 160 | 83.5 ± 2.5aA | 200 | 48.6 ± 2.3aB |
| LONxLON | 41 | 74.8 ± 5.0aA | 32 | 55.0 ± 5.6aB |
| 2007 | | | | |
| BARxBAR | 20 | 49.8 ± 9.7cA | 63 | 32.5 ± 5.5eA |
| BARxF ₁ | 68 | 74.0 ± 5.3bA | 80 | 29.6 ± 4.9eB |
| F ₁ xBAR | 68 | 77.2 ± 5.3bA | 88 | 35.2 ± 4.6deB |
| BARxLON | 3 | 23.0 ± 25.2cA | 7 | 15.1 ± 16.5eA |
| LONxBAR | 54 | 83.5 ± 5.9abA | 68 | 64.5 ± 5.3abB |
| F ₁ xF ₁ | 82 | 81.5 ± 4.8abA | 53 | 48.5 ± 6.0cdB |
| F ₁ xLON | 55 | 87.0 ± 5.9abA | 48 | 40.2 ± 6.3cdeB |
| LONxF ₁ | 60 | 95.7 ± 5.6aA | 84 | 50.6 ± 4.8bcB |
| LONxLON | 50 | 95.7 ± 6.2aA | 59 | 66.2 ± 5.7aB |

Means presented are least-squares means (LSMEANS). Interaction between cross and gender was significant both years (2006: $F = 9.48$; $df = 2, 819$; $P < 0.0001$; 2007: $F = 1.78$; $df = 8, 992$; $P = 0.08$). Within columns, means followed by the same lowercase letter are not significantly different; within rows, means followed by the same uppercase letter are not significantly different ($P > 0.05$).

Table 6. Mean head capsule widths (in mm), by cross and gender, for individuals in 2006

| Cross (♀ × ♂) | N | ♀ Head capsule width (mm) ± SE | N | ♂ Head capsule width (mm) ± SE |
|---------------|----|--------------------------------|----|--------------------------------|
| BARxBAR | 52 | 1.11 ± 0.01aA | 48 | 1.14 ± 0.01cA |
| LONxBAR | 51 | 1.14 ± 0.01aB | 49 | 1.19 ± 0.01aA |
| LONxLON | 57 | 1.13 ± 0.01aB | 43 | 1.16 ± 0.01bA |

Means presented are least-squares means (LSMEANS). Interaction between cross and gender was significant ($F = 2.38$; $df = 1, 294$; $P = 0.09$). Within columns, means followed by the same lowercase letter are not significantly different; within rows, means followed by the same uppercase letter are not significantly different ($P > 0.05$).

vided an opportunity to directly compare and contrast key life-history parameters of the closely related species. Within the constraints of this study, survival to the adult stage was similar among the species (Figs. 1 and 2); and reproductive potential, as measured by fecundity and net reproductive rate, was greater for *D. barberi* than *D. longicornis* (Figs. 1 and 2; Table 8). Fecundity here (Figs. 1 and 2) was comparable to that reported for both field-collected wild-type (Naranjo and Sawyer 1987, Boetel and Fuller 1997) or lab-reared *D. barberi* (Dominique and Yule 1983), and lab-reared *D. longicornis* (Golden and Meinke 1991).

Woodson and Jackson (1996) reported that the mean head capsule width of male *D. barberi* was larger than that of female *D. barberi*. This trend also was recorded for both *D. barberi* and *D. longicornis* in this study (Tables 6 and 7). Golden and Meinke (1991) suggested that *D. longicornis* might have a shorter life span than *D. barberi*, but, in that study, *D. longicornis* and *D. barberi* were not compared directly. In addition, *D. longicornis* adults were fed zucchini squash, which contains cucurbitacins that have been shown to reduce longevity in a related *virgifera* group species

Table 7. Mean head capsule widths (in mm), by cross and gender, for individuals in 2007

| Cross (♀ × ♂) | Gender | N | Head capsule width (mm) ± SE |
|--------------------------------|--------|-----|------------------------------|
| BARxBAR | | 30 | 1.15 ± 0.01c |
| BARxF ₁ | | 25 | 1.19 ± 0.01ab |
| F ₁ xBAR | | 30 | 1.18 ± 0.01abc |
| BARxLON | | 5 | 1.07 ± 0.03d |
| LONxBAR | | 30 | 1.20 ± 0.01a |
| F ₁ xF ₁ | | 29 | 1.17 ± 0.01bc |
| F ₁ xLON | | 29 | 1.19 ± 0.01ab |
| LONxF ₁ | | 29 | 1.17 ± 0.01bc |
| LONxLON | | 30 | 1.17 ± 0.01bc |
| | ♀ | 121 | 1.15 ± 0.01b |
| | ♂ | 117 | 1.18 ± 0.01a |

Means presented are least-squares means (LSMEANS). Effect of cross was significant ($F = 4.93$; $df = 8, 220$; $P < 0.0001$); effect of gender was also significant ($F = 14.71$; $df = 1, 220$; $P = 0.0002$). Thirty individuals (15 females and 15 males) each of BARxBAR, F₁xBAR, F₁xLON, LONxBAR, and LONxLON were measured; 29 individuals (15 females and 14 males) of F₁xF₁ and LONxF₁ were measured. Only 25 individuals (15 females and 10 males) of the BARxF₁ cross, and one female and four males of the BARxLON cross were available to be measured. The interaction of cross and gender was not significant ($F = 1.28$; $df = 8, 220$; $P = 0.25$). Within cross and within gender comparisons, means followed by the same lowercase letter are not significantly different ($P > 0.05$).

Table 8. Net reproductive rate (R_0) and intrinsic rate of population increase per day (r_m) for each cross, based on proportion of individuals that survived to emergence and 10 d postemergence

| Cross ($\text{♀} \times \text{♂}$) | Emergence | | 10 d postemergence | |
|--------------------------------------|---------------------|--------------------|--------------------|--------------------|
| | $R_0 \pm SE$ | $r_m \pm SE$ | $R_0 \pm SE$ | $r_m \pm SE$ |
| BARxBAR | 71.4 \pm 18.2bc | 0.12 \pm 0.02ab | 68.7 \pm 17.8bc | 0.13 \pm 0.02ab |
| BARxF ₁ | 81.7 \pm 22.8bc | 0.14 \pm 0.01a | 78.4 \pm 23.3bc | 0.14 \pm 0.01a |
| F ₁ xBAR | 101.1 \pm 23.5abc | 0.10 \pm 0.01bc | 94.9 \pm 22.5ab | 0.10 \pm 0.01bc |
| BARxLON | 1.7 \pm 1.7d | 0.03 \pm 0.02e | 1.3 \pm 1.3d | 0.02 \pm 0.02e |
| LONxBAR | 84.9 \pm 18.0bc | 0.10 \pm 0.01bcd | 79.7 \pm 17.0bc | 0.10 \pm 0.01bcd |
| F ₁ xF ₁ | 152.5 \pm 26.6a | 0.12 \pm 0.01ab | 151.8 \pm 26.4a | 0.12 \pm 0.01ab |
| F ₁ xLON | 105.9 \pm 19.7ab | 0.10 \pm 0.01bc | 93.4 \pm 16.8ab | 0.10 \pm 0.01bc |
| LONxF ₁ | 93.5 \pm 26.1abc | 0.08 \pm 0.01d | 89.0 \pm 25.6abc | 0.08 \pm 0.01d |
| LONxLON | 52.8 \pm 14.0c | 0.09 \pm 0.01cd | 49.0 \pm 12.9c | 0.09 \pm 0.01cd |

Within columns, parameters followed by the same lowercase letter are not significantly different ($P > 0.05$).

Diabrotica virgifera virgifera LeConte (Ferguson et al. 1985). In this study, *D. longicornis* individuals consistently lived longer than *D. barberi* when adults of both were fed a diet of corn and lettuce (Tables 4 and 5).

This study also provides the first intensive evaluation of LONxBAR and BARxLON hybrid fitness, including the potential of hybrids and F₂ backcrossed individuals to contribute to population growth. The fitness of LONxBAR hybrids, F₂ hybrids (from F₁ LONxBAR cross), and backcrosses (LONxBAR backcrossed with LON or BAR parents) as estimated by key life-history parameters (reproduction, survival, gender ratio, developmental time, longevity, and head capsule width) was similar or greater than that of the parental species (Figs. 1 and 2; Tables 2–7). Additionally, the viability of offspring from LONxBAR F₁ hybrids was consistently similar to that of hybrid parents and of the backcrossed parent species (Figs. 1 and 2; Tables 2–7). The LONxBAR cross and their offspring also had similar, or greater, R_0 and r_m compared with the parental species (Table 8). These results collectively indicate that LONxBAR individuals and their offspring are likely to be viable and fit if LONxBAR matings occur in the field.

In contrast to the results from the LONxBAR hybrids, the fitness of BARxLON F₁ hybrids was poor (Figs. 1 and 2; Tables 2–8). Few eggs were laid by BARxLON pairs, BARxLON eggs were rarely viable, survival of BARxLON individuals was lower than that of all other crosses, and BARxLON individuals were smaller than all other crosses. The low number of viable eggs is consistent with the results of Krysan et al. (1983), who reported low rates of successful insemination in pairs of female *D. barberi* and male *D. longicornis*. Even when BARxLON fitness parameters were not significantly different from the other crosses, the cumulative low viability of BARxLON individuals would have a strong biological effect. For example, the low survival to the adult stage (Figs. 1 and 2) and the shortened life span (Table 5) would prevent most BARxLON individuals from reproducing under lab or field conditions. Additional evidence demonstrating the low reproductive potential of BARxLON individuals can be seen with the very low R_0 and r_m , compared with all other crosses (Table 8). Because R_0 for the BARxLON cross is slightly >1 , regardless of how

survival to reproductive age is calculated (Table 8), a BARxLON female should be able to replace herself under experimental conditions in the lab but probably would not contribute significantly to population growth in the field (Gotelli 2001).

Differences in larval eclosion and net replacement rate need to be interpreted with caution. The three *D. barberi* and *D. longicornis* colonies used in this study all exhibited the extended egg diapause trait, and some eggs eclosed after two to four simulated winters (Campbell 2009). Because net replacement rates and intrinsic rates of population increase were calculated for only one cycle of eclosion, the R_0 and r_m values reported here (Table 8) represent a conservative estimate of a female's lifetime production of offspring. The only exception was the BARxLON cross, in which no eggs were viable after one hatching period. Despite confounding by extended diapause, the conservative population growth rates for all crosses but BARxLON suggest the potential for substantial population growth (Table 8).

In the future, larger initial colony sizes and sampling from a greater geographic range could be used to examine variation in the life-history parameters presented here. Unfortunately, when *D. longicornis* collections were made for this study, much of western Nebraska and the Southwest were experiencing prolonged drought (CPC 2009). Population densities of *D. longicornis* were very low, compared with densities reported in the literature (Krysan et al. 1983, Krysan and Smith 1987, Golden 1990). In addition, obtaining beetles to document geographic variation in parameters was outside the scope of this initial study. Because both species exhibit geographic clines in color (Krysan et al. 1983, Krysan and Smith 1987) and *D. barberi* can be highly diverse within populations (Krafsur et al. 1993), specific life-history parameters of parental species and hybrid crosses could vary for beetles from different geographic areas. In addition, it is possible that *D. barberi* and *D. longicornis* populations in the area of sympatry exhibit greater divergence in life-history characteristics than those in allopatry.

The reason for the unidirectionality of hybridization between *D. barberi* and *D. longicornis* is currently unknown, but multiple factors could be involved. Ver-

tically inherited intracellular bacteria, including *Wolbachia*, can cause cytoplasmic incompatibility, which can lead to reproductive isolation (Werren et al. 2008). *Wolbachia* has been found in other species of the *virgifera* group of *Diabrotica* (Giordano and Jackson 1999, Clark et al. 2001b, Segura-León 2004), and multiple strains of *Wolbachia* have been documented in the eastern part of the range of *D. barberi* (Roehrdanz et al. 2003, Roehrdanz and Levine 2007). However, to date, *Wolbachia* has not been found in either *D. longicornis* or western populations of *D. barberi* (Clark et al. 2001b, Roehrdanz et al. 2003), but this may be due to limited sampling.

Unidirectional incompatibility also could be the result of differences in mating behavior or mate recognition between *D. barberi* and *D. longicornis*. Mating behavior experiments (Campbell 2009) have revealed that, under lab conditions, *D. barberi* males seem to be more aggressive and receptive to mating than *D. longicornis* males. In addition, *D. longicornis* males transferred significantly larger spermatophores than *D. barberi* males did (Campbell 2009). *D. barberi* and *D. longicornis* also have been shown to have strong quantitative differences in cuticular hydrocarbon profiles (Golden et al. 1992). Mate choice in many insects can be influenced by species-specific cuticular hydrocarbons (Coyne et al. 1994, Peterson et al. 2007), so cuticular hydrocarbons could play some role in isolating *Diabrotica* species. Although there are no physical barriers to mating between *D. barberi* and *D. longicornis* (Krysan and Smith 1987), small behavioral and physiological differences may play a part in male or female receptivity to mating.

Although successful mating and potential gene flow between taxa are unidirectional, fitness data from this study (i.e., hybrid viability and positive contribution to population growth, Figs. 1 and 2; Tables 2–8) support the working hypothesis that natural hybridization between the two taxa in the area of sympatry is likely. The relative fitness of hybrids and hybrid progeny raises further questions about the possible role of hybrids in the area of sympatry. Hybridization between *D. barberi* and *D. longicornis* has the potential to affect the evolution and pest status of either taxon. Population densities of *D. barberi* in corn have been increasing during the last decade in northeastern and east central Nebraska, but densities of *D. barberi* remain low in southeast Nebraska, which is in the area of sympatry (L.J.M., unpublished data). It is currently unclear whether this is related to introgression, which could change the habitat and ovipositional preferences of the populations in the area, or to other factors. A shift in habitat or ovipositional preferences of introgressed progeny toward behavioral preferences often exhibited by *D. longicornis* may effectively reduce the densities of *D. barberi* found in corn. Further ecological and genetic work will help to elucidate the frequency of natural hybridization and its relative importance to the evolution and pest status of *D. barberi* and *D. longicornis* in Nebraska.

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