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# Inheritance of an extended diapause trait in the Northern corn rootworm, *Diabrotica barberi* (Coleoptera: Chrysomelidae)

## Abstract

Diapause is an adaptive trait that delays development or reproduction under unfavourable circumstances. The northern corn rootworm, *Diabrotica barberi* Smith & Lawrence, an important maize, *Zea mays* L., pest in the *Diabrotica* species complex, overwinters in diapause during the egg stage. Some NCR populations are adapted to crop rotation by expressing an extended diapause (ED) trait that delays embryonic development for 2 years. This ED trait has increased in frequency and geographic distribution since first reported in Illinois in 1932. Reciprocal single pair crosses among beetles from a laboratory colony with the ancestral 1-year diapause trait and field collected beetles with the 2-year ED trait indicated that ED females laid significantly more ED eggs than did females with the 1-year diapause trait regardless of male genotype. The ED trait was highly heritable [realized heritability ( $h^2$ ) =  $0.698 \pm 0.314$ ], with genetic dominance ( $D$ ) of the trait strongly influenced by female genotype. Selection of the ED trait and maintenance of polymorphic diapause phenotypes within maize-soybean cropping systems is discussed in relation to response to a fluctuating environment and as a potentially advantageous life history adaptation.

## Keywords

insect, maize pest, maternal effects, overwinter, pest management

## Disciplines

Agronomy and Crop Sciences | Animal Sciences | Biology | Entomology | Systems Biology

## Comments

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## ORIGINAL CONTRIBUTION

**Inheritance of an extended diapause trait in the Northern corn rootworm, *Diabrotica barberi* (Coleoptera: Chrysomelidae)**B. W. French<sup>1</sup>, B. S. Coates<sup>2</sup> & T. W. Sappington<sup>2</sup>

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**Introduction**

Diapause is an arrest in development, often utilized by insects to increase potential for individual survival when environmental conditions are unsuitable for growth and/or reproduction (Danks 2007; Denlinger 2008). For the pernicious pest species of Diabroticite leaf beetles, diapause occurs during the egg stage, lasts throughout a single winter season and terminates when climate becomes favourable for continued embryonic development (Chiang 1973). Diapause induction and duration are adaptive traits that are genetically heritable and show phenotypic plasticity in response to changing environmental conditions (Tauber et al. 1986). Fluctuating conditions can lead to divergent selection of life history traits within the same geographic region (Travis 1994), such that polymorphic phenotypes adapted to different niches can

**Abstract**

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evolve within a population (Levins 1968). These environmental niches thus often lead to the maintenance of more than one adaptive variant within the same geographic region (Gillespie 1972). For instance, dimorphic diapause durations have evolved within sympatric populations of the lepidopteran species, *Ostrinia nubilalis* (Hübner) (Showers 1993), where genetic differentiation may be reinforced by the presence of asynchronous mating periods (Eckenrode et al. 1983; Dopman et al. 2010).

The *Diabrotica* (Coleoptera: Chrysomelidae) species complex that attacks maize, *Zea mays* L., is comprised of the western (WCR) *Diabrotica virgifera virgifera* LeConte, Mexican (MCR) *Diabrotica virgifera zea* Krysan & Smith, southern (SCR) *D. undecempunctata howardi* Barber, and northern corn rootworms (NCR) *D. barberi* Smith & Lawrence. Corn rootworms have adapted to feeding on the root tissues of a limited number of

grasses including maize (Krysan and Branson 1983; Oyediran et al. 2004a,b, 2008), and in the United States alone cause greater than \$1 billion US dollars in annual economic loss because of reduced crop yield and input costs (Metcalf 1986; Sappington et al. 2006). The main damage on maize results from the feeding of larvae on root tissues. Severe pruning reduces the plant's ability to absorb soil nutrients, reduces shoot photosynthesis and compromises structural stability (Kahler et al. 1985; Riedell and Reece 1999). Larval feeding damage can be suppressed by systemic seed treatments, soil-applied insecticides or transgenic maize hybrids that express *Bacillus thuringiensis* (Bt)-derived insecticidal proteins. However, seed treatments appear ineffective in suppressing corn rootworm abundance, and their ability to evolve resistance to many of the insecticides and Bt toxins is well documented (Chio et al. 1978; Levine and Oloumi-Sadeghi 1991; Meinke et al. 1998; Wright et al. 2000; Furlan et al. 2006; Lefko et al. 2008; Meihls et al. 2008, 2011; Gassmann et al. 2011; Oswald et al. 2011).

A 2-year crop rotation in the United States between maize and soybean, *Glycine max* (L.) Merrill, has been highly successful in controlling corn rootworm populations in most regions for a century (Gillette 1912; Pedigo 1989; Levine et al. 2002). This success is because of female fidelity for oviposition in the soil of maize fields in late summer followed by an overwintering diapause in the egg stage, and the specialized feeding of larvae on maize roots in the subsequent spring. Within this crop rotation scheme, the cultivation of soybeans in years following maize presents hatching corn rootworm larvae with plant roots unsuitable for feeding, and population suppression occurs because larvae fail to develop into adults. However, two independent adaptations within the corn rootworm complex have circumvented crop rotation practices. Female WCR have lost the fidelity for laying eggs in maize fields and appear equally likely to oviposit near other plants including soybean (Shaw et al. 1978; Sammons et al. 1997; O'Neal et al. 2002), leading to emergence of larvae the following year in a maize crop. In the case of NCR, a portion of individuals within the population show an extended diapause (ED) trait that causes eggs to overwinter for 2 years and to a lesser extent even 3–4 years (Levine et al. 1992). Extended diapause allows the hatched NCR larvae to feed on maize roots the year following cultivation of soybean in the second year of a 2-year rotation scheme (Bigger 1932; Chiang 1965, 1973; Krysan et al. 1984, 1986; Levine et al. 1992, 2002; Steffy et al. 1992; Gray et al. 1998). Although some NCR

females may lay a few eggs near volunteer maize plants growing in fields other than maize, these numbers are insignificant compared to the number of extended diapause eggs responsible for larval emergence in first-year maize fields, which is clearly the mechanism of rotation resistance in this species (Shaw et al. 1978; Levine et al. 1992). These adaptations limit the effectiveness of a 2-year crop rotation as a management practice in the US Corn Belt (Gray et al. 2009), and likely are examples of phenotypic divergence in an environment containing strong selection pressures along two or more different evolutionary trajectories.

The frequency and distribution of the extended diapause trait remain poorly defined in NCR populations across the US Corn Belt. However, Levine et al. (1992) found that extended diapause ranged from approximately 14–51% in Illinois and was strongly related to geographic location (county) and percentage of maize planted in a 2-year rotation with soybean. Krysan et al. (1984) found that approximately 40% of the eggs from an NCR population near Brookings, South Dakota exhibited ED traits, which is similar to the proportions estimated nearly 20 years later at the South Dakota corn rootworm area-wide management site near Brookings (French et al. 2004; B. W. French, unpublished data). In WCR, diapause appears to be a polygenic trait, based on response to selection for a non-diapausing colony (Branson 1976; Krysan and Branson 1977), but those studies also showed a strong female influence on diapause duration. The objectives of the current study were to determine the genetic basis of the extended diapause trait in NCR, ascertain the pattern and dominance of diapause inheritance and estimate the narrow sense heritability of diapause duration. The results are important for understanding the genetic basis of diapause duration and the extended diapause trait in NCR.

## Materials and Methods

### Collecting beetles

We established mating crosses for all combinations of NCR beetles emerged from eggs that diapaused for 1 or 2 years. Diapause trait (D) beetles were obtained as pupae from a laboratory colony that had been selected for 1-year diapause over 10 years. The D colony originated from adult insects collected from maize fields near Brookings, SD in 1996, and only 1-year diapause eggs were used to maintain the colony. Beetles with a 2-year extended diapause trait (ED) were collected as

pupae from maize fields that had been in a 2-year rotation of maize and soybean for several years. These fields were part of the South Dakota corn rootworm area-wide management site (French et al. 2004). French and Hammack (2010) give a detailed account on obtaining, indentifying, handling and housing individual pupae and adults. Briefly, to prevent premature mating, all pupae were sexed (Krysan 1986), given a unique number, and housed individually in a 7-ml plastic bioassay cup containing approximately 5 ml dried and sifted (80-mesh) soil dampened with 2.5 ml of distilled water to prevent desiccation. Pupae were placed in a dark environmental chamber at 25°C and 60% RH, and checked daily for eclosion. All recently enclosed adults were removed from the bioassay cups within 1 day after their cuticles had hardened and placed in a 1.15-l plastic container with plenty of fresh food and water (Branson and Jackson 1988; French and Hammack 2010). All containers were placed in a growth chamber at 25°C and 60% RH under a 14:10 (L:D) h photoperiod.

#### Establishing matings and egg collection

We established 40  $F_1$  pedigrees from single pair matings between NCR individuals in diapause for 2 years (extended diapause; ED) and individuals in diapause for 1 year (D), with 10 replicates of each ♀D × ♂D, ♀D × ♂ED, ♀ED × ♂D and ♀ED × ♂ED cross. For each pairing, a male and female were placed in a 1.15-l plastic container provisioned with fresh food and water and placed in a growth chamber for 1 week with a 14:10 (L:D) h photoperiod at 25°C and 60% RH, after which the male was removed, flash frozen in liquid nitrogen and stored at -80°C. The female was provided with an oviposition dish (15- × 35-mm Petri dish) containing soil thoroughly moistened with distilled water as described by French and Hammack (2010). Egg dishes were replaced at weekly intervals for 6 weeks, after which females were flash frozen in liquid nitrogen and stored at -80°C. Following routine rearing procedures for WCR and NCR, each egg dish was placed in a growth chamber at 25°C for 2 weeks to allow for pre-diapause egg development and then into a growth chamber at 8°C for 5–8 months to synchronize hatching (Chiang 1973; Jackson 1986). After cold storage, we collected the eggs by washing them through a No. 60 standard mesh sieve. We counted the eggs and visually determined their potential viability, eliminating those with physical damage or discoloration (Boetel and Fuller 1997). The viable eggs were plated out in a 10 × 10 grid onto moistened filter paper inside a

10 cm × 10 cm × 2 cm plastic sandwich box. The box with eggs was placed back into the growth chamber at 25°C for 45 days allowing sufficient time for incubation and hatching considering median egg hatch occurs at about 21 days under these conditions (Apple et al. 1971). We monitored at least twice weekly for hatching. Eggs hatching during this 45-day period were considered to have a 1-year diapause trait (D). Eggs that did not hatch were considered extended diapause (ED) eggs.

#### Variance and inheritance of extended diapause in NCR pedigrees

Significant differences in the estimated mean proportions of egg with the ED trait among  $F_1$  progeny from the four crosses (table 1) were tested through randomization and resampling. The Student's *t*-test statistics were approximated by calculating the difference between the group (cross) means, which were standardized by dividing by the standard error of the difference. Mean differences were calculated from 5000 random assignments of  $F_1$  individuals to cross type, which were subsequently used to generate a distribution of mean differences with the programme Resample (<http://www.uvm.edu/~dhowell/StatPages/Resampling/Resampling.html>; David C. Howell unpublished data). A total of six *t*-test statistics was generated in a pairwise fashion between the four cross types, and each used to test the null hypothesis ( $H_0$  = no difference in proportion of  $F_1$  progeny with the ED trait) with a critical value of  $\alpha = 0.05$ .

Additionally, the Spearman's rank correlation coefficient ( $\rho$ ) was estimated between male or female parent diapause trait and the proportion of ED eggs produced among their resulting  $F_1$  progeny. To accomplish this, randomization tests were applied to this bivariate data with the dependent variable (male or female phenotype) held constant, while the independent variable (proportion of  $F_1$  progeny) was randomly assigned to the dependent variable group. Specifically, the null hypothesis of  $\rho = 0$  was tested by creating a sampling distribution of *r* from randomization of the proportion of ED progeny among parental phenotype and was iterated 5000 times. The number of these 5000 randomizations that exceeded  $\pm$  the obtained *r*-value was used to approximate the associated P-value and were evaluated using a critical value of  $\alpha = 0.05$ .

Genetic dominance of the ED trait was calculated using a modification of the method described by Stone (1968), a method widely used to determine the

**Table 1** Egg production by different crosses of NCR adults that developed from 1-year diapause (D) or 2-year extended diapause (ED) eggs. Depicted for each cross are the number of samples (n), mean  $\pm$  SD and range for total good (i.e. fertile and viable) eggs, 1-year diapause (D) eggs, 2-year ED eggs and per cent 2-year ED eggs

Cross	n	Total good eggs		1-year diapause eggs		2-year diapause eggs		Per cent ED eggs	
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range
♀D $\times$ ♂D	10	268 $\pm$ 105	114–398	236 $\pm$ 94	102–365	33 $\pm$ 28	5–89	12 $\pm$ 9	3–26
♀D $\times$ ♂ED	4	183 $\pm$ 52	161–271	160 $\pm$ 47	104–202	23 $\pm$ 16	9–42	13 $\pm$ 8	5–23
♀ED $\times$ ♂D	7	198 $\pm$ 117	92–387	136 $\pm$ 74	47–284	62 $\pm$ 74	4–213	27 $\pm$ 23	4–63
♀ED $\times$ ♂ED	5	239 $\pm$ 164	109–499	155 $\pm$ 139	50–381	84 $\pm$ 39	32–118	41 $\pm$ 19	24–69

genetic dominance of resistance traits in insects (Liu and Tabashnik 1997; Tabashnik et al. 2002; Germano et al. 2010; Nair et al. 2010; Kaur and Dilawari 2011; Jagadeesan et al. 2012). Based on the formula of Stone (1968), we calculated the degree of dominance ( $D$ ) for both the 1-year and 2-year diapause traits using:  $D_D = (2(\text{Htz}) - \text{Hmz}_D - \text{Hmz}_{ED}) / (\text{Hmz}_D - \text{Hmz}_{ED})$  and  $D_{ED} = (2(\text{Htz}) - \text{Hmz}_{ED} - \text{Hmz}_D) / (\text{Hmz}_{ED} - \text{Hmz}_D)$ ; where  $\text{Hmz}_{ED}$  = homozygote with extended diapause (♀ED  $\times$  ♂ED),  $\text{Htz}$  = heterozygote for diapause,  $\text{Hmz}_D$  = homozygote for 1-year diapause (♀D  $\times$  ♂D). For both  $D_D$  and  $D_{ED}$ , we used mean proportion ED eggs from the respective crosses. Both reciprocal crosses for heterozygotes were used in the calculations of  $D$ . For  $D_D$ , we used the mean proportion ED eggs from ♀D  $\times$  ♂ED, and for  $D_{ED}$ , we used the mean proportion ED eggs from ♀ED  $\times$  ♂D. Using both reciprocal heterozygote crosses in the calculations of  $D$  helps determine the influence of gender on degree of dominance. With this formula, dominance values range from  $-1$  to  $+1$ . When  $D = 0$ , there is no dominance; when  $D = 1$ , there is complete dominance, when  $0 < D < 1$  there is incomplete dominance; when  $-1 < D < 0$  there is incomplete recessiveness, and finally when  $D = -1$  there is complete recessiveness. The calculations for  $D$  were performed on  $\log_{10}$  transformed means.

Realized heritability ( $h^2$ ) was used as an estimate of narrow sense heritability and was calculated by rearranging the function  $R = h^2 \times S$  (Falconer and MacKay 1996). Calculations used the phenotypic response to divergent environmental selection for the extended diapause (ED) trait compared to the ancestral 1-year diapause (D) trait. Specifically, this calculation used  $R$ , the response to selection [ $R = (\text{mean proportion of ED progeny produced from the ♀D} \times \text{♂D cross}) - (\text{mean proportion of ED progeny produced by ♀ED} \times \text{♂ED crosses})$ ] and the selection differential ( $S$ ) [ $S = (\text{mean proportion of ED progeny produced from the ♀D} \times \text{♂D cross}) - (\text{mean proportion of ED progeny produced by each individual ♀ED} \times \text{♂ED$

cross)]. Narrow sense heritability was calculated for  $F_1$  egg data from each ♀ED  $\times$  ♂ED cross, and the final estimate was taken as the mean across  $h^2$  estimates from all ♀ED  $\times$  ♂ED crosses. Values of  $h^2 > 0.6$  were interpreted as indicating a proportion of ED phenotypes among offspring derived from selected ED parents differing from the original diapausing population as much as their parental genotypes.

## Results

### Phenotypic variance and inheritance of extended diapause traits in NCR

A total of 7481  $F_1$  eggs were collected from 26 female NCR in the 40 initial crosses (65% successful mate pairs), of which 6001 eggs (80.2%) were fertile and viable. The means  $\pm$  SD for total good eggs, 1-year diapause, 2-year diapause and per cent ED eggs are in table 1. The ♀ED  $\times$  ♂ED crosses produced significantly higher proportions of  $F_1$  eggs exhibiting the ED trait than observed among progeny originating from ♀D  $\times$  ♂D crosses and ♀D  $\times$  ♂ED crosses, but not the proportions observed among the ♀ED  $\times$  ♂D crosses (table 2). Proportion of ED eggs produced by the ♀D  $\times$  ♂D crosses did not differ significantly ( $\alpha = 0.05$ ) from that produced by the ♀D  $\times$  ♂ED crosses or from the ♀ED  $\times$  ♂D crosses (table 2). The sample distributions involving the ♀ED  $\times$  ♂D crosses showed two families with 0.525 and 0.634 of  $F_1$  eggs that expressed the ED trait, compared to the five remaining families with proportions  $\leq 0.262$  (fig. 1a). When compared with data from the ♀D  $\times$  ♂ED cross (fig 1b), these data suggest that  $F_1$  hybrid progeny derived from the ♀ED  $\times$  ♂D cross have greater variability in expressing the diapause trait, which indicates that interfamily variance of ♀ED effects may be present.

Based on randomization tests, the frequency of the ED trait among progeny was significantly correlated with crosses that were derived from a ♀ED parent

**Table 2** Pairwise comparisons of ED egg production by different crosses of adult NCR developing from 1-year diapause (D) or 2-year extended diapause (ED) eggs. Randomization estimates of *t*-tests are shown below the diagonal. Randomization tests of Spearman's rank correlations between samples are shown above the diagonal

Cross	♀D × ♂D	♀D × ♂ED	♀ED × ♂D	♀ED × ♂ED
♀D × ♂D	–	–1.491	0.434*	–0.754‡
♀D × ♂ED	–0.116	–	0.362	–0.723§
♀ED × ♂D	–1.865*	–1.168	–	–0.336
♀ED × ♂ED	4.133‡	3.294†	1.128	–

\**P* < 0.1; §*P* < 0.05; †*P* < 0.01; ‡*P* < 0.001.

(table 2;  $R^2 \geq 0.523$ ,  $P = 0.022$ ). In contrast, a reduced presence of the ED trait among progeny was not significantly correlated with crosses involving a ♀D parent (table 2;  $R^2 \leq 0.188$ ,  $P \geq 0.076$ ). A bimodal distribution of Spearman's rank correlation coefficients ( $\rho$ ) was also observed from the randomization tests for calculations involving the ♀ED parent.

The slope of the regression line of proportion of ED progeny on crosses with increasing proportions of genetic background with the ED trait ( $m = 0.2863$ , fig. 1a) is consistent with a weak additive genetic component of the trait. If the phenotypic value of the homozygote from the ♀ED × ♂ED cross is  $2a = 1.000$ , then the corresponding phenotypic value of the progeny derived from the ♀ED × ♂D cross is  $a = 1 - (\text{mean proportion of ED eggs from the ♀ED × ♂D cross} / \text{mean proportion of ED eggs from the ♀ED × ♂ED cross}) = 1 - (0.266 / 0.406) = 0.345$ . This compares to an expected phenotypic value for the heterozygote class of 0.5 for purely additive traits. Values closer to 1.0 are indicative of dominance effects. Calculations of dominance genetic variance also suggested that the ED trait inherited from the ♀ED parent shows incomplete dominance ( $D_{ED} = 0.30$ ). A similar calculation of dominance for

the 1-year diapause trait from the ♀D parent indicates that it is strongly dominant over the ED trait originating from the ♂ED ( $D_D = 0.92$ ).

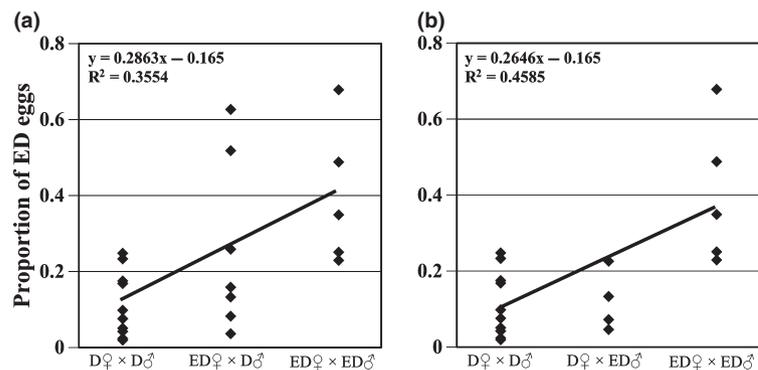
The corresponding estimate of realized heritability ( $h^2$ ) was calculated using the response to environmental selection (R) for the ED trait within natural populations. Using this statistic, estimates of  $h^2$  ranged from 0.4145 to 1.162 across ♀ED × ♂ED crosses with a mean of  $0.698 \pm 0.314$ .

**Discussion**

Species in the corn rootworm complex have shown a remarkable ability to adapt to field control tactics, and crop damage caused by this pest complex remains a source of reduced yield throughout major maize producing regions of the United States (Sappington et al. 2006). NCR eggs that express the 2-year extended diapause (ED) trait are capable of circumventing the popular maize-soybean rotation scheme as a cultural control method, and the population frequency of this phenotype has increased and spread geographically over the years (Bigger 1932; Levine et al. 1992; French et al. 2004; Geisert 2011). Our data show that parents derived from ED eggs produce a significantly higher proportion of ED progeny than parents derived from D eggs, suggesting that the ED trait has a genetic basis. Furthermore, realized heritability estimates indicate substantial genetic variation for this trait is present in the populations tested. Differences in the proportion of ED eggs produced by the parental groups also indicate that local environments where maize-soybean rotations are prevalent may impose differential selection pressures on this trait affecting NCR survival.

The historically more prevalent 1-year diapause is presumed to be the ancestral state in NCR. However, even the low proportion of ED eggs obtained

**Fig. 1** Inheritance of the NCR extended diapause (ED) trait in  $F_1$  progeny derived from reciprocal crosses of adults that developed from 1-year diapause (D) or 2-year extended diapause (ED) eggs. (a) ♀ED × ♂D and (b) ♀D × ♂ED.



from laboratory crosses involving parents selected for 1-year diapause ( $\text{♀D} \times \text{♂D}$ ) suggests that genetic variability for diapause may be maintained in natural populations, allowing the potential to respond to fluctuating selection pressures. This maintenance of genetic variability or population polymorphism can be adaptive in unstable environments or fluctuating landscapes (Philippi and Seger 1989), and for female fitness trade-offs may be considered a type of diversified bet-hedging (Seger and Brockmann 1987). Bet-hedging favours the persistence of life history traits that support a temporal and/or spatial distribution of survival risks across a set of potential environmental conditions, relying on genetic and phenotypic variance among offspring. (Hubbell 1979; Levins 1979; den Boer 1981; Stearns and Crandall 1981; Strong 1986; Tauber et al. 1986; Hopper 1999). For NCR, a bet-hedging strategy for spreading diapause duration over multiple years may distribute the risk of larval emergence over unfavourable years of drought or other compromised environmental situations. For example, small proportions of field collected NCR eggs are known to hatch without entering diapause, whereas some eggs remain in diapause up to 4 years (Patel and Apple 1967; Krysan et al. 1984; Levine et al. 1992). In WCR, a small proportion of eggs express a dominant non-diapause trait and fail to enter embryonic developmental arrest (Branson 1976; Krysan and Branson 1977), a further indication that genetic variation in diapause traits may be the rule in the rootworm complex.

Although extended diapause may be a mechanism by which populations adapt to variable environmental conditions (Menu et al. 2000; Danks 2007), prolonged diapause durations are often associated with decreased survival rates and fecundity (Sims 1983; Bradshaw et al. 1998; Ellers and Van Alphen 2002). Reduced survivorship could occur through increased exposure to predation (Murphy and Lill 2010), parasitism (Gouge et al. 1999; Corley et al. 2004), desiccation and exhaustion of metabolic energy reserves (Hahn and Denlinger 2007). Decrease in per cent survival to adulthood was found for a natural population of NCR expressing prolonged diapause over 3 years (Levine et al. 1992). Despite these tradeoffs, stochastic modelling of extended diapause in the chestnut weevil was predicted to be selectively advantageous under assumptions of the bet-hedging strategy (Menu et al. 2000). Fitness costs and overall selective advantage of the NCR ED trait were not explored in the current study, but the persistence and

increasing proportions of the ED trait in NCR populations suggest that it is favoured at least in some generations over significant parts of its geographic range (Bigger 1932; Levine et al. 1992; French et al. 2004; Geisert 2011).

Expression of D and ED traits by  $F_1$  NCR eggs was significantly influenced by the maternal phenotype and was similar to the maternally influenced polygenic 1-year diapause vs. non-diapause trait in WCR (Branson 1976; Krysan and Branson 1977). In the case of sex linkage, the phenotype of a male offspring is completely dictated by the maternal allele inherited on the X chromosome (Morgan 1909). In contrast, a maternal effect is not necessarily linked to the sex chromosome and is transmitted across generations based on genetic and environmental conditions experienced by the female parent (Mousseau and Dingle 1991). The onset and duration of diapause is usually a function of environmental cues (Danks 1987) interacting with genetic programming (Mousseau and Roff 1987). However, egg diapause traits seem to be influenced by maternal effects (Fulda 1951; Zaslavsky and Umarova 1982) in the form of maternally provided hormones (Yamashita 1996) or differential egg provisioning with mRNAs that regulate specific gene pathways (Denlinger 1998; Xu et al. 2011). The mechanism underlying the observed maternal effects on NCR diapause traits remains an intriguing question for future research.

In conclusion, our results show that the ED trait in NCR is heritable and strongly influenced by the female parent, representing another example of strong maternal effects on diapause traits in some insect species (Denlinger 2002). A bet-hedging scenario may be a plausible explanation for maintenance of genes giving rise to polymorphic diapause traits among progeny, which serves to distribute among offspring the risk associated with variable year-to-year environmental conditions, in this case, farmers who do and do not engage in crop rotation. Further research will be needed to determine the gene(s) involved in regulation of diapause duration in NCR and to evaluate the comparative selective advantage/disadvantage within current cropping environments.

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