

## ORIGINAL ARTICLE

# Biological characteristics of a non-photoperiodic-diapause strain of the cabbage beetle *Colaphellus bowringi* (Coleoptera: Chrysomelidae)

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## Abstract

Comparison of biological characteristics between diapausing and non-diapausing strains of insects provides some insights into the mechanisms regulating diapause. In this study, biological characteristics, especially diapause characteristics and life-history traits, of a non-photoperiodic-diapause (NPD) strain of the cabbage beetle *Colaphellus bowringi* were compared with those of a normal, high-diapause (HD) strain that enters diapause in response to either long day length or low temperature. The NPD strain did not enter diapause at any photoperiod at 22°C or higher, but still had a capacity to enter diapause at low temperatures. Although diapause could be induced in both strains by exposure to 20°C, the proportion of individuals having shorter diapause duration was greater in the NPD strain compared to the HD strain. The NPD strain had significantly lower hatching and larval survival rates, longer developmental duration of immature stages, smaller body size and lower longevity and female fecundity compared to the HD strain. The NPD strain of this species is a promising model for investigating diapause regulation in insects in general.

**Key words:** diapause induction, diapause termination, life-history traits.

## INTRODUCTION

Diapause is a strategy to adapt to seasonal environments. In many insects and mites, diapause is regulated by environmental stimuli such as photoperiod and temperature, and their responses to environmental stimuli often show intra- and inter-population variation (Henrich & Denlinger 1982; Tauber *et al.* 1986; Danks 1987; Saunders 2002). Non-diapause strains are examples of such variation and are excellent materials to analyze diapause characteristics and mechanisms (Tauber *et al.* 1986; Danks 1987; Riihimaa & Kimura 1988; Ito 2009; Coleman *et al.* 2014). For example, diapause intensity of a low-diapause strain is weaker than that of a high-diapause strain in *Papilio zelicaon*, suggesting a possibility

that diapause incidence and diapause intensity are linked (Sims 1980). As well, comparison of biological characteristics such as life-history traits between non-diapause and diapause strains could provide insights into the physiological and molecular mechanisms between diapause and life-history traits (Scheiner & Istock 1991).

Strains so far named as “non-diapause” are not always truly non-diapausing, but they are often insensitive to only one of the diapause-inducing stimuli. For example, non-photoperiodic-diapause strains that are insensitive to diapause-inducing action of photoperiod could enter diapause at low temperatures (Riihimaa & Kimura 1988; Hodkova & Socha 1995). In such cases, comparison of photoperiod-induced diapause and low-temperature-induced diapause could provide a cue to understanding diapause characteristics and mechanisms. Quantifying such differences between non-diapause and diapause strains is an essential step for improving our understanding of the mechanisms regulating diapause.

The cabbage beetle, *Colaphellus bowringi* Baly (Coleoptera: Chrysomelidae), is a serious pest of cruciferous vegetables in the mountainous areas of China (Xue *et al.* 2002a). The Xiushui population (29°1'N,

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114°4'E) of this pest has two distinct peaks of occurrence with one generation appearing in spring and one to three generations in autumn (Xue *et al.* 2002a). This beetle typically enters diapause as adults in soil in response to either long day lengths or low temperatures; diapause is induced by long day length at temperatures above 20°C, but by temperature alone below 20°C (Xue *et al.* 2002b; Wang *et al.* 2004). In April, when the daily mean temperature is below 20°C, the first-emerging adults enter summer diapause mainly in response to low temperature. However, adult beetles that emerged after early May when the daily mean temperature becomes higher than 20°C enter summer diapause mainly in response to long day length. Adults of late-autumn generations enter winter diapause in response to low temperatures (Xue *et al.* 2002b).

This paper describes key biological characteristics of a previously reported non-photoperiodic-diapause (NPD) strain (Ma *et al.* 2011) of *C. bowringi*, particularly differences in responses to photoperiod and temperature, diapause intensity and life-history traits between the NPD and high-diapause (HD) strains of this species. The results suggest that the NPD strain of *C. bowringi* could be a suitable model species for further investigation of diapause regulation in insects.

## MATERIALS AND METHODS

### High-diapause strain

More than 1000 *C. bowringi* individuals were collected in Xiushui County (29°1'N, 114°4'E), Jiangxi Province, China, in late November 2008. Post-diapause adults that had emerged from the soil were moved to transparent plastic containers (height × length × width = 7.5 cm × 7.5 cm × 6 cm) for mass rearing (Xue *et al.* 2002a). The offspring of these beetles reared under diapause-averting conditions (25°C under conditions of 12 h light:12 h dark (LD 12:12) was regarded as a high-diapause (HD) strain and reliably entered diapause in response to long photoperiods at temperatures above 20°C.

### Non-photoperiodic-diapause strain

Another 500–600 *C. bowringi* individuals had previously been collected at Xiushui in late November 2006. Post-diapause adults were reared under weak diapause-inducing conditions (22°C and LD 13:11) and non-diapause individuals were obtained. This non-diapause founder population was then kept under diapause-inducing conditions (22°C and LD 16:8). By continuing to rear non-diapausing individuals for 22 generations, a non-photoperiodic-diapause (NPD) strain

was obtained. This strain is still maintained under the same condition in our laboratory.

### Insect rearing conditions

All larvae and adults used in this study were kept in incubators (SPX–250IC; Boxun Medical Instruments, Shanghai, China) and fed on fresh radish leaves (*Raphanus sativus* var. *longipinnatus*), which were provided daily (Ma *et al.* 2011).

### Comparison of diapause between strains

The effect of photoperiod was studied in each strain by examining diapause incidence under LD 8:16, LD 10:14, LD 12:12, LD 14:10 and LD 16:8 at 22°C, and the effect of temperature was studied by examining diapause incidence at 18, 20, 22, 24 and 26°C under constant darkness. All the individuals were reared under either of the above experimental conditions from the egg stage until their diapause attributes were determined at the adult stage. The determination of diapause attributes were made after adult feeding for 14 days at 18°C, 12 days at 20°C, 9 days at 22°C, and 5–6 days at 24 and 26°C in the transparent plastic containers filled with soil (Ma *et al.* 2011). These experiments were replicated three times with a sample size of 60–140 per replicate.

Diapausing adults of the HD and NPD strains were obtained by rearing them at a low temperature, 20°C, under constant darkness (Ma *et al.* 2011). These beetles were placed in large glass bottles with soil to allow them to burrow into soil, and then they were kept under natural conditions from 21 January 2011 (Xue *et al.* 2002b). Diapause termination in each strain was then measured by counting the number of post-diapause adults of each strain that had emerged from the soil each day; newly emerged adults were removed daily from 2011 to 2013. Emergence was monitored until 10 November 2013.

### Comparison of life-history traits

The following life-history traits of the HD and NPD strains were investigated under diapause-averting conditions (25°C and LD 12:12; Xue *et al.* 2002b).

#### *Hatching rate and larval and pupal survivorship*

Newly laid eggs of each strain were randomly sampled, incubated on wet filter paper in a plastic Petri dish (diameter 7.0 cm) and the number of larvae hatched was recorded daily. Newly hatched larvae of each strain were reared on fresh radish leaves in a transparent plastic container until pupation, and the numbers of individuals that pupated and emerged as adult were recorded. This

experiment was replicated three times, with each replicate comprising 100–150 individuals.

#### Developmental duration of immature stages

The number of larvae hatched was recorded for each strain at a 12 h interval. Newly hatched larvae of each strain were reared in transparent plastic containers containing fresh radish leaves until pupation, and pupae were then placed in individual tubes until adult emergence. The numbers of individuals that pupated and emerged as adults were recorded at a 12 h interval. The overall sample size was approximately 150–200.

#### Body weight of pupae and adults

Experimental individuals were sexed at the pupal stage (Wang *et al.* 2006). Approximately 80 3-day-old pupae and 40 4-day-old adults were weighed for each strain. Thereafter, they were dried at 80°C for 24 h and then measured for dry weight.

#### Adult longevity and fecundity

Thirty-six adult females were individually paired with a male and reared in plastic Petri dishes. The total number of eggs laid by female adults and survival of both sexes were recorded daily until all adults died.

#### Statistical analysis

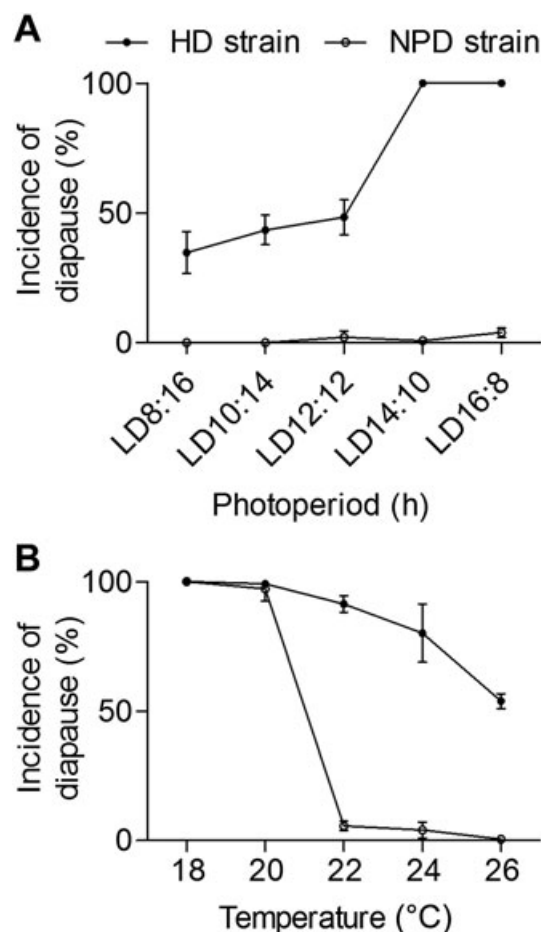
Data analyses were conducted using SPSS 11.5 (SPSS Inc., Chicago, IL, USA). Percentage data were normalized by arcsine square-root transformation prior to analysis. Differences in life-history parameters between the NPD and HD strains were analyzed using a Student's *t*-test ( $P < 0.05$ ).

## RESULTS

### Diapause induction and termination

At 22°C, the incidence of diapause of the HD strain was lower than 50% at LD 12:12 or shorter day lengths but reached to almost 100% at LD 14:10, whereas almost all individuals of the NPD strain did not enter diapause irrespective of photoperiod (Fig. 1A). The diapause incidence gradually decreased with the increase of temperature in the HD strain, but abruptly dropped between 20 and 22°C in the NPD strain (Fig. 1B).

When beetles were kept under continuous darkness at 20°C, almost all adults of both strains entered diapause (Fig. 1B), and thereafter diapause was terminated when they were kept under natural conditions (Fig. 2). The course of diapause termination was stepwise and much different between the HD and NPD strains; 87% of the

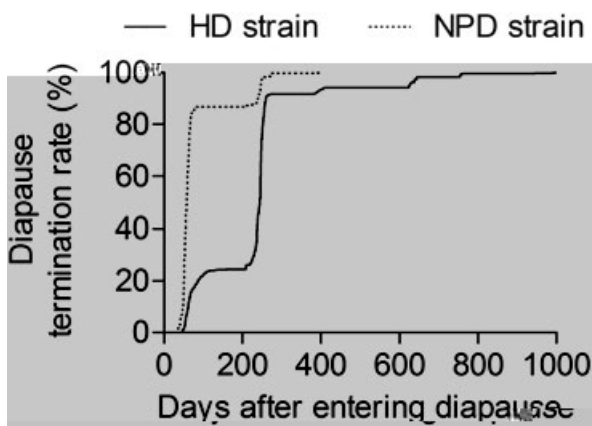


**Figure 1** Photoperiodic (A) and thermal (B) responses of the high-diapause (HD) and non-photoperiodic diapause (NPD) strains of *Colaphellus boursini*. Photoperiodic response was examined at 22°C and thermal response was examined under continuous darkness. Error bars indicate standard deviation. The experiment was replicated three times,  $n = 60$ –140 per replicate.

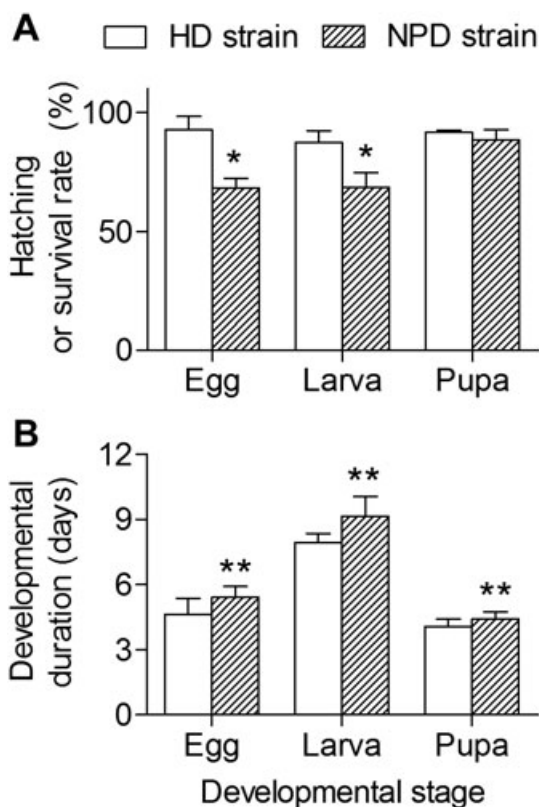
NPD strain terminated diapause within 100 days after the induction of diapause, but only 24% of the HD strain terminated in 100 days.

### Life-history traits

Differences in the life-history traits of the NPD and HD strains under LD 12:12 at 25°C are shown in Figures 3–6. The hatching and larval survival rates of the NPD strain were significantly lower than those of the HD strain (egg hatching:  $t = -4.214$ ,  $P < 0.05$ ; larval survival:  $t = -4.031$ ,  $P < 0.05$ ) (Fig. 3A), whereas there was no difference in the pupal survival ( $t = 1.094$ ,  $P = 0.335$ ). The immature period was significantly longer in the NPD strain than in the HD strain (egg:  $t = 12.659$ ,  $P < 0.01$ ; larva:  $t = 22.697$ ,  $P < 0.01$ ; pupa:  $t = 6.181$ ,  $P < 0.01$ ) (Fig. 3B). Body size of the NPD strain was



**Figure 2** Diapause termination of the high-diapause (HD) and non-photoperiodic-diapause (NPD) strains of *Colaphellus bowringi*. All adults entered diapause at 20°C under continuous darkness on 19–21 January 2011, and then they were placed under natural conditions.  $n=612$  (NPD strain) and 1359 (HD strain).



**Figure 3** Hatching rate of eggs, and survival rates of larvae and pupae (A) and duration of development of immature stages (B) of the high-diapause (HD) and non-photoperiodic-diapause (NPD) strains of *Colaphellus bowringi*. All insects were reared at 25°C under LD 12:12. Error bars indicate standard deviation. Each experiment was replicated three times,  $n=100$ –150 (A) and 150–200 (B) per replicate. \* $P < 0.05$ , \*\* $P < 0.01$ .

significantly smaller than that of the HD strain (Fig. 4); the average fresh and dry weights of 3-day-old pupae (fresh weight: ♀,  $t=-7.690$ ,  $P < 0.01$ ; ♂,  $t=-9.281$ ,  $P < 0.01$ . dry weight: ♀,  $t=-6.679$ ,  $P < 0.01$ ; ♂,  $t=-9.106$ ,  $P < 0.01$ ) and 4-day-old adults (fresh weight: ♀,  $t=-4.852$ ,  $P < 0.01$ ; ♂,  $t=-7.702$ ,  $P < 0.01$ ; dry weight: ♀,  $t=-6.809$ ,  $P < 0.01$ ; ♂,  $t=-6.551$ ,  $P < 0.01$ ) of the NPD strain were significantly lower than those of the HD strain. The longevity of female and male adults of the NPD strain was also significantly lower than that of the HD strain (female:  $t=-3.034$ ,  $P < 0.01$ ; male:  $t=-2.613$ ,  $P < 0.05$ ) (Fig. 5). Fecundity (total egg production:  $t=-3.940$ ,  $P < 0.01$ ; daily egg production:  $t=-3.313$ ,  $P = 0.01$ ) was significantly lower in the NPD strain than in the HD strain (Fig. 6).

## DISCUSSION

Photoperiodic diapause is an essential ecological adaptation in a wide range of temperate-zone insects (Saunders 1990; Syrová *et al.* 2003). At moderate temperatures, photoperiod appears to be the main diapause-inducing cue in many insects (Saunders 2002). Sensitivity to photoperiod may change with temperature to the extent that insects may become insensitive to photoperiod at extreme high or low temperatures, in which case temperature itself may become the main diapause-inducing cue (Tauber *et al.* 1986; Danks 1987). Non-photoperiodic-diapause strains of several insects, such as *Chymomyza costata* and *Pyrrhocoris apterus*, have almost lost the tendency to enter diapause at high temperatures, but most individuals still enter diapause at low temperature, irrespective of photoperiod (Riihimaa & Kimura 1988; Hodkova & Socha 1995). In our study, very few individuals of the NPD strain entered diapause at temperatures between 22 and 26°C, but almost all entered diapause between 18 and 20°C. These results suggest the responsiveness to low temperatures in the induction of diapause was not eliminated in the NPD strain but weakened compared to the HD strain. In this study, the HD strain was sensitive to photoperiod at 22°C, but the NPD strain was completely insensitive to photoperiod at this temperature. Previous studies suggest that insensitivity to photoperiod in diapausing insects may be caused by a mutation in a gene associated with the photoperiodic clock (Goto 2009). If so, the NPD strain of cabbage beetle could be useful for investigating the molecular mechanisms underlying photoperiodic diapause in insects.

Diapause termination is generally recognized as a measure of diapause intensity (Danks 1987; Masaki 2002). Late diapause termination is indicative of high

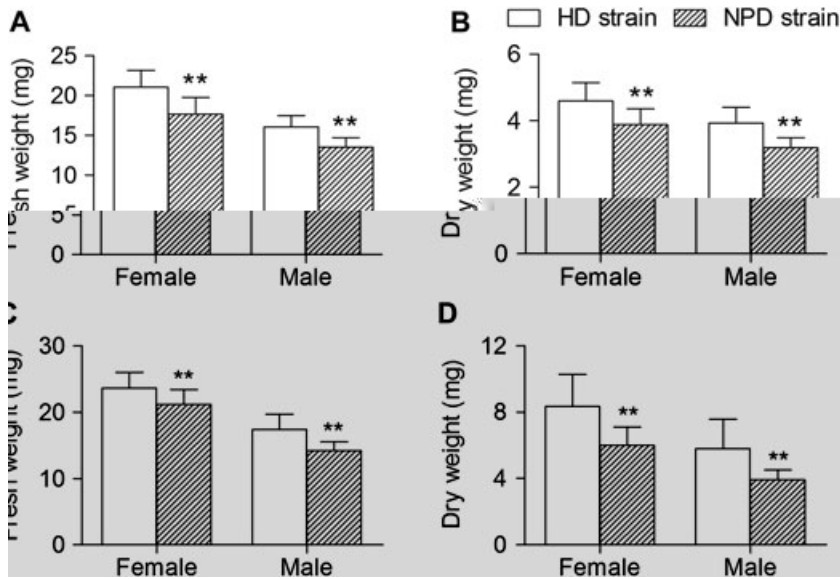


Figure 4 Fresh and dry weights of 3-day-old pupae (A,B) and 4-day-old adults (C,D) of the high-diapause (HD) and non-photoperiodic-diapause (NPD) strains of *Colaphellus bowringi*. All insects were reared at 25°C under LD 12:12. Error bars indicate standard deviation.  $n=80$  (3-day-old pupae) and 40 (4-day-old adults) for each strain.  $**P < 0.01$ .

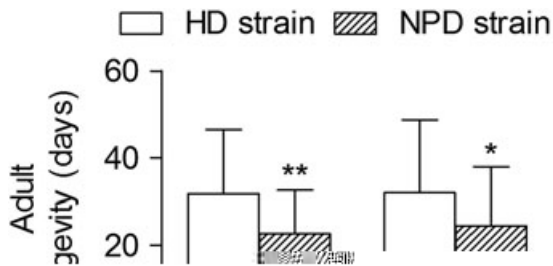


Figure 5 Adult longevity of the high-diapause (HD) and non-photoperiodic-diapause (NPD) strains of *Colaphellus bowringi*. All insects were reared at 25°C under LD 12:12. Error bars indicate standard deviation.  $n=36$  for each strain.  $*P < 0.05$ ,  $**P < 0.01$ .

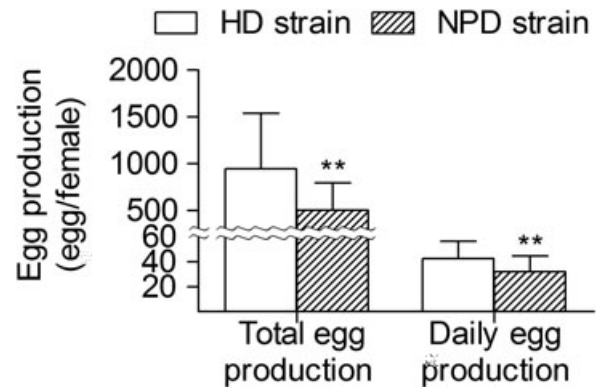


Figure 6 Egg production of the high-diapause (HD) and non-photoperiodic-diapause (NPD) strains of *2:12pof*

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diapause intensity (Tauber & Tauber 1972; Chen *et al.* 2014). Previous studies indicate that non-diapause strains of *Diabrotica virgifera* (Krysan *et al.* 1977) and *Oncopeltus fasciatus* (Dingle *et al.* 1977) had shorter diapause durations than diapause strains of these species. In this study, we found that the course of diapause termination was stepwise and much different between the HD and NPD strains; 87% of the NPD strain terminated diapause within 100 days after the induction of diapause but only 24% of the HD strain terminated in 100 days when they were kept under natural conditions. These results may suggest that the NPD and HD strains contain individuals with different diapause intensities; the proportion of individuals with higher

diapause intensity is lower in the NPD strain. The cabbage beetle enters summer diapause in response to either low temperature or long day length, but enters winter diapause in response to low temperature irrespective of day length (Xue *et al.* 2002b). The rate of incidence of summer and winter diapause may differ between the NPD and HD strains, and this may be reflected in the difference in the course of diapause termination between them. Thus, the NPD strain is very useful to characterize summer and winter diapause.

The NPD strain had significantly lower hatching and larval survival rates, longer developmental duration, smaller body size and lower fecundity than the HD strain. These life-history traits are key aspects of insect biology

(Nylin & Gotthard 1998) and may be directly related to environmental adaptability of insects (Scheiner & Istock 1991). Previous studies have shown that non-diapause and high-diapause strains of the same insect species have different life-history traits. Compared with high-diapause strains, non-diapause strains generally have longer developmental duration (Hoy & Knop 1978), smaller body size and lower fecundity (So & Takafuji 1991). However, one study found that the fecundity of a low-diapause strain of mite was higher than that of a high-diapause strain (Ito 2009). We found that the immature period is significantly longer and the hatching and larval survival rates, adult weight and fecundity are significantly lower in the NPD strain of *C. bowringi* than in the HD strain, suggesting a possibility that these life-history traits are genetically linked with photoperiodic response. However, during selection for the non-diapause strain, genes involved in these life-history traits might be unintentionally changed (Tauber *et al.* 1986; Danks 1987). More lines of NPD strains should be selected to verify this possibility.

Our study indicates that the NPD strain of *C. bowringi* has different biological characteristics from the HD strain, such as diapause intensity, thermal response in the induction of diapause, survival rate, developmental time and body size. These differences will benefit using the NPD strain of *C. bowringi* to analyze the diapause regulation mechanism in insects.

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