

## RESEARCH

## Adaptation of the Egg of the Desert Beetle, *Microdera punctipennis* (Coleoptera: Tenebrionidae), to Arid Environment

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**ABSTRACT.** *Microdera punctipennis* Kaszab (Coleoptera: Tenebrionidae) is an endemic species in Guerbantonggut desert in China. To explore the ways that *M. punctipennis* egg adapts to dry desert environment, morphological characteristics of the egg was investigated along with the egg of the nondesert beetle *Tenebrio molitor* (Coleoptera: Tenebrionidae). Water loss rate and survival rate of these eggs under different dry treatments (relative humidity 0, 10, and 20%) were measured to evaluate the desiccation resistance of the eggs at different developmental stages (day 0, 2, and 5 eggs). Our results showed that the 50-egg weight in *T. molitor* was heavier than *M. punctipennis*, while the 50-first-instar larva weight in *T. molitor* was almost the same as in *M. punctipennis*. The water loss rate of *M. punctipennis* egg under dry conditions was significantly lower than *T. molitor*, and the egg survival rate was significantly higher than *T. molitor*. The estimated developmental threshold temperature of *M. punctipennis* egg was 18.30°C, and the critical thermal maximum of *M. punctipennis* egg is above 39°C. These features partly account for the adaptability of *M. punctipennis* to desert environment in egg stage.

**Key Words:** morphological characteristic, water loss rate, survival rate, developmental threshold

Key ecological factors such as temperature and water influence the distribution and population dynamics of arthropods (Verhoef 1977, Reynolds et al. 2012, Ricalde et al. 2012). Beetles (especially tenebrionid) are among the most successful animals of the desert, and are called “indicators of desertization” (Ren and Yu 1999). Insect structure, behavior, and physiological mechanism, including forming subelytral cavity (Draney 1993, Gorb 1998), low cuticular water permeability (Zachariassen 1991), burying body deeply in the substrate (Cloudsley-Thompson 1990) and taking up fog-water as water source (Seely 1979), have been linked to adaptation processes in arid environments. Egg is an essential stage in the development of insect, mortality is higher at egg stage than other developmental stages, which decides the size of population. On the other hand, egg is the most damageable stage because of its inability to move to avoid environmental insults. Micropyle, aeropyle, water vapor uptake, and cold tolerance of eggs had been studied (Yoder and Denlinger 1992, Cönsoli et al. 1999, Gauvin et al. 2001, Shintani and Ishikawa 2007, Sukontason et al. 2007). However, the mechanisms of egg’s resistance to desiccation and high temperature remain unknown. Without the ability to move or feed in response to environmental conditions, eggs of desert insects must be self-sufficient to avoid dehydration and thermal injury.

*Microdera punctipennis* Kaszab (Coleoptera: Tenebrionidae), a small flightless beetle, is an endemic species in Guerbantonggut desert (Huang et al. 2005), the second largest desert in the northwest of China. The annual average air temperature there is 5–7.5°C. The temperature difference between day and night in summer and between winter and summer is huge. The highest air temperature is more than 40°C and the lowest is lower than –40°C (Wei and Liu 2000, Qian et al. 2004). *M. punctipennis* produces antifreeze proteins to survive such low temperatures (Zhao et al. 2005, Qiu et al. 2010). The way this beetle adapts to the extremely dry and fluctuated temperatures includes night active, living in sands, and having subelytral cavity (Wang et al. 2011). In addition, the egg of this beetle has a sticky layer usually covered with sand, which may be another adaptation for desiccation resistance. Similar

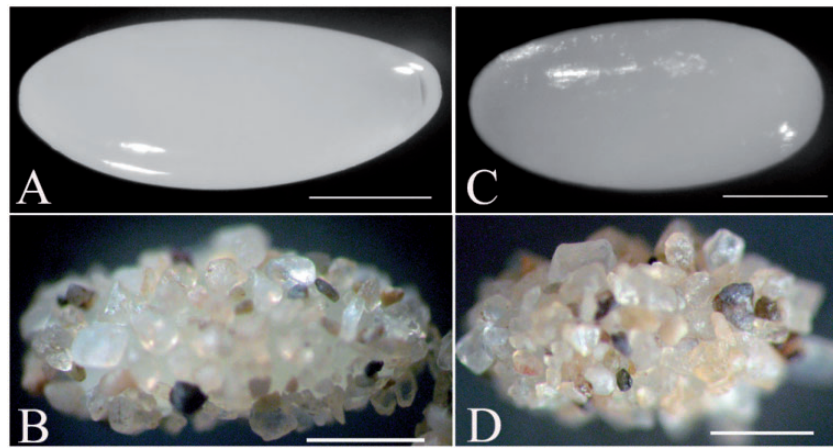
structure had been found in the egg of desert beetle *Gyriosomus kingi* (Pizarro-Araya et al. 2007). Investigating the effects of dryness and temperature on the egg of *M. punctipennis* may help to further understand the adaptation of this darkling beetle to desert environment.

*Tenebrio molitor* (Coleoptera: Tenebrionidae), a nondesert beetle, lives in barns where the temperature and humidity are relatively constant. Its egg also has sticky layer. In this study, *T. molitor* egg was compared with the *M. punctipennis* egg to shed light on the adaptation mechanism of the egg of the desert beetle. The present work aims to investigate: 1) the morphological characteristics of *M. punctipennis* egg; 2) the difference of water loss rate and survival rate in the eggs between the desert *M. punctipennis* and the nondesert *T. molitor*; 3) the functionality of egg’s sticky layer in desiccation resistance; and 4) the critical temperature for *M. punctipennis* egg development.

### Materials and Methods

**Adult Beetle Collection and Maintenance.** *M. punctipennis* adults were collected in March from Wujiaqv (N 44° 29′, E 87° 31′, 410 m), which is about 100 km northeast of the geological center of Asia. *T. molitor* adults, used as a nondesert beetle for the comparison in this study, were obtained from laboratory breeding. These beetles (six groups for each, 30 pairs of male and female per group) were reared in large plastic beakers containing dry sands at 30 ± 0.5°C, 35 ± 6% relative humidity (RH), 2000 Lux light intensity and 16:8 (L:D) h photoperiod conditions in an incubator (GXZ, Southeast Instrument Ltd., Dongguan, China). The beetles were fed with fresh cabbage. Adults were allowed to mate freely and lay eggs in the sands.

**Egg Collection and Observation.** Eggs were daily collected by sifting the eggs from the sands. Egg production was counted every day. The fresh eggs were placed in Petri dishes (15 cm in diameter), and maintained at 30 ± 0.5°C, 35 ± 6% RH, 2000 Lux light intensity and 16:8 (L:D) h photoperiod conditions in an incubator (GXZ, Southeast Instrument Ltd., Dongguan, China). Hatching time length and hatched eggs were daily recorded. The freshly laid eggs were designated as “day



**Fig. 1.** *M. punctipennis* eggs (left) and *T. molitor* eggs (right). (A) Washed egg of *M. punctipennis*; (B) egg of *M. punctipennis* attached with sands; (C) washed egg of *T. molitor*; (D) egg of *T. molitor* attached with sands. Bars represent 500  $\mu\text{m}$ .

**Table 1.** Egg characteristics of *M. punctipennis* and *T. molitor*

| Species                | Egg length (mm)               | Egg width (mm)                | S/V                            | 50 eggs weight (mg)             | 50-first-stage larvae weight (mg) |
|------------------------|-------------------------------|-------------------------------|--------------------------------|---------------------------------|-----------------------------------|
| <i>M. punctipennis</i> | 1.7303 $\pm$ 0.0760a (n = 98) | 0.6663 $\pm$ 0.0237a (n = 98) | 36.0680 $\pm$ 1.2347a (n = 98) | 56.1222 $\pm$ 1.8167a (n = 900) | 22.1056 $\pm$ 1.5872 (n = 703)    |
| <i>T. molitor</i>      | 1.6690 $\pm$ 0.0774b (n = 54) | 0.7364 $\pm$ 0.0275b (n = 54) | 32.6373 $\pm$ 1.3190b (n = 54) | 68.7889 $\pm$ 1.2128b (n = 900) | 21.8800 $\pm$ 1.0843 (n = 521)    |

Values with the different letters are significantly different (independent samples *t*-test,  $P < 0.05$ ) for comparisons within a parameter.

0”); those incubated in the incubator for 2 or 5 d were designated as “day 2” and “day 5” eggs, respectively.

Day 0 eggs were cleaned singly with a drop of distilled water (Kučerová and Stejskal 2008), and then dried with filter paper. Egg shell structure was observed under scanning electronic microscopic (LEO1430VP, LEO, Beijing, www.zeiss.com). Egg length ( $a$ ) and width ( $b$ ) were measured under stereomicroscope equipped with Elements 3.0 software (Nikon SMZ-800, www.nikon.com). The surface area ( $S$ ) of these ellipsoid eggs was calculated as  $S = 4\pi ab$ ; the volume ( $V$ ) was calculated as  $V = (1/6)\pi ab^2$  (Gauvin et al. 2001). Day 0 eggs were weighed per 50 on a fine electronic scale (0.1 mg, AL104, Mettler Toledo, Shanghai, China).

**Dry Treatment and Water Loss Determination.** Eggs (50 per group) were dry treated with various RH at 25  $\pm$  1°C. The near 0% RH was generated in a sealed glass desiccator ( $d = 60$  cm) containing dried crystals of self-indicating silica gel (1,000 g); 10% RH was generated by 200 g of NaOH + 7 g of water, and 20% RH by 200 g of NaOH + 40 g of water (Wu and Shao 1991). The air humidity in the desiccators was measured with a hygromicrograph ( $\pm 1.5\%$  RH; MT6600, Microtest Instruments Ltd., Beijing, China).

Eggs at different developmental stages (day 0, 2, and 5) in each treatment were placed, respectively in Parafilm boxes (1.5 cm by 1.5 cm by 1 cm). Each empty box was weighed in advance and the total weight of the box and eggs was daily weighed till the first larva emerged. Total larvae emerged from each treatment were recorded to calculate survival rate. In addition, day 0 eggs were washed and then treated at 20% RH to study the function of the outside sticky layer in desiccation tolerance.

The water loss was determined by  $m_t/m_0$ , where  $m_0$  is the initial fresh weight of 50 eggs,  $m_t$  is the weight of the same eggs at time  $t$ . The slope of  $m_t/m_0$  plotted against time is the water loss rate per day (Pelletier et al. 1995). Six replicates were performed for each experimental group.

**Temperature Treatment.** Day 0 eggs ( $n > 60$ ) were placed in glass dishes and held in incubators at constant temperatures (22, 25, 27, 30, 33, 36, 39°C), with 35  $\pm$  6% RH, 2000 Lux light intensity and a photoperiod of 16:8 (L:D) h. Egg hatching time and the number of the

emerged larvae in each dish were recorded. Egg survival rate was calculated as the ratio of hatched egg to total egg in each treatment. Duration of the embryonic development is defined as the period between egg deposition and the time 50% of the eggs hatched (Delobel 1983). Developmental threshold temperature and accumulated degree days for egg development were determined as described in literatures (Choi et al. 2000, Jallow and Matsumura 2001). Each treatment group has more than six repeats ( $n > 60$  per group).

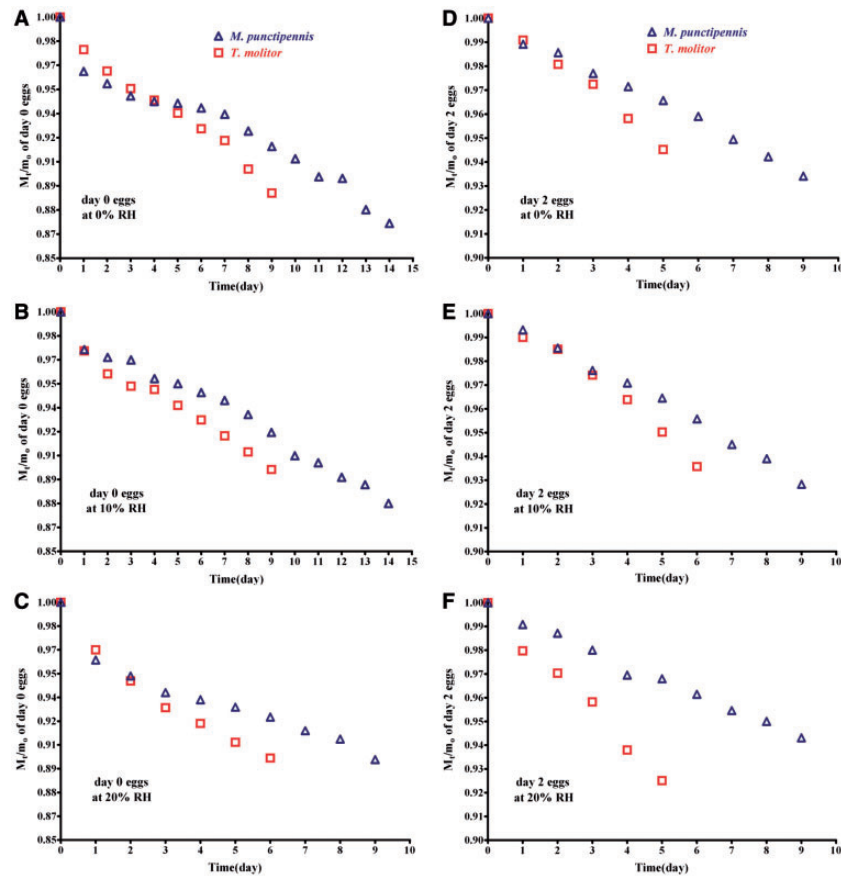
**Statistical Analysis.** Water loss curve was built by using curve fitting analysis. Data were submitted to independent samples *t*-test, and one way analysis of variance (ANOVA). Data for egg survival rate were subjected to arcsine transformation prior to analysis. Data analysis was conducted by GraphPad Prism 5 software.

## Results

**Egg Morphology of *M. punctipennis* and *T. molitor*.** Eggs of *M. punctipennis* (Fig. 1A and B) and *T. molitor* (Fig. 1C and D) were both elliptic, creamy white, and sticky with sands often attached. The eggshells were homogeneous and compact under scanning electronic microscope (6,000 $\times$ ). No micropyles or aeropyles were observed on egg surface. *M. punctipennis* egg was longer than *T. molitor* egg, and the  $S/V$  (surface area/volume) ratio of *M. punctipennis* egg is bigger than *T. molitor* egg. Egg size difference between these two species was significant ( $P < 0.05$ ; Table 1). Interestingly, the weight of 50 eggs of *M. punctipennis* was significantly smaller than that of *T. molitor*, but the weight of 50-first-instar larvae was bigger than that of *T. molitor*, though not significant.

Under the condition of 25°C and 30  $\pm$  6% RH, the developmental duration of *M. punctipennis* egg was 11.04  $\pm$  0.06 d ( $n = 2,930$ ), *T. molitor* egg was 8.67  $\pm$  0.52 d ( $n = 1,735$ ). Egg hatchability (%) of *M. punctipennis* and *T. molitor* was 72.73  $\pm$  5.83% ( $n = 2,930$ ) and 85.26  $\pm$  4.16% ( $n = 1,735$ ), respectively.

**Water Loss Rate of Day 0 and 2 Eggs.** Water loss rate of day 0 and 2 eggs of the two beetles under dry conditions were measured to compare the water holding capacity of the eggs. The slope of  $m_t/m_0$  plotted against time is the water loss rate per day. The results showed that



**Fig. 2.** Water loss rates by day 0 eggs (left) and day 2 eggs (right) of *M. punctipennis* and *T. molitor* at different relative humidities. (A) day 0 eggs at 0% RH; (B) day 0 eggs at 10% RH; (C) day 0 eggs at 20% RH; (D) day 2 eggs at 0% RH; (E) day 2 eggs at 10% RH; (F) day 2 eggs at 20% RH. For clarity, error bars are omitted.

*M. punctipennis* eggs had lower water loss rate than *T. molitor* with the time prolonged, though it was high in day 0 eggs at early times (Fig. 2A–C).

For day 0 eggs (Table 2), at 0% RH the slope (linear regression) of *T. molitor* eggs was  $-0.0109$  which was about 1.5 times of *M. punctipennis* that was  $-0.0074$ . At 10% the ratio of these two slopes was 1.2. At 20% the ratio was 1.76, indicating that *T. molitor* egg had higher water loss rate than *M. punctipennis* egg. Besides, at 20% RH water loss rate was better described in exponential model than linear by comparing  $R^2$  and SSE of the fitting curves, which means an uneven water loss rate with fast speed in early days and slow speed in later days. In the exponential model, the parameters in *T. molitor* egg were both larger than those in *M. punctipennis* egg.

Day 2 eggs' water loss rates of these two beetles showed similar profiles as day 0 eggs in that *T. molitor* eggs lose water faster than *M. punctipennis* eggs (Fig. 2D–F). Water loss ( $m_t/m_0$ ) and exposed days under dry conditions follow linear models at all relative humidities. The ratio of slopes (linear regression) of *T. molitor* and *M. punctipennis* was 1.6 at 0% RH, 1.3 at 10% RH, and 2.35 at 20%, suggesting that water loss rate of *T. molitor* day 2 eggs was faster than *M. punctipennis* day 2 eggs under dry conditions (Table 3).

The developmental duration of day 5 eggs at room temperature was 2–3 d, so the data were not enough to use in regression analysis.

**Water Loss Rate of Washed Day 0 Eggs.** To evaluate the function of the outside sticky layer of the egg in desiccation tolerance, day 0 eggs of *M. punctipennis* and *T. molitor* were washed prior to dry treatment. The results showed that both washed eggs had higher water loss rate than unwashed eggs at laboratory temperature, 20% RH (Fig. 3). The water loss rate of washed *M. punctipennis* day 0 eggs was 3.27 times of

the unwashed eggs at 20%RH, whereas the washed *T. molitor* eggs was 3.02 times of the unwashed ones (Table 4). The washed *T. molitor* eggs lost water faster than washed *M. punctipennis* eggs. The water loss rate was 1.63 times of the *M. punctipennis* eggs.

The water loss rate for washed eggs at 20% RH was better described in exponential model, which is different from the unwashed eggs. The coefficients were greatly increased. The unwashed *M. punctipennis* was  $Y = 0.0952e^{-0.2260X} + 0.8972$ , whereas the washed one was  $Y = 0.3059e^{-0.2491X} + 0.6693$ . Similarly, the unwashed *T. molitor* egg was  $Y = 0.1234e^{-0.2492X} + 0.8756$ , whereas the washed one was  $Y = 0.3714e^{-0.4572X} + 0.6152$ . It was noticeable that change of the exponential coefficients in *M. punctipennis* before and after the out sticky layer removal was almost the same (from  $-0.2260$  to  $-0.2491$ ), while this change in *T. molitor* eggs was obvious from  $-0.2492$  to  $-0.4572$ . These results suggest that *M. punctipennis* egg shell itself has a stronger water loss resistance. In this way the water loss rate was fast on first days and slow on the following days.

**Effect of Drought on Egg Survival Rate.** Figure 4 shows the survival data of various developmental stage eggs (day 0, 2, and 5) in *M. punctipennis* and *T. molitor* under  $25 \pm 1^\circ\text{C}$  and different relative humidities. In general, *M. punctipennis* had significant higher survival rate than *T. molitor* ( $P < 0.05$ ) in all chosen developmental stages under each dry treatment.

For *M. punctipennis*, there was no significant difference in survival rates among different developmental eggs under 0 and 20% RH, except that day 0 egg at 10% RH was significantly lower than old eggs ( $P < 0.05$ ). For *T. molitor*, there was no significant difference in survival rates among different developmental eggs under 10% RH, while at 0 and 20% RH, different developmental eggs showed significant

**Table 2. Curve fitting analysis for water loss rate of day 0 eggs as a function of the time spent at laboratory temperature and different relative humidities**

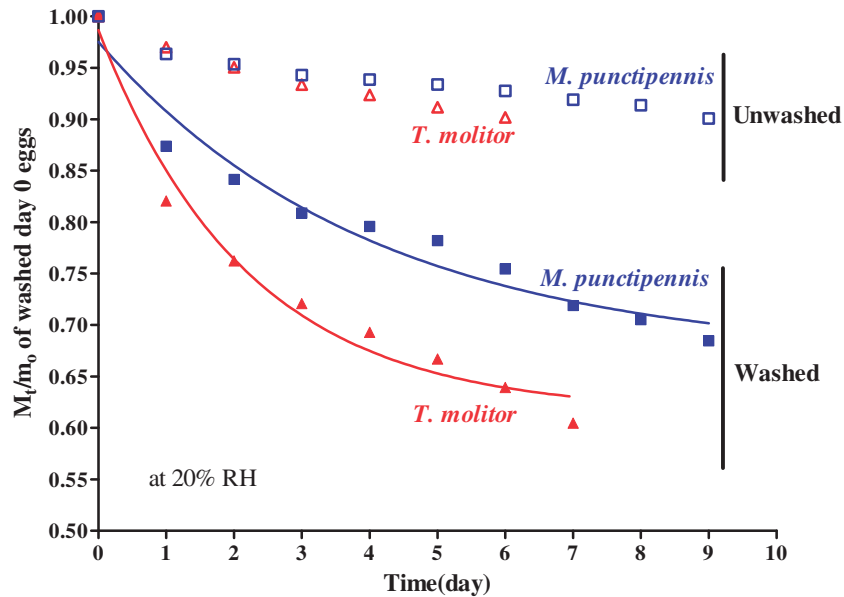
| Species                | RH          | Regression type                   | Equation                          | $R^2$  | $P$      | SSE      |
|------------------------|-------------|-----------------------------------|-----------------------------------|--------|----------|----------|
| <i>M. punctipennis</i> | 0%          | Linear                            | $Y = -0.0074x + 0.9831$           | 0.9466 | 0.1492   | 8.77E-04 |
|                        |             | Exponential                       | $Y = 0.9839e^{-0.0080x}$          | 0.9452 | 0.1492   | 8.99E-04 |
|                        | 10%         | Linear                            | $Y = -0.0079x + 0.9929$           | 0.9828 | 0.5136   | 3.04E-04 |
|                        |             | Exponential                       | $Y = 0.9938e^{-0.0084x}$          | 0.9813 | 0.5136   | 3.29E-04 |
| <i>T. molitor</i>      | 0%          | Linear                            | $Y = -0.0089x + 0.9793$           | 0.9032 | 0.0397   | 6.99E-04 |
|                        |             | Exponential                       | $Y = 0.0952e^{-0.2260x} + 0.8972$ | 0.9537 | 0.1905   | 3.34E-04 |
|                        | 10%         | Linear                            | $Y = -0.0109x + 0.9932$           | 0.9825 | 0.1905   | 1.76E-04 |
|                        |             | Exponential                       | $Y = 0.9940e^{-0.0116x}$          | 0.9826 | 0.1905   | 1.75E-04 |
|                        | 20%         | Linear                            | $Y = -0.0097x + 0.9887$           | 0.9683 | 0.1905   | 2.54E-04 |
|                        |             | Exponential                       | $Y = 0.1917e^{-0.0677x} + 0.8011$ | 0.9741 | 0.1167   | 2.07E-04 |
| 20%                    | Linear      | $Y = -0.0157x + 0.9885$           | 0.957                             | 0.2    | 3.09E-04 |          |
|                        | Exponential | $Y = 0.1234e^{-0.2492x} + 0.8756$ | 0.9982                            | 0.5429 | 1.32E-05 |          |

$R^2$  is the coefficient of determination,  $Y$  stands for water loss rate ( $m_t/m_0$ ), and  $x$  stands for the day until the egg hatched,  $P$  is the measure of deviation from curve fitting model, and SSE represents the residual of curve fitting.

**Table 3. Curve fitting analysis for water loss rate of day 2 eggs as a function of the time spent at laboratory temperature and different relative humidities**

| Species                | RH          | Regression type          | Equation                 | $R^2$  | $P$      | SSE      |
|------------------------|-------------|--------------------------|--------------------------|--------|----------|----------|
| <i>M. punctipennis</i> | 0%          | Linear                   | $Y = -0.0070x + 0.9991$  | 0.9947 | 0.6429   | 2.24E-05 |
|                        |             | Exponential              | $Y = 0.9994e^{-0.0073x}$ | 0.994  | 0.6429   | 2.46E-05 |
|                        | 10%         | Linear                   | $Y = -0.0078x + 1.0010$  | 0.9958 | 0.8333   | 1.51E-05 |
|                        |             | Exponential              | $Y = 1.0010e^{-0.0081x}$ | 0.9949 | 0.8333   | 2.76E-05 |
| 20%                    | Linear      | $Y = -0.0062x + 0.9982$  | 0.9922                   | 1.0000 | 2.50E-05 |          |
|                        | Exponential | $Y = 0.9985e^{-0.0064x}$ | 0.9927                   | 1.0000 | 2.33E-05 |          |
| <i>T. molitor</i>      | 0%          | Linear                   | $Y = -0.0109x + 1.0020$  | 0.9906 | 0.4000   | 1.97E-05 |
|                        |             | Exponential              | $Y = 1.0020e^{-0.0111x}$ | 0.9892 | 0.3000   | 2.28E-05 |
|                        | 10%         | Linear                   | $Y = -0.0105x + 1.0030$  | 0.9818 | 0.2000   | 5.74E-05 |
|                        |             | Exponential              | $Y = 1.0030e^{-0.0108x}$ | 0.9794 | 0.2000   | 6.47E-05 |
|                        | 20%         | Linear                   | $Y = -0.0146x + 0.9985$  | 0.9901 | 0.7000   | 3.73E-05 |
|                        |             | Exponential              | $Y = 0.9988e^{-0.0152x}$ | 0.9898 | 0.7000   | 3.86E-05 |

$R^2$  is the coefficient of determination,  $Y$  stands for water loss rate ( $m_t/m_0$ ), and  $x$  stands for the day until the egg hatched,  $P$  is the measure of deviation from curve fitting model, and SSE represents the residual of curve fitting.



**Fig. 3.** Water loss rates by washed day 0 eggs of *M. punctipennis* (blue) and *T. molitor* (red) as a function of the time spent at  $25 \pm 1^\circ\text{C}$  and 20% RH. For clarity, error bars are omitted.

differences in survival rate ( $P < 0.05$ ). At 0% RH, day 5 eggs had significant lower survival rate ( $P < 0.05$ ) than day 0 and 2 eggs, suggesting that *T. molitor* eggs soon before hatching were sensitive to extreme dryness. On contrary, day 5 eggs showed significant higher survival rate at

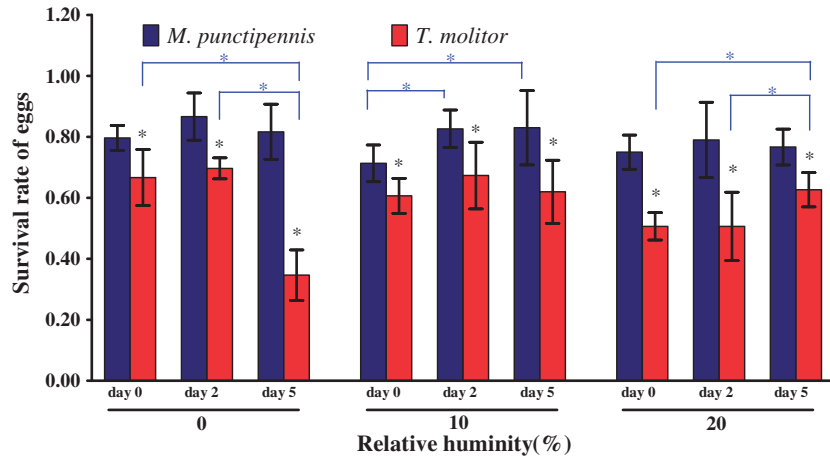
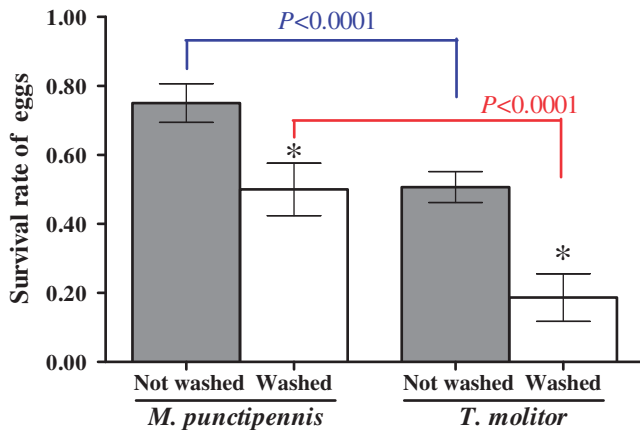
20% RH than day 0 and 2 eggs, which indicated that mild dryness could increase the survival rate of the eggs soon before hatching.

Egg hatching rates (survival rates) of day 0 eggs of *M. punctipennis* and *T. molitor* at  $25 \pm 1^\circ\text{C}$  and 20% RH were 75 and 50%, respectively

**Table 4.** Curve fitting analysis for water loss rate of washed day 0 eggs at 20% RH

| Species                | Regression type | Equation                          | $R^2$  | $P$    | SSE    |
|------------------------|-----------------|-----------------------------------|--------|--------|--------|
| <i>M. punctipennis</i> | Linear          | $Y = -0.0291x + 0.9276$           | 0.8934 | 0.3571 | 0.0084 |
|                        | Exponential     | $Y = 0.3059e^{-0.2491x} + 0.6693$ | 0.9567 | 0.1905 | 0.0034 |
| <i>T. molitor</i>      | Linear          | $Y = -0.0475x + 0.9046$           | 0.8531 | 0.1429 | 0.0163 |
|                        | Exponential     | $Y = 0.3714e^{-0.4572x} + 0.6152$ | 0.9784 | 0.4286 | 0.0024 |

$R^2$  is the coefficient of determination,  $Y$  stands for water loss rate ( $m_t/m_0$ ), and  $x$  stands for the day until the egg hatched,  $P$  is the measure of deviation from curve fitting model, and SSE represents the residual of curve fitting.

**Fig. 4.** Survival rates of eggs of *M. punctipennis* and *T. molitor* at  $25 \pm 1^\circ\text{C}$  and various relative humidities. Bars indicate standard deviation. *M. punctipennis* had significant higher survival rate than *T. molitor* (independent samples  $t$ -test,  $P < 0.05$ ) in all developmental stages under each dry treatment (black asterisks). The blue asterisk denotes a significant difference relative to the developmental stage (independent samples  $t$ -test,  $P < 0.05$ ).**Fig. 5.** Survival rates of washed and unwashed day 0 eggs in *M. punctipennis* and *T. molitor* at  $25 \pm 1^\circ\text{C}$  and 20% RH ( $n = 300$ ). Bars indicate standard deviation. The asterisk on the columns denotes a significant difference relative to the washing treatment (independent samples  $t$ -test,  $P < 0.0001$ ).

(Fig. 5). This result was inconsistent with the above water loss rate experiments. After removal of the outside sticky layer, both beetles' eggs showed significant decrease in hatching rate ( $P < 0.05$ ). Noteworthy, the decrement in *T. molitor* was 31.3%, while it was 25% in *M. punctipennis*. This may suggest that *M. punctipennis* egg shell itself has a higher water loss resistance.

**Effect of Temperature on *M. punctipennis* Egg Development.** The developmental time of the egg stage at 22, 25, 27, 30, 33, 36, and  $39^\circ\text{C}$  was  $23.2083 \pm 0.3219$  ( $n = 776$ ),  $11.0430 \pm 0.0572$  ( $n = 2,930$ ),  $9.5215 \pm 0.1054$  ( $n = 1,453$ ),  $7.0699 \pm 0.0298$  ( $n = 2,559$ ),

$5.0833 \pm 0.0615$  ( $n = 1,089$ ),  $4.6607 \pm 0.0795$  ( $n = 1,465$ ), and  $4.9444 \pm 0.0398$  ( $n = 780$ ) days, respectively.

The egg developmental rate in relation to temperature is linear except at  $39^\circ\text{C}$  (Fig. 6), so data for this temperature were not included in our analysis. The linear regression equation is  $Y = 0.0125X - 0.2291$  ( $R^2 = 0.9857$ ,  $P < 0.0001$ ), where ( $Y$ ) is the reciprocal of the number of days and ( $X$ ) temperature ( $^\circ\text{C}$ ). It means that  $1^\circ\text{C}$  caused a growth rate of 0.0125 1/d. A developmental threshold of  $18.2987^\circ\text{C}$  was estimated from this equation, and a thermal constant (effective accumulated temperature) of 80.4518 degree days was estimated as the effective temperature sum for the development of the egg.

The results of the influence of temperature on the survival rate of *M. punctipennis*, showed that from 22 to  $33^\circ\text{C}$  the survival rate of *M. punctipennis* eggs increased steadily, from 60% to the peak of 85.8% at  $33^\circ\text{C}$  (Fig. 7). When the temperature was higher than  $33^\circ\text{C}$ , egg survival rate began to decrease. At  $39^\circ\text{C}$  egg survival rate was significantly declined ( $P < 0.05$ ). There was no significant difference in survival rate between temperatures from 27 to  $36^\circ\text{C}$ , though 30 and  $33^\circ\text{C}$  were more suitable for egg hatching in this study.

## Discussion

Egg is an essential stage in the development of insects (Yoder and Denlinger 1992, Reynolds et al. 2012). Studies on the desert environmental adaptation in the view point of egg may help us better understand why beetles are among the most successful living things in desert regions. Egg characteristics of desert beetle *M. punctipennis* and non-desert beetle *T. molitor* showed certain difference. Though egg shape of these two species was both elliptic, *M. punctipennis* egg was longer than *T. molitor* egg. The  $S/V$  in *M. punctipennis* was larger than that of *T. molitor* egg. This was not consistent with other reports that eggs have a decreased egg surface to volume ratio in other desert tenebrionid (Marshall 1985). *M. punctipennis* egg may adopt other strategies to

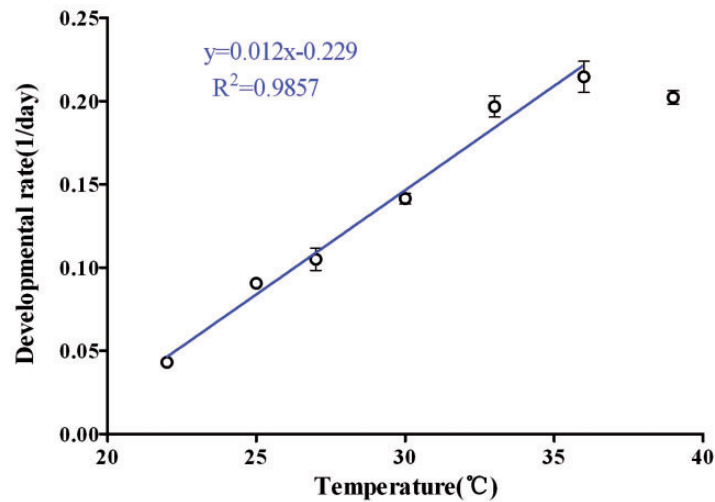


Fig. 6. Relationship between egg developmental rate (1/day) of *M. punctipennis* and temperature. Bars indicate standard deviation.

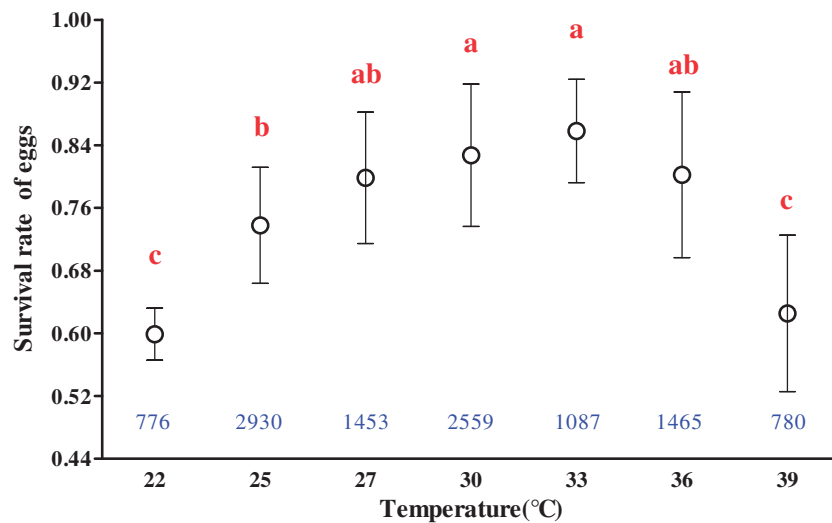


Fig. 7. Survival rates of *M. punctipennis* eggs at different temperatures. Numbers of individuals examined in each test are shown in graph (blue). Bars indicate standard deviation. Bars with the same red letter are not significantly different (independent samples *t*-test,  $P < 0.05$ ).

protect water evaporation. We noticed that the 50-egg weight in *T. molitor* was heavier than *M. punctipennis*. However, the 50-first instar weight in *T. molitor* was almost the same as in *M. punctipennis*. This may suggest that *M. punctipennis* egg stored more nutrients for hatching in stead of water. Besides, we observed that female *M. punctipennis* kicked sand to bury eggs after oviposition, which may be another type of adaptation that *M. punctipennis* employs for desiccation resistance.

Openings, such as micropyles and aeropyles, on egg surface of these two beetles were not observed under electronic microscopy in our study, which are frequently present in insect egg shell (Cônsoi et al. 1999, Wolf and Reid 2001). In another darkling tenebrionid beetle, *Sternoplax souvorowiana* Reitt, we observed micropyles and aeropyles on the surface of egg, which was laid in wet sand and had little outside sticky layer. We proposed that the homogeneous and compact egg shell may help *M. punctipennis* egg to reduce water loss.

Desiccation resistance is essential for desert insect eggs. In this study, *M. punctipennis* egg in all ages (day 0, 2, 5) presented a significant lower water loss rate and higher survival rate than the nondesert *T. molitor* eggs at 25°C and dry treatments, showing that *M. punctipennis* egg has a higher desiccation resistance.

Because of the existence of the outside sticky layer on both *M. punctipennis* and *T. molitor* eggs, we investigated its function in protecting eggs from desiccation. Removal of the sticky layer showed significant effect on both insect eggs in increasing water loss rates and decreasing egg survival rates. However, the degree of this influence in *T. molitor* eggs rather than *M. punctipennis* was much prominent, suggesting that the outside sticky layer only play a partial role in the desiccation resistance of desert beetle *M. punctipennis* egg. Punzo and Mutchmor (1980) discussed that eventual resistance to water loss for egg is the result of waxy layer which is laid down on the inside of the chorion, also the waxy layer' breakdown was influenced by high temperatures, but desiccation resulted for *T. molitor* happened at 12% RH regardless of whether the temperature was high (35°C), optimal (25°C), or low (10°C). Thus, the texture of egg shell itself may be essential for desiccation resistance.

The relationship between egg water loss rate and exposed days under dry treatments was fitted by linear and exponential curves. Under severe dry (0% RH) and mediate dry (10%) conditions linear curve was the best fitting, which means water loss rate was constant with respective coefficients. Although under 20% RH, exponential curve described

the water loss process much better. This means that under mild dry condition the eggs lost water more like a physical diffusion process. The difference in egg water lose pattern at different environmental humidities may suggest that under extreme dry conditions, living eggs can manage to hold the water fast from losing rapidly. Although at mild drought, the exponential decay of water lose might be due to the lost of absorbed outside water vapor. *M. punctipennis* and *T. molitor* eggs with the removal of outside sticky layer also showed exponential way of water loss at 20% RH, but with much higher coefficients than the unwashed ones. These increases in water lose coefficients indicated that the water lose happened in the physical diffusion process was enhanced, and the lost water should be from inside the egg due to the removal of the sticky surface. Meanwhile, the washed eggs showed significant lower survival rate than unwashed ones. These results strongly suggest that the sticky layer surface play important roles in protecting the eggs from losing water. Interestingly, the nondesert *T. molitor* egg even showed more dependency on the sticky outside layer for survival than *M. punctipennis*, this suggests that the desert *M. punctipennis* egg itself, probably the egg shell structure, may provide other protections. Similar exponential curve are reported in other insects (Pelletier 1995, Yoder et al. 2004, Elnitsky et al. 2008).

Temperature is another key factor that determines the survival of desert insects. The developmental threshold temperature of *M. punctipennis* was about 18.30°C, which is higher than that of *T. molitor* reported as 10.46°C (Chen and Liu 1992). Our outdoor observation found that when the air temperature was above 19°C, *M. punctipennis* began to oviposit. So, *M. punctipennis* is a thermophilic insect. The effective accumulated temperature of *M. punctipennis* egg was ~80.45 degree days, lower than *T. molitor*'s 123.5 degree days (Chen and Liu 1992). This means that desert beetle *M. punctipennis* eggs need higher temperature to start embryo development, but requires lower heat units than *T. molitor*. This may also be an adaption of *M. punctipennis* to desert environment.

In our studies, temperatures from 22 to 36°C are suitable for *M. punctipennis* eggs development, and the optimal temperature was 33°C. At 39°C egg survival rate was significantly decreased but still above 60%. It was reported that the optimal temperature for *T. molitor* was 25°C (Bowler 1967, Punzo and Mutchmor 1980), and 23–28°C (Yinon and Shulov 1970). Chen and Liu (1992) found that 28°C was proper for *T. molitor* eggs, and when temperature exceeded 30°C, developmental rate would be weakened. At 35°C, female adults could not lay eggs (Chen and Liu 1992). *T. molitor* eggs failed to hatch at 38–40°C (Peng and Huang 1993). By comparing to these data, we conclude that the critical thermal maximum to *M. punctipennis* egg is above 39°C; to *T. molitor* egg is below 38°C. It means that the egg thermal tolerance of desert beetle *M. punctipennis* is much stronger than that of barn beetle *T. molitor*. In the field, we found that during the laying season of *M. punctipennis*, the temperature on the sand surface layer usually exceeded 40°C.

In conclusion, *M. punctipennis* egg shell is homogeneous and compact with no micropyles and aeropyles, which may help the egg to reduce water loss. Under dry conditions, the water loss rate of *M. punctipennis* egg was lower than the nondesert beetle *T. molitor*, and the egg survival rate was higher than *T. molitor*. In addition, the developmental threshold temperature for *M. punctipennis* egg was around 18.30°C and the effective accumulated temperature was ~80.45 degree days. The critical thermal maximum to *M. punctipennis* egg is above 39°C. Thus, *M. punctipennis* is a thermophilic insect. Burying eggs after oviposition might be another method for *M. punctipennis* to protect their eggs from desiccation. These features partly account for the adaptability of *M. punctipennis* to desert environment in egg stage.

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