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Hibernation Durations Affect Life-history Traits of *Gymnopleurus mopsus* (Coleoptera: Scarabaeidae), an Endangered Dung Beetle

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ABSTRACT

The dung beetle, *Gymnopleurus mopsus* (Coleoptera: Scarabaeidae), is one of endangered species in South Korea. It was last recorded in 1971. To restore this species, we introduced *G. mopsus* populations from eastern and southern regions of Mongolia in July 2019 and August 2019, respectively. One of the main tasks for the restoration of endangered insects is to develop breeding techniques to eventually incorporate these insects into the restoration system. In a series of laboratory experiments, we investigated effects of short -term hibernation periods on life-history traits of *G. mopsus*. Adult *G. mopsus* that had hibernated for 30, 60, and 90 days had lower survival rates than adults that had hibernated for 120 days. We also compared developmental time of these four experimental groups and found a significant difference in the egg - phase. However, the duration of hibernation did not affect the fecundity, brood-ball size, or body size of F1 adults. Follow-up studies are currently being conducted to further investigate the effect of a short-term hibernation period on population growth of *G. mopsus* under laboratory conditions.

Keywords: Captive breeding, Developmental time, Ecological restoration, Fecundity, Laboratory rearing

Introduction

Due to rapid industrialization and urbanization through the nineteenth to twenty-first centuries, global environment has changed significantly, leading to habitat fragmentations, nitrogen depositions, and climate changes (Butchart *et al*., 2010; Ceballos *et al*., 2017; Maxwell *et al*., 2016; Sánchez-Bayo & Wyckhuys, 2019). Human activities can also negatively affect threatened or ecologically important species in various environments. Threatened species worldwide are gradually disappearing because they are highly vulnerable to rapid changes in the ecosystem. Threatened species in the Red List of International Union for Conservation of Nature (IUCN) are important members for biodiversity. Vié *et al*. (2009) reported that of 44,837 species assessed in 2008, at least 17,038 (38%) were classified as threatened and 804 (1.8%) species were classified as extinct. Recently, over 130,000 species are on the Red List and almost 40,000 species have been classified as threatened (IUCN, 2021). In the Korea Red Data Book, 6.6% of threatened species were classified based on evaluation of the risk of extinction for targeted 8,037 species (National Biodiversity Center, 2020). Salomão *et al*. (2019) have demonstrated that the abundance of coprophagous species and species diversity indices of ball-rolling dung beetles are negatively affected by urbanization.

In the present study, we focused on *Gymnopleurus mopsus* Pallas, an endangered dung beetle widely distributed in grasslands and deserts throughout Eurasia (Kang *et al*., 2018). This species has gradually disappeared in many countries. In particular, there is a sharp decline in *G. mopsus* populations in the Iberian Peninsula (including Portugal and Spain) and Western European countries such as Italy and France (Carpaneto *et al*., 2007; Lobo, 2001; Sánchez-Bayo & Wyckhuys, 2019). In South Korea, it was one of the most dominant dung beetles before 1970 (Paik, 1976). After the last report of *G. mopsus* in 1971, it has not been reported in South Korea. The Ministry of Enviro-

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nment (2013) reported that populations of *G. mopsus* had declined rapidly owing to habitat destruction, a decrease in grazing livestock, and the abuse of chemicals such as pesticides. In addition, it is an important detritivore for livestock dung in pasture environments. Knowledge on the influence of abiotic factors such as hibernation, which is directly involved in the life cycle of *G. mopsus*, is an important part of the restoration process. Hibernation period is one of crucial factors for the reproduction and survival of *G. mopsus*. Thus, we focused on effects of hibernation periods on life-history traits of *G. mopsus* in the present study to determine whether the survival, fecundity, and offspring of *G. mopsus* could be affected by hibernation periods by comparing the survival, fecundity, larval developmental time, and body sizes of F1 adults under different hibernation periods.

Materials and Methods

Collection and introduction of dung beetles

In this study, we investigated a Mongolian species mainly because of its genetic homogeneity (Yoon *et al*., 2017). We collected a total of 200 *G. mopsus* adults from two grazing lands, Saikhandulaan (44°58′18.8″N, 110°05′53.5″E) and Khankhongor (43°39′37.91″N, 104°35′40.58″E) in Mongolia between July 2019 and August 2019. Dung beetles were collected by hand or forceps and placed into a plastic jar to prevent escape. These collected beetles were brought to the laboratory. Small invertebrates (e.g., acari species) attached to each dung beetle's body were removed using a brush. When importing *G. mopsus* colonies into South Korea, dung beetles should be inspected under strict quarantine processes in accordance with quarantine laws and procedures so that exotic pests are not included in the body of a dung beetle.

Dung beetle culture

A starter population of *G. mopsus* has been cultured at the National Institute of Ecology (Yeongyang, South Korea) since July 2019. Dung beetles were maintained in ventilated 60 L plast ic boxes with 40 L of artificial soil. Artificial soil was prepared by mixing river sand and vermiculite (Verminuri, Green Fire Chemicals, Hongseong, South Korea) at a 1:1 ratio. Fresh horse dung (300 g) was provided every 2 d as food. These food items were collected from a horse farm located on Jeju Island. We also checked dung food resource for the presence of pesticides and antibiotics. Distilled water was sprayed onto the artificial soil to maintain moisture content. Food items were stored at -24°C in a freezer for more than 8 d before use to minimize dung-borne pests. Eight dung beetles were reared in each plastic box. The culture was maintained under laboratory conditions at temperature of 25 ± 1°C [16:8 (L:D)] with a relative humidity of 45 ± 10%.

Hibernation

In September 2019, before the beginning of hibernation, dung beetles were sequentially exposed to low temperatures of 22 ± 1 °C [14:10 (L:D)], 20 ± 1 °C [12:12 (L:D)] and 18 ± 1 °C [10:14 (L:D)] for 14 d to adapt to low temperature conditions. After the adaptation process, dung beetles grown in plastic boxes were transferred to an incubator (MLR-352H, Panasonic, Japan) maintained at 5 ± 1 °C [0:24 (L:D)] with a relative humidity of 60 ± 10%. We began our experimental hibernation trials with a dung be etle colony of 32 dung beetles (sex ratio, 1:1) divided into four equal groups according to hibernation periods (30, 60, 90, and 120 d). After the hibernation course, dung beetles were re-transferred from the incubator to the laboratory. They were then sequentially exposed to high temperatures of 18 ± 1 °C [10:14 (L:D)], 20 ± 1 °C [12:12 (L:D)], and 22 ± 1 °C [14:10 (L:D)] for 14 d each in reverse order of the initial temperature exposure.

Survival and fecundity under different hibernation periods

This trial was conducted to investigate life history traits of *G. mopsus* under different hibernation periods with a focus on adult survival and fecundity. Fresh horse dung (150 g) was placed in a customized transparent plastic culture vessel (17.0 cm in diameter, 40 cm in height) covered with a ventilated nylon mesh (Fig. 1). Culture vessels were filled with artificial soil (2.5 L) that provided a habitat for dung beetles. Water was supplied adlibitum to an artificial soil to maintain soil moisture content. One pair of dung beetles raised on horse dung were placed in each culture vessel. To obtain eggs, we collected dung balls made by *G. mopsus* and buried them in the ground at one day after confirming the behavior of dung beetles making dung balls (Fig. 1). Data collection was terminated when dung beetles reached the adult stage.

Dung beetle life history traits: Larval development and morphological characters

This trial was conducted to investigate basic life history traits of *G. mopsus* feeding on horse dung with a focus on larval development and adult growth (body size). Frozen horse dungs were thawed at 25 °C for 8 h before use. Thawed horse dung was divided into 10 g and placed in a customized transparent plastic bottle (2.8 cm in diameter, 3.5 cm in height). One egg laid on a dung ball was placed in a plastic bottle filled with horse dung using a small spoon. When transferring an egg, a portion of the dung ball surrounding the egg was also transferred together. This is because the maternal gift made by adult females is

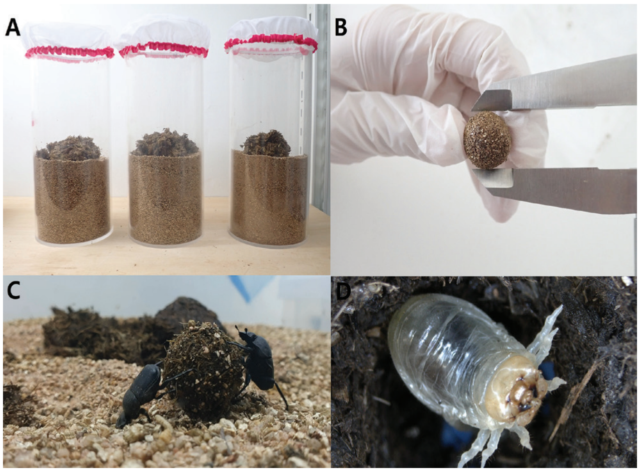

Fig. 1. Experimental design and *Gymnopleurus. mopsus*. A: Culture vessels, B: Dung chamber measurement, C: A pair of *G. mopsus* (left, female, right, male) with dung chamber, D: 3rd instar larvae of *G. mopsus*.

known to be an important factor for larval growth (Shukla *et al*., 2016). Daily monitoring was performed. The upper part of the dung chamber was artificially opened so that the inside of the plastic-dung bottle was visible for the observation of larval growth. In addition, we artificially re-opened the upper part of the dung chamber on each day of survey because when we opened the chamber, larvae always re-blocked the hole. We determined developmental time based on larval head width. Body length and thorax width were measured when dung beetle larvae became adults. Morphological traits of larval development and adult dung beetles were measured using a dissecting microscope connected to a digital camera (Tcapture 5.1.1 software, TrueChrom IIS, Tucsen Photonics Co., Ltd., China). In the case of larval development monitoring, we worked partly with 30-, 60-, and 90-d treatments. However, some parts of 60-, 90-d, and 120-d treatments were not monitored because larval mortalities were high in 30-, 60-, and 90-d treatment groups. We presumed that such high mortalities were caused by the daily artificial opening of the chamber. Data collection was terminated when dung beetles reached the adult stage.

Data analysis

Adult survival was assessed using the Kaplan–Meier method. A log-rank test was applied to test for significant differences among the four hibernation period groups. Sizes and weights of dung beetle-made brood balls and newly emerged adults and the duration of larval developmental time were analyzed using one-way ANOVA followed by Tukey's HSD tests. All data were evaluated using the Shapiro–Wilk W test and Levene's test for assumptions of normality and homogeneity, respectively. All statistical analyses were conducted using STATISTICA v. 8.0 (Statsoft, USA).

Results and Discussion

Effects of hibernation periods on adult dung beetle survival

In our study, the survival of hibernated *G. mopsus* adults was reduced by approximately 40% in 30-, 60-, and 90-d hibernation groups compared to that in the 120-d hibernation group (100% survival). However, no statistical difference was found because of a low number of replicates (log-rank test, p = 0.389, Fig. 2). Our results indicate that hibernation duration is an important factor for the survival of *G. mopsus*. This finding is consistent with previous studies showing that insects such as ladybugs, bees, and dung beetles need hibernation for their survival (Bang *et al*., 2004; Bosch & Kemp, 2003; Jean *et al*., 1990). Bosch and Kemp (2003) have reported that the survival of 30-d hibernated *Osmia lignaria* is lower than that of the group that has hibernated for longer periods of time. In addition, Bang *et al*. (2004) have found that larvae of the dung beetle (*Copris ochus*) without a hibernation are not developed and dead.

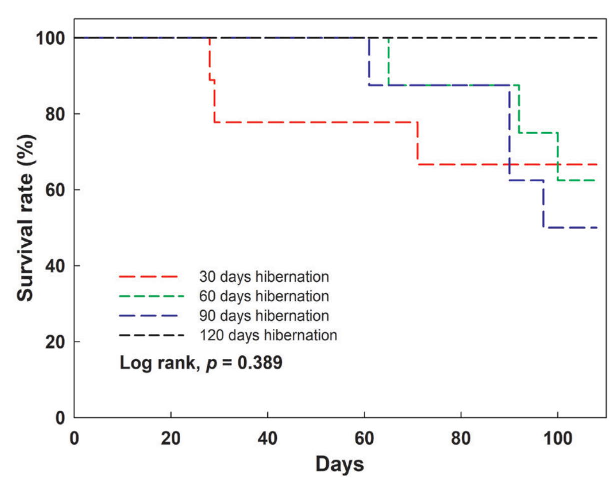

Fig. 2. Overall survival of *Gymnopleurus mopsus*, an endangered dung beetle, under different hibernation periods (30-d, red; 60-d, green; 90-d, purple; and 120-d, black). Data were derived from Kaplan-Meier curves (N = 8). Adult beetles surviving for more than 108 days are censored.

Effects of hibernation periods on dung beetle fecundity

The total number of eggs in the 30-d hibernation group was only 45%, 32%, or 62% of that in the 60-, 90-, or 120-d hibernation group, respectively. However, the difference was not significant owing to the low number of replicates (p = 0.086, Fig. 3).

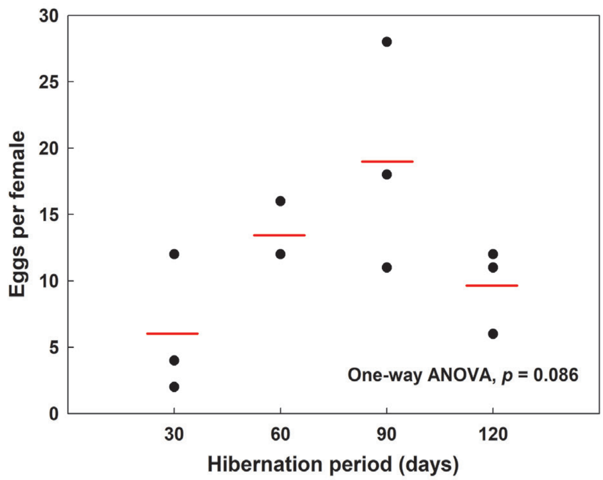

Fig. 3. Number of eggs per female of *Gymnopleurus mopsus* when hibernated at 5 ± 1°C for 30, 60, 90, or 120 d. Black circles represent individual values. Red vertical lines represent means (N = 3).

Many Coleopteran species are known to require hibernation during the winter season for ovarian maturation (Gittings & Giller, 1997; reviewed in Hodek, 2012; Sakurai *et al*., 1992). Sakurai *et al*. (1992) have reported that ovarian maturation of the ladybug (*Harmonia axyridis*) is rapidly induced during hibernation. Gittings and Giller (1997) have also demonstrated that two *Aphodius* dung beetle species (*A. depressus* and *A. erratticus*) do not have ovarian development before overwintering. Our preliminary study confirmed that non-hibernated *G. mopsus* adults did not lay eggs. In contrast, Jang *et al*. (2018) have reported that the fecundity of *Protaetia brevitarsis* is not affected by the duration of hibernation under laboratory conditions. In the case of *P. brevitarsis*, it overwinters as 3rd instar larvae, whereas *G. mopsus* overwinters as the adult. Therefore, these differences might be due to differences in species and growth stage.

Effects of hibernation periods on sizes of offspring

Brood-ball sizes (length, width, and weight) and adult sizes (body length, thorax width, and weight) were similar among the four hibernation treatment groups (Table 1). Tukey's HSD tests showed that the developmental time of the egg stage was significantly delayed in the 60-d treatment group compared to that in the 30- or 90-d treatment group. In the case of the 2nd instar, developmental time was significantly delayed after the 30-d treatment compared to that with the 60-d treatment. In addition, the duration of hibernation did not affect the developmental time in the 1st instar, 3rd instar, pupation, or egg to adult stages (Table 2). When considered as a whole (i.e., egg to adult), hibernation period had no effect on development time. Differences in developmental time in some developmental stages might be caused by other factors such as handling effects and diet quality rather than hibernation periods of their parents. However, this still clearly demonstrated the development time of each instar for *G. mopsus* from egg stage to adult stage. To the best of our knowledge, this is the first report of a detailed life history of *G. mopsus*.

Conclusions

In this study, the number of replicates was relatively low to determine significant differences between experimental groups. Most individuals of the dung beetle population collected from Mongolia were not included in this study. We hypothesized that the short hibernation period could have a negative effect on dung beetles. Moreover, shortening of the hibernation period could be a key to increase its rearing efficiency. Our laboratory experiment with hibernation periods, horse dung, and *G. mopsus* provides a useful design for the establishment of a recovery system and guidelines for an endangered dung beetle. Results of this study indicate that *G. mopsus* can develop on horse dung as an exclusive diet from eggs to adults. Our preliminary study showed that eight adults consumed approximately 300 g of horse dung every 2 d and made dung balls for oviposition. Therefore, *G. mopsus* can be reared successfully exclusively on horse dung prey. Effects observed under conditions of a short hibernation period might negatively affect the survival and fecundity of *G. mopsus*. These findings from the life history traits and the mode of hibernation of *G. mopsus* might provide important information for establishing successful breeding techniques for the restoration of endangered dung beetles.

Conflict of Interest

The authors declare that they have no competing interests.

Acknowledgments

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Table 1. Measurements of the external size and weight of brood balls and newly emerged adults of *Gymnopleurus mopsus* with different hibernation periods

Factors	30 days	60 days	90 days	120 days	*F*	*p*-value
Ball length (mm)	19.48±1.25 (13)	19.73±0.38 (36)	19.99±0.32 (57)	18.84±0.31 (28)	0.865	0.461
Ball width (mm)	17.38±0.86 (13)	17.51±0.30 (36)	18.28±0.27 (57)	17.09±0.21 (28)	1.631	0.185
Ball weight (g)	3.42±0.52 (13)	3.44±0.16 (36)	3.84±0.17 (57)	3.13±0.16 (28)	0.926	0.430
Adult body length (mm)	11.00±0.03 (3)	11.94±0.42 (11)	11.58±0.33 (22)	11.81±0.30 (14)	0.490	0.691
Adult thorax width (mm)	7.73±0.82 (3)	8.38±0.36 (11)	8.15±0.25 (22)	8.74±0.24 (14)	1.170	0.330
Adult weight (g)	0.14±0.01 (3)	0.20±0.03(11)	0.22±0.05 (22)	0.22±0.02 (14)	1.903	0.142

Values are presented as mean ± SE (N). N is the number of replicates. All factors were analyzed using one-way ANOVA.

Table 2. Time (days) spent as each instar of development for *Gymnopleurus mopsus* from egg to adult emergence.

Factors	30 days	60 days	90 days	120 days	*F*	*p*-value
Egg	4.7±0.3(9)^{a}	6.5±0.6(15)^{b}	4.1±0.2(15)^{a}	-	9.878	<0.001
1st instar	2.0±0.3(8)^{a}	2.0±0.3(13)^{a}	2.0±0.3(12)^{a}	-	0.0	1.0
2nd instar	4.9±0.5(7)^{b}	3.4±0.3(13)^{a}	3.8±0.4(12)^{ab}	-	3.148	0.058
3rd instar	11.0±3.3(4)^{a}	15.1±1.1(8)^{a}	16.3±2.9(3)^{a}	-	2.075	0.168
Pupation	18.5±(3)^{a}	20.5±3.0(4)^{a}	16.0(1)^{a}	-	0.633	0.569
Egg to adult	39.5±4.5(3)^{a}	41.0±0.9(12)^{a}	40.2±1.1(25)^{a}	40.3±2.0(15)^{a}	1.016	0.393

Values are presented as mean ± SE (N). N is the number of replicates. All factors were analyzed using one-way ANOVA. Values with the same letter are not significantly different (Tukey's HSD test, $p > 0.05$).

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