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Original research article

Oxygen Consumption as a Measurement of Diapause Termination in the Korean Native Bumblebee, *Bombus ignitus*

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Abstract Bumblebees are a commercially important pollinator of both agricultural crops and natural plants, particularly tomato, which require huge, buzzing pollinators. The artificial rearing of these bees is increasing to meet the growing demand, and breaking diapause is a critical element in this process. As the pre-diapause *Bombus ignitus* queen has a higher metabolic rate than that of the post-diapause *B. ignitus* queen, we hypothesized that there would be a difference in the oxygen consumption of the queen before and after wintering. Therefore, we measured the rate of oxygen consumption of *B. ignitus* queens to create a barometer for evaluating diapause termination. The O_2 uptake of *B. ignitus* queens was 39.0 μL/mg wt./min just after emergence and increased rapidly from 186.3 μL/mg wt./min at 6 h after emergence to 354.2 μL/mg wt./min one day after emergence. The rates of oxygen uptake of the queens during the first 6 days after emergence raised from 354.8 μL/mg wt./min at one day to a maximum level of 494.7 μL/mg wt./min in the 5-day-old queens, which corresponds to 3.4-fold increase from that just after emergence. When evaluating the oxygen consumption of queens that are presumed to have completed hibernation in the nature, we found that the oxygen consumption was 265.1~306.2 μL/mg wt./min, similar to that of the queen bees in this study the first day after hibernation. Therefore, we defined the standard for termination of dormancy of *B. ignitus* queen bees in the range of 250~300 μL/mg wt./min. Furthermore, our results indicate that oxygen consumption can provide important information for the indoor rearing of *B. ignitus*. During the wintering period, the oxygen consumption of the *B. ignitus* queen bees clearly increased as time passed, and the oxygen consumption value of the queen bees sampled at the 4th month of hibernation was 195.7 μL/mg wt./min, which was close to the oxygen consumption value at the end of hibernation. The queen bees, which were stored at 2.5°C for artificial wintering, showed the highest oxygen consumption value after the end of hibernation. Based on these findings, we concluded that oxygen consumption of the bumblebee can provide rare insight for artificially raising *B. ignitus* as a barometer for measuring diapause termination in this species.

Keywords Bumblebee, *Bombus ignitus*, Queen, Diapause, Oxygen uptake

INTRODUCTION

Bees are critical for the pollination of natural vegetation and crop plants, including fruits, natural products, vegetables, seed plants, consumable oil crops, garden blossoms, and significant search crops (Morandin and Winston, 2005; Greenleaf and Kremen, 2006; Winfree *et al*., 2007). Bumblebees are especially skilled at pollinating species in the family Solanaceae, including tomato and eggplant (Banda and Paxton, 1991; Free, 1993). In addition, bees are both generally assorted and abundant, with 16,325 species currently distinguished around the world (Michener, 2000). Commercially managed bees are available for pollination

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services, and are utilized in enormous business commercial fields, small gardens, and enclosures such as greenhouses and screen houses (Free, 1993; Dag and Kammer, 2001). The use of bumblebees for pollination in nurseries has become unavoidable, and the demand for bumblebees builds every year (Yoon *et al*., 2021).

Bombus bees are eusocial insects with short-lived colonies that are primarily found in humid or temperate regions of East Asia. Queens in the *Bombus* genus usually overwinter (enter diapause), while the workers and males perish in the pre-fall and early pre-winter, respectively. In the late winter, queens that have overwintered withdraw from hibernation. The queen develops a store of pollen, then lays her initial clump of eggs into the pollen mass after tracking down a reasonable site for the establishment of a colony. When the workers of the primary brood have emerged, they take over the foraging duties from the queen, after which she predominantly spends her energy laying eggs. In the pre-fall, numerous males and new queens are produced. Only the mated queens rest and emerge in the spring (Heinrich, 1979; Duchateau and Velthusis, 1988). Bumblebees customarily produce one generation each year. One of the vital stages in the year-round rearing of bumblebees is breaking diapause.

Diapause is the essential mechanism by which insects in temperate regions synchronize their life cycle with seasonal changes. Diapause might be succinctly characterized as a genetically modified, neurohormonally mediated, dynamic condition of low metabolic action during which morphogenesis stops or essentially slows down (Tauber *et al*., 1986; Dank, 1987). Furthermore, during the diapause stage in the improvement of specific animals, morphological development might be suspended or extraordinarily impeded (Andrewartha, 1952; Mansigh, 1971). The programming of diapause involves the development of specific behavioral, morphological, and physiological plans that interestingly prepare the diapause-destined insect for a time of formative capture (Denlinger, 2002). Oxygen consumption is considered a key element in these plans.

Oxygen consumption is impacted by several factors, such as movement, temperature, nutrition, body size, stages in the life cycle, season, time of day, and genetic background (Prosser and Brown, 1961). In insects whose oxygen consumption is modified regardless of the surrounding temperature, the flight strength can be modified to use the previous ride to elevate its temperature to the extent needed for flight (Heinrich, 1975; Abrol, 1986). Kammer and Heinrich (1974) showed that the oxygen consumption of a flying bee (*Bombus vosnesenskii*) is modified to 42~50 times greater than that of a resting bee at a thoracic temperature of 25°C, with a <two-fold enlargement in oxygen consumption for every 5°C growth in thoracic temperature in resting bees. Pre-diapause bumblebee queens display an improved metabolic fee compared to that of post-diapause queens, likely due to the build-up of fat reserves that are vital for hibernation (Beekman and Stratum, 1999). Evaluations of oxygen intake in bumblebees have primarily focused on flight pastime and frame temperature (Komai, 2001). Bumblebees consume less energy per g thorax than honeybees at a similar weight, and oxygen consumption increases less steeply with an extra burden in the mid-region (Wolf *et al*., 1989). The oxygen consumption of bumblebees depends strongly on movement level and surrounding temperature (Crailsheim *et al*., 1999). Oxygen consumption of dynamic bees fluctuates broadly depending on encompassing temperature and level of action; however, it does not contrast among foragers (>18 days) and middle-aged honeybees (7~10 days) (Stabentheiner *et al*., 2003).

Oxygen intake of prepupae seven days prior to reaching the duvet segment of *Megachile rotundata* averages 20% that of those efficiently looking after or turning structures, and is usually dissimilar to the O_2 intake stages of prepupae misleadingly adapting at 14°C fourteen days after the fact, and from that discovered in prepupae which have completed seven months of wintering at a constant 4°C (Kemp *et al*., 2004). The U-formed oxygen intake curve and static weights of the wintering prepupae of *M. rotundata* is similar to that in other Hymenoptera (Kemp *et al*., 2004). *Osmia lignaria*, which overwinters as adult, exhibits stepwise increases in oxygen consumption and continuous weight loss throughout the wintering period. This species also displays proportionally greater oxygen consumption levels during the pre-wintering and wintering periods (Kemp *et al*., 2004). In bumblebees, oxygen consumption per muscle potential is two-fold higher in rambles than that in workers. However, oxygen consumption for warming the thorax is relatively similar in workers and drones.

Thoracic temperature influences the amount of oxygen consumed per muscle potential (Goller and Esch, 1991).

Oxygen consumption in insects is influenced by numerous variables. In particular, as the pre-diapause *B. ignitus* queen has a higher metabolic rate than that of the post-diapause *B. ignitus* queen, we hypothesized that there would be a difference in the oxygen consumption of the queen before and after wintering. In addition, we theorized that the oxygen consumption of the queen bee could be used as an indicator of the end of hibernation. To verify this hypothesis, we investigated the change in oxygen consumption of *B. ignitus* queens with the passage of time immediately after hibernation.

MATERIALS AND METHODS

1. Origin of experimental insects

This study was carried out over three consecutive years (2018~2020) using a total of 897 bumble bee queens, 731 of which were mated while the other 166 were virgins. Queens were collected from 14 different colonies (4 colonies in 2018, 4 in 2019, and 6 in 2020). The insects utilized in the experiment were second and third generation queens acquired from *B. ignitus* colonies that were reared year-round in a climate-controlled room (27°C, 65% relative humidity, and continuous darkness) at the Division of Applied Entomology, Department of Agricultural Biology, National Academy of Agricultural Science, Republic of Korea.

2. Indoor rearing

The basic colony-rearing technique used in this study followed that described by Yoon *et al*. (2002). The queens were reared in three types of plastic boxes for nest initiation ($10.5 \times 14.5 \times 6.5$ cm^3), colony foundation ($21.0 \times 21.0 \times 15.0$ cm^3), and colony maturation ($24.0 \times 27.0 \times 18.0$ cm^3). Queens were first restricted separately to small boxes for colony initiation and remained there until oviposition. To stimulate egg laying, two narcotized *B. ignitus* workers matured 10~20 days after development were added to each box with a queen (Yoon and Kim, 2002). When the adults emerged from the first brood, the nest was moved to a medium box for colony foundation and remained there until the number of workers present reached 50. The nest was then moved to a large box for additional colony development. A 40% sugar solution with 0.3% sorbic acid and pollen dough were provided ad libitum (Yoon *et al*., 2005). The pollen dough was made from a sugar solution and pollen (v : v = 1 : 1).

3. Measurement of oxygen uptake

To create a barometer for measuring diapause termination, we estimated the rate of oxygen utilization of *B. ignitus* queens. The oxygen take-up method utilized in the present study was similar to that previously described by Yoon and Kim (2004). The oxygen uptake of *B. ignitus*, *Bombus ardens*, and *B. terrestris* queens was estimated in a volumetric system with an O_2 Up tester (Daiyo Scientific Industrial Co., Tokyo, Japan). Both hibernated and non-hibernated bumblebee queens were utilized for this investigation. Individual queens were placed in the 20 mL main chamber of each of the vessels. CO_2 was absorbed into a strip of filter paper saturated with 0.5 mL 20% potassium hydroxide, with the queen separated from the absorbing agent by a porous polyethylene membrane. The oxygen uptake tester was set in a room maintained at 24~25°C, and the chamber containing the queen was submerged in a 25°C water bath. Readings were taken every 10 min for 1 h. All the queens were maintained at 25°C until transferred for measurement. In the case of the chilled queens, O_2 uptake was measured 1 h after the queens were transferred to 25°C. Oxygen uptake was expressed as μL/mg body wt./min. Each reported oxygen uptake value represents the mean of 10 replicates.

4. Changes in the O_2 uptake of *B. ignitus* queens during the first day after emergence

To explore the progression in O_2 uptake of *B. ignitus* queens during the day immediately after emergence, we utilized second-generation *B. ignitus* queen which had been stored at 5°C for 4 months. O_2 uptake was measured at 1 h after transfer to 25°C. Oxygen measurements were carried out every 3 h for a total of 24 h; each value for the oxygen uptake is the mean of three *B. ignitus* queens, replicated three times per queen. A regression analysis was performed to confirm the relationship between changes in the O_2 uptake value of *B. igni-*

tus queens and the number of hours after emergence.

5. Changes in the O_2 uptake of *B. ignitus* queens during the first 6 days following emergence

To investigate the change in O_2 uptake for 6 days after emergence, the O_2 uptake of second-generation *B. ignitus* queens that had been stored at 5°C for 4 months was measured every day at 10 am using a similar test technique to that used in section 2.3. Each value for the oxygen uptake is the mean of three *B. ignitus* queens, replicated ten times per queen. A regression analysis was performed to confirm the relationship between the changes in the O_2 uptake value of *B. ignitus* queens and the time after emergence.

6. O_2 uptake of queen bees after diapause

To explore the O_2 uptake value of queen bees after diapause, we collected hibernated *B. ignitus* and *B. ardens* queens in springtime from the Jeongseon-gun region, Kangwon-do, Korea (37°19′47″N, 128°47′09″E). *B. terrestris* queens stored for 4 months at 5°C were utilized as a hibernated control. Oxygen uptake was measured at 25°C with 10 replications each per queen.

7. Changes in the O_2 uptake value of the queen bees under various conditions

To evaluate the effects of the timing of cold temperature application on diapause break in *B. ignitus* queens, the following environmental conditions were established. The hibernation conditions were a constant temperature of 5°C and 80% humidity. After mating (Yoon *et al*., 2008), the mated *B. ignitus* were individually preserved in bottles filled with perlite in a perforated plastic box containing perlite to prevent mold growth and stored for 4 months in a different chilling chamber under continuous darkness. The O_2 uptake of the queens was measured each month. Thirty queens were utilized in this trial. Due to the demise of the queen during hibernation, 30 queen bees were measured at 1 month, 20 queen bees at 2 months, 15 queens at 3 months, and 10 queens at 4 months. A regression analysis was performed to elucidate the relationship between the wintering period and the O_2 uptake value of the bees.

To inspect the impacts of chilling temperature on the O_2 uptake of *B. ignitus* queens, the following environmental conditions were established. The chilling temperature systems were 0°C, 2.5°C, 5°C, and 7°C under a constant humidity at more than 80%. After hibernation for 4 months following the exploratory technique described above, O_2 uptake was estimated for each temperature of hibernation at 1 h after of the queen from 5°C to 25°C.

To analyze changes in the O_2 uptake of *B. ignitus* queens in different mating states, mated and non-mated bees were hibernated per the above technique for 4 months. The O_2 uptake of each mated and non-mated queen was then measured, with 10 replicates per queen.

To compare the O_2 uptake of *B. ignitus* queens in different hibernation locations, we hibernated *B. ignitus* queens both outside of and inside soil (Yoon *et al*., 2004). The time, temperature, and period of chilling treatment of the *B. ignitus* queens were one month after mating, 5°C, and 4 months, respectively. Oxygen uptake was measured at 25°C, with 10 replicates per queen.

To compare the O_2 uptake of *B. ignitus* queens under CO_2 treatment, a CO_2 narcosis group was exposed to almost 99% CO_2 for 30 min daily during two consecutive days in a flask. The time, temperature, and period of chilling treatment of the *B. ignitus* queens were one month after mating, 5°C, and 3 months, respectively. Oxygen uptake was measured at 25°C for both the CO_2 narcosis and non-treatment groups, with 10 replicates per queen.

8. Statistical analysis

A t-test was performed to compare the O_2 uptake of *B. ignitus* queens under different mating status, hibernation locations, and CO_2 treatments. The effect of different hibernation temperatures on the O_2 uptake value were analyzed using a one-way ANOVA test. Significant differences identified by the ANOVA and Tukey's test were used for post-hoc analysis. In the correlation analysis, after performing Pearson correlation analysis, the regression equation was derived by regression analysis if a significant correlation was confirmed. All statistical analysis was performed using the SPSS PASW 22.0 package for Windows (IBM, Chicago, IL, USA).

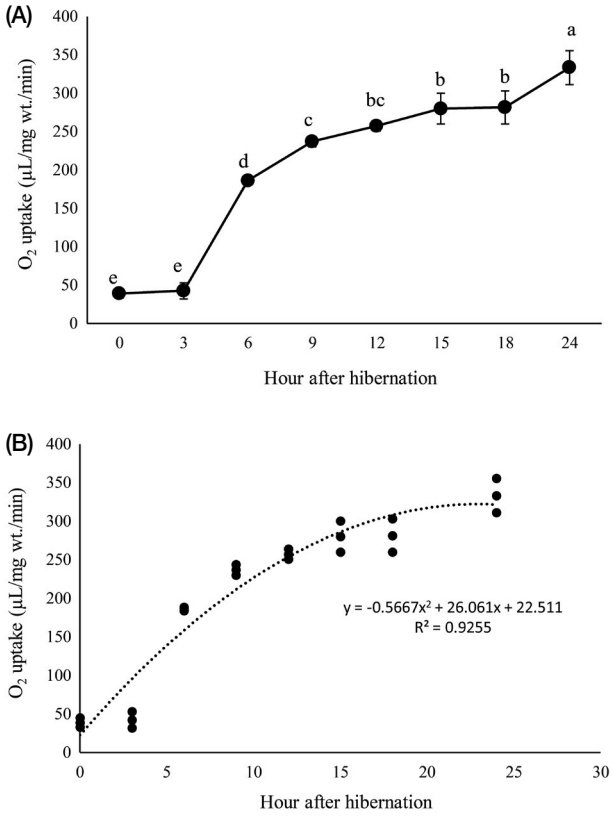

Fig. 1. Changes in the O_2 uptake of *B. ignitus* queens during the first day after hibernation (A); regression analysis between the O_2 uptake value and hour after hibernation (B). In (A), Different letters indicate significant differences among a particular hour after hibernation based on the one-way ANOVA and Tukey's honestly significant difference results ($p<0.05$).

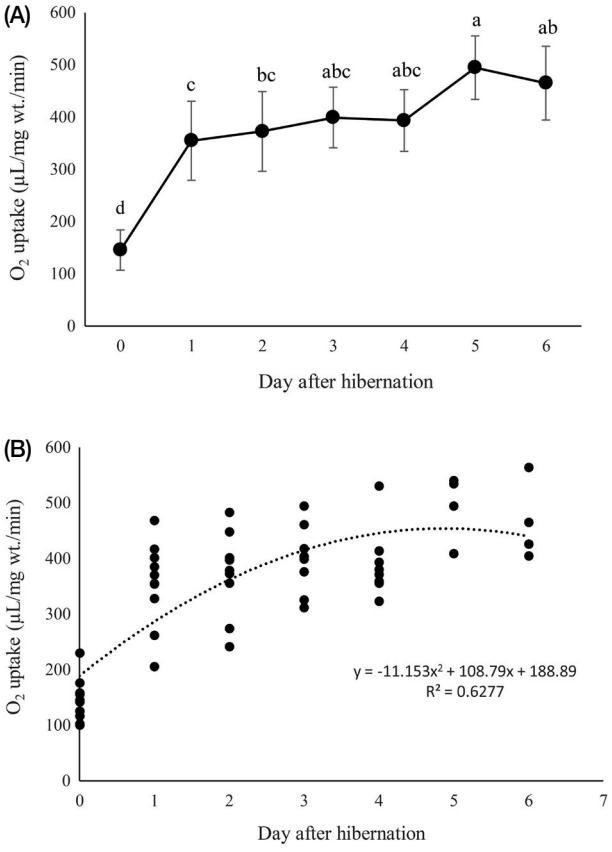

Fig. 2. Changes in O_2 uptake of queens of *B. ignitus* during 6 days from just after hibernation (A), and regression analysis between O_2 uptake value and day after hibernation (B). In (A), Different letters indicate significant differences among days from just after hibernation based on the one-way ANOVA and Tukey's honestly significant difference results ($p<0.05$).

RESULTS

1. Changes in the O_2 uptake of *B. ignitus* queens during the first day after emergence

The O_2 uptake values of the *B. ignitus* queens showed a significant difference as the time post-hibernation increased (one-way ANOVA test: $F_{7, 16}=184.663$, $p=0.0001$; Fig. 1A). Specifically, following hibernation and 3 h after hibernation showed a similar level at 39.1~42.5 μL/mg wt./min. However, this increased significantly to 4.4 times that level at 6 h (186.3 μL/mg wt./min) after hibernation. From that point onward, the highest O_2 uptake value was at 24 h (333.3 μL/mg wt./min). A significant positive correlation was confirmed between the hour after hibernation and the O_2 uptake value ($r=0.914$, $p=0.0001$). Based on this, a significant second order regression equation was determined as follows: $y=-0.5667x^2+26.061x+22.511$, ANOVA test: $F_{2, 21}=130.521$, $p=0.0001$, $R^2=0.9255$, $DW=0.678$ (Fig. 1B).

2. Changes in the O_2 uptake of *B. ignitus* queens during the first 6 days after emergence

The O_2 uptake value of the *B. ignitus* queens showed a significant difference with day ($F_{7, 16}=184.663$, $p=0.0001$; Fig. 2A). Immediately following hibernation until 24 h after emergence, the O_2 uptake value increased rapidly by 2.4 occasions from 145.4 to 354.8 μL/mg wt./min. From the first day after hibernation, the O_2 uptake value repeatedly increased and decreased within the range of 350~450 μL/mg wt./min. A signifi-

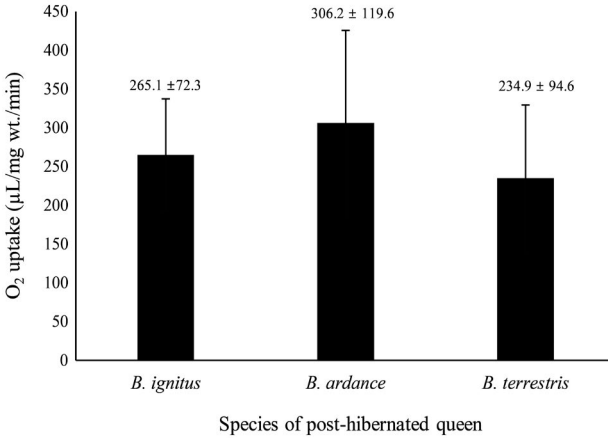

Fig. 3. Comparison of O_2 uptake of different species of bumblebee queens which were ended a hibernation. The hibernated *B. ignitus* and *B. ardens* queens were collected in springtime. *B. terrestris* queens stored for 4 months at 5°C were utilized as a hibernated control. There was no statistically significant difference in O_2 uptake value between the different species of bumblebee.

cant positive correlation was confirmed between the day after hibernation and the O_2 uptake value of the *B. ignitus* queens ($r=0.792$, $p=0.0001$). In view of this, a significant second order regression equation was derived as follows: $y=-11.153x^2+108.79x+188.89$, ANOVA test: $F_{2,52}=43.842$, $p=0.0001$, $R^2=0.628$, $DW=1.300$ (Fig. 2B).

3. O_2 uptake of the queen bees after diapause

Fig. 3 shows the results of contrasting the O_2 uptake value for each species of bumblebee queen after hibernation. The O_2 uptake values for the *B. ignitus* and *B. ardens* queens, which were collected in spring and were assessed to have ended hibernation, were 265.1 and 306.2 μL/mg wt./min, respectively. The O_2 uptake value for the *B. terrestris* reared indoors was 234.9 μL/mg wt./min, which was slightly lower than that of the other two species of bees. However, there was no statistically significant difference in the O_2 uptake values of the different species of bumblebee ($p>0.05$). Considering the O_2 uptake values according to time after hibernation and those of bumblebees post-hibernation in nature, we determined that a *B. ignitus* queen bee is awakened from hibernation when it shows a O_2 uptake value of 250~300 μL/mg wt./min.

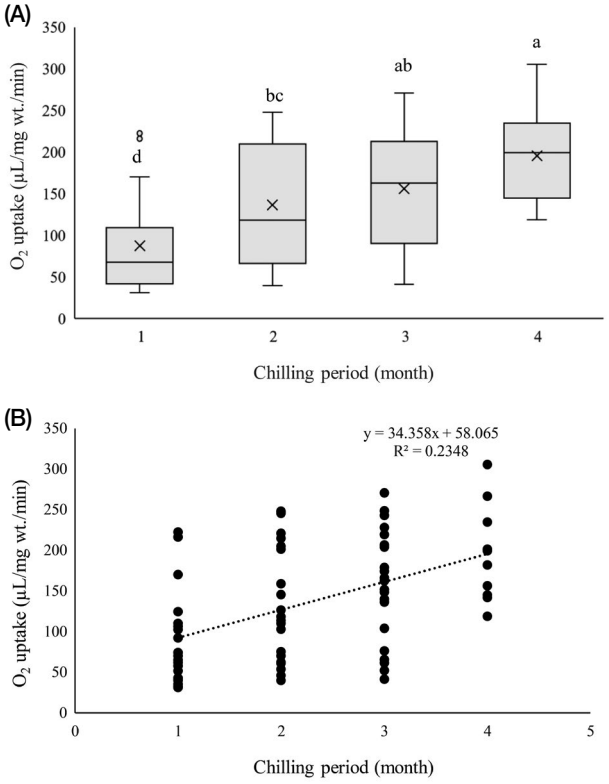

Fig. 4. Change of O_2 uptake of *B. ignitus* queens by chilling period (A); regression analysis between the O_2 uptake value and chilling period (B). In (A), different letters indicate significant differences among chilling periods based on the one-way ANOVA and Tukey's honestly significant difference results ($p<0.05$). Gray boxes indicate the interquartile range, the bold line in the boxes indicates the median, x is the mean, and empty cycles are the outliers.

4. Changes in the O_2 uptake values of queen bees under various conditions

1) Examination of the O_2 uptake of *B. ignitus* queens by chilling period

To affirm the properties of the O_2 uptake of queen bees in the life cycle of *B. ignitus*, we investigated the O_2 uptake value of the queen bees during the chilling period. The O_2 uptake value of the *B. ignitus* queens showed a significant difference between the different chilling periods ($F_{3,69}=7.363$, $p=0.0001$; Fig. 4A). The longer the chilling period, the greater the O_2 uptake of the *B. ignitus* queens ($r=0.485$, $p=0.0001$). At 4 months after hibernation, the O_2 uptake value was 195.7, over two times that immediately after hibernation. Thus, a significant first-order regression equation was derived between the chilling period and O_2 uptake values of the *B. ignitus* queens: $y=34.358x+58.065$, ANOVA test:

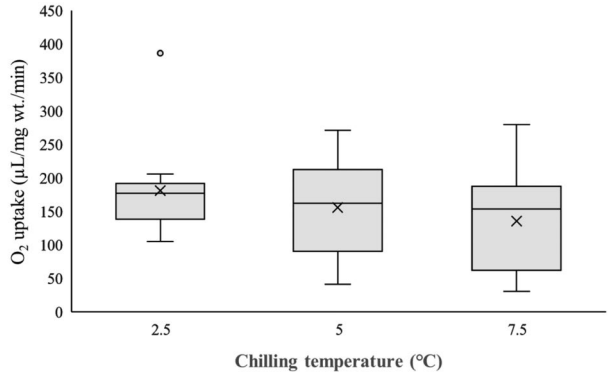

Fig. 5. Comparison of the O_2 uptake of *B. ignitus* queens at different chilling temperatures. Gray boxes indicate the interquartile range, the bold line in the boxes indicates the median, x is the mean, and empty cycles are the outliers. There was no statistically significant difference in the O_2 uptake values at different chilling temperatures.

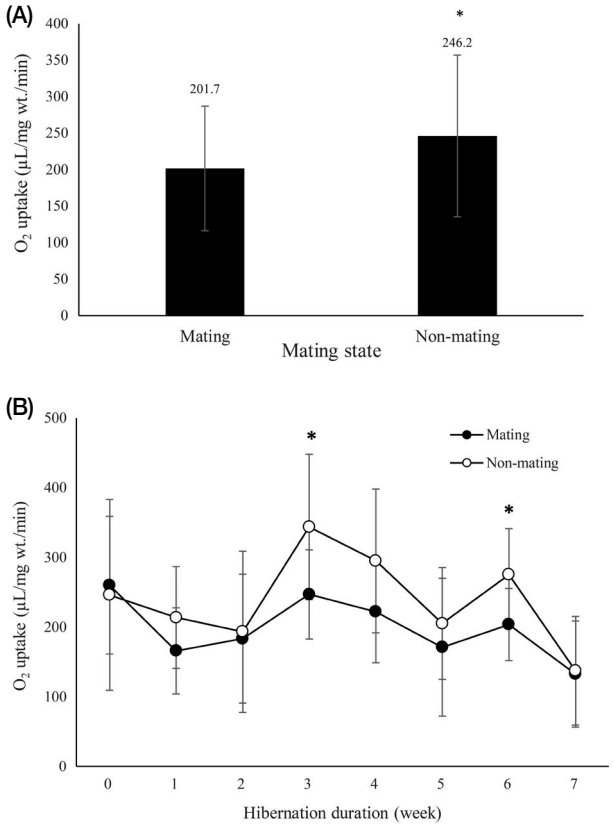

Fig. 6. Comparison of the O_2 uptake between mated and non-mated *B. ignitus* queens (A); changes in the O_2 uptake of mated and non-mated *B. ignitus* queens over 7 weeks following hibernation (B). * indicates that the data were significantly different according to a t-test ($p<0.05$).

$F_{1,71}=21.785$, $p=0.0001$, $R^2=0.235$, $DW=0.531$ (Fig. 4B).

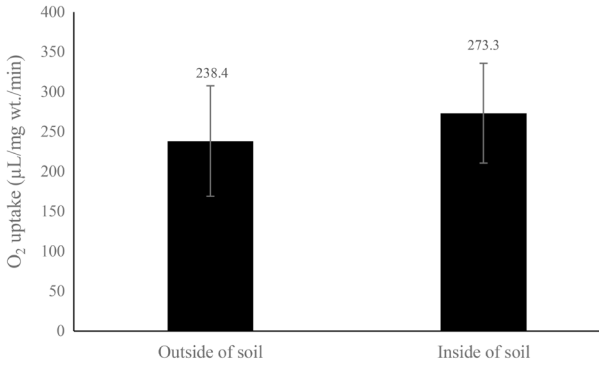

Fig. 7. Comparison of the O_2 uptake of *B. ignitus* queens in varying hibernation locations. The location of hibernation of queens was a mating cage in a field net house. The time, temperature, and period of chilling treatment of the *B. ignitus* queens were one month after mating, 5°C, and 4 months, respectively. Oxygen uptake was measured at 25°C with 10 replicates per queen. There was no statistically significant difference in the O_2 uptake values between the hibernation locations.

2) Examination of the O_2 uptake of *B. ignitus* queens at different chilling temperatures

The O_2 uptake of *B. ignitus* queens at different chilling temperatures is shown in Fig. 5. There was no significant difference in the O_2 uptake value of the queens at the different chilling temperatures ($p>0.05$). Nevertheless, the O_2 uptake value of the queens at 2.5°C, the lowest chilling temperature, was 1.2 and 1.3 times higher than those at 5°C and 7.5°C, respectively (Fig. 5).

3) Examination of the O_2 uptake of *B. ignitus* queens with different mating statuses

The mated *B. ignitus* queens showed a significantly greater respiration rate of 1.2 times that of the non-mated *B. ignitus* queens (t-test: $t_{153}=-2.787$, $p=0.006$; Fig. 6A). The non-mated queens showed a higher O_2 uptake value than the mated queens during the chilling period (Fig. 6B); specifically, the O_2 uptake value of the non-mated queens (3rd week: 343 μL/mg wt./min; 6th week: 275.3 μL/mg wt./min) was 1.4 times greater than that of the mated queens at the 3rd (246 μL/mg wt./min) and 6th (275.3 μL/mg wt./min) week of wintering (3rd week: $t_{16}=-2.532$, $p=0.020$; 6th week: $t_{16}=-2.516$, $p=0.023$).

4) Examination of the O_2 uptake of *B. ignitus* queens in different hibernation locations

Fig. 7 shows the O_2 uptake of the *B. ignitus* queens in different hibernation locations. There was no signif-

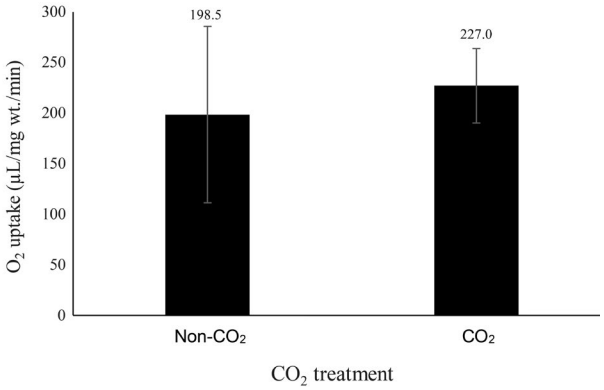

Fig. 8. Oxygen uptake of chilled *B. ignitus* queens after CO_2 treatment. The CO_2 narcosis-treated queens were exposed to 99% CO_2 for 30 min daily during two consecutive days in a flask. The time, temperature, and period of chilling treatment of the *B. ignitus* queens were one month after mating, 5°C, and 3 months, respectively. Oxygen uptake was measured at 25°C with 10 replicates per queen. There was no statistically significant difference in the O_2 uptake values between the control and CO_2 treatments.

icant difference in the O_2 uptake values of the *B. ignitus* queens between the different hibernation locations ($p > 0.05$). Overall, the O_2 uptake values of the queens were 10% higher in the within soil group than those in the external soil group (Fig. 7).

5) Examination of the O_2 uptake of chilled *B. ignitus* queens after CO_2 treatment

We investigated whether the O_2 uptake value of the queens is changed by CO_2 narcosis treatment, which influences the diapause cycle of the bumblebee queen (Fig. 8). However, no significant difference in O_2 uptake value could be confirmed. Nevertheless, the O_2 uptake value of the queen bee group treated with CO_2 narcosis was 10% higher than that of the non-CO_2 treatment group.

DISCUSSION

Oxygen consumption in insects is influenced by several variables, such as activity, temperature, nutrition, body size, stages in the life cycle, season, time of day, and genetic background (Prosser and Brown, 1961). In particular, as the pre-diapause *B. ignitus* queen has a higher metabolic rate than that of the post-diapause *B. ignitus* queen, we hypothesized that there would be a difference in the oxygen consumption of the queen before and after wintering. In addition, we theorized that the oxygen consumption of the queen bee could be used as an indicator of the end of hibernation. To verify this hypothesis, we investigated the changes in oxygen consumption of *B. ignitus* queens with the passage of time immediately after hibernation. The oxygen consumption of the queen increased by more than 4 times within 6 h after hibernation (Fig. 1) and showed a tendency to slightly increase from the 1st to the 6th day immediately after hibernation. However, it repeatedly increased and then decreased within 350~450 μL/mg wt./min during this period (Fig. 2). Thus, we determined that hibernation was terminated within the range of 237~282 μL/mg wt./min within 1 day of hibernation. By comparing oxygen consumption using bumblebee queens that are presumed to be post-hibernation in nature, we found that the oxygen consumption was 265.1 ± 72.3~306.2 ± 119.6 μL/mg wt./min, similar to that of the experimental queen bee after the first day post-hibernation (Fig. 3). Considering these results, we defined the standard for termination of dormancy of the queen bee of *B. ignitus* in the range of 250~300 μL/mg wt./min. This result can provide important information for indoor rearing of *B. ignitus*. In particular, when terminating hibernation of a rearing queen for oviposition in artificial wintering, it can be used as a marker to determine whether they have properly terminated hibernation. *B. ignitus* queens that do not wake up from diapause do not oviposition and therefore cannot form a normal colony (Beekman and Van Stratum, 2000; Yoon *et al*., 2010). In addition, we derived two regression equations between the time since the end of hibernation and oxygen consumption in the above experiment. By applying the regression equation for hour after hibernation and oxygen consumption, it is possible to estimate the appearance time of the queen after overwintering with a high probability of over 90% ($R^2 = 0.93$) in a queen that has been post-hibernation for less than 1 day. In addition, the age of the queen within 7 days after the end of hibernation can be estimated with a 63% probability ($R^2 = 0.628$) using the regression equation for days after wintering and oxygen consumption. This result can be used for determining the appropriate timing for the treatment process for colony formation by the queen after hibernation and adjusting the oviposition time of the queen.

In addition to this, oxygen consumption can provide various information necessary for the indoor rearing of *B. ignitus*. First, it is possible to obtain information on the appropriate hibernation period required for artificial rearing. During the wintering period, the oxygen consumption of the *B. ignitus* queen bees clearly increased as time passed (Fig. 4), and the oxygen consumption value of the queen sampled during the 4th month of hibernation was 195.7 μL/mg wt./min, which was close to the oxygen consumption value at the end of hibernation. This indicates that the queen bee is ready to end hibernation soon after more than 4 months of wintering. In addition, the appropriate oxygen consumption for each month during the hibernation period can be defined as 60~114 μL/mg wt./min for 1 month, 103~170 μL/mg wt./min for 2 months, 125~188 μL/mg wt./min for 3 months, and 157~234 for 4 months. We obtained a significant first-order regression equation from this result. Although the prediction probability (R^2=0.235) is not high for this equation, the hibernation time of the queen can be judged by the oxygen consumption value. Second, it informs the effective storage temperature for the queen bee during artificial hibernation (Fig. 5). The queens, which were stored at 2.5°C for artificial wintering, showed the highest oxygen consumption values after the end of hibernation. This may be taken as one piece of evidence indicating that the optimum diapause temperature for *B. ignitus* queen bees is 2.5°C (Yoon *et al*., 2013). Third, the effect of CO_2 narcosis, which can replace the dormant process, was confirmed. CO_2 narcosis is an important indoor rearing technique that allows bumblebee queens to form colonies without hibernation (Yoon and Lee, 2014). The queens treated with CO_2 narcosis in this study showed higher oxygen consumption than that of the non-CO_2 treated queens, and the O_2 consumption values were close to the oxygen consumption standard at the end of hibernation defined in this study (Fig. 8). Therefore, it is possible to determine whether the CO_2 narcosis treatment was performed correctly by measuring the amount of oxygen consumption.

In addition, the oxygen consumption of *B. ignitus* provides interesting information on the life cycle of this species. For example, wild *B. ignitus* queens mainly hibernate in the ground (Beekman *et al*., 1998; Yoon *et al*., 2010); in our results, the *B. ignitus* queen bees that wintered inside the soil also exhibited slightly higher oxygen consumption than that of the queen bees that wintered outside the ground (Fig. 7). These results show that the more advantageous wintering site for *B. ignitus* is underground. In addition, the non-mated queen bees exhibited increased oxygen consumption compared to that of the mated queen bees during the dormancy period we determined for the end of diapause (Fig. 6). Even during hibernation, the oxygen consumption of the non-mating queen bee was ≥300 μL/mg wt./min, indicating a higher value than the oxygen consumption standard for the end of dormancy. Thus, an unmated queen does not overwinter properly. This result indicates that a queen which failed to mate in the fall could be eliminated naturally due to poor diapause (Baer and Schmid-Hempel., 2005; Bogo *et al*., 2017).

We defined the criteria for determining whether hibernation is complete by measuring the oxygen consumption of *B. ignitus* queens. In addition, we confirmed that the measurement of oxygen consumption can provide various information regarding the process leading to hibernation and oviposition of the queen. Our oxygen consumption standards can confirm whether diapause is required for rearing *B. ignitus* queens more quickly and conveniently than the current methods of dissection or genetic markers. We are confident that our results can provide an important key to the use of domestic bumblebees as commercial pollinators by improving indoor artificial rearing. However, it is difficult to perfectly determine the termination of hibernation of bees based solely on the oxygen consumption. In general, hibernation and the termination of hibernation in insects are caused by the interaction of various genes, proteins, and hormones related to environmental factors such as temperature, illuminance, and humidity (Doorn, 1989; Beekman *et al*., 1998; Yoon *et al*., 2004). Therefore, in future studies, it is necessary to verify the correlation with various markers (the number of related genes, proteins, or hormones) related to hibernation when the oxygen consumption changes after the end of hibernation. Such studies will help appropriately determine whether evaluating the termination of hibernation using oxygen consumption is physiologically valid. In addition, when the dormancy of wild *B. ignitus* is delayed due to climate change such as global warming or an air tempera-

ture rise during hibernation occurs, it will be possible to expand the respiration volume modeling study for various physiological changes.

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