

Breeding of the Korean Native Bumblebee, *Bombus ignitus* Queens

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Running title : Breeding of the bumblebee *Bombus ignitus*

Abstracts

Many bumblebee species have declined in number in recent decades, particularly in developing regions. Widespread declines of bumblebee species threaten the pollination levels of both wildflowers and crops. Here, we investigated the body weight and colony-developmental characteristics of Korean native bumblebee (*B. ignitus*) queens collected from 2000 to 2010 for conservation of native bumblebees for breeding. The average weight of 6,852 queens was 0.77 ± 0.44 g. The weight of *B. ignitus* queens collected in 2005 was the greatest, 0.87 ± 0.12 g, which was 1.0–1.3-fold heavier than any other year. The average oviposition rate was $81.6 \pm 10.7\%$, and 2004 showed the highest rate, 95.0%. This value corresponded to 1.1-1.6-fold increases over the queens collected in the other years. The average rate of colony foundation was $60.9 \pm 11.0\%$. Queens in 2008 exhibited the best performance, 75.4%, which was 1.0-1.9-fold higher than the other years. The rate of progeny-queen production averaged $27.0 \pm 9.4\%$ and peaked in 2001 at 43.2%; this value was 1.1–4.7-fold higher than other years. The average number of queens produced and number of generations begotten by queens was $27.6 \pm 10.1\%$ and 4.8 ± 2.0 , respectively. Queens in 2000 averaged 9 generations of offspring, which was 1.1-3.1-fold greater than other years. These results indicate that the colony-developmental characteristics of the collected queens changed significantly between 2000 and 2010. In addition, there was no correlation between body weight and number of queens collected, although body weight was affected by collection year. Since 2008, the colony-developmental characteristics of queens have worsened.

Key words: Bumblebee, *Bombus ignitus*, Breeding, Colony development, Generation.

Introduction

Bees are diverse and abundant, with 16,325 species identified throughout the world (Michener, 2000). Arguably, the most important activity of bees is their pollination of natural vegetation and agricultural plants. Pollination is an ecosystem service in that wild pollinators, in particular wild bees, contribute significantly to the pollination of a large array of crops (Morandin and Winston, 2005; Greenleaf and Kremen, 2006; Winfree *et al.*, 2007). Commercially managed bees are also available for pollination services and are used in large commercial fields, small gardens, or enclosures such as greenhouses and screen houses (Free, 1993; Dag and Kammer, 2001).

The introduction of bumblebees into greenhouses for pollination has become widespread in recent years, and demand increases annually. Bumblebees provide farmers the opportunity to decrease their pollination labor costs and promise a good crop yield, both in quantity and in quality (reviewed by Velthuis and van Doorn, 2006). It has been estimated that the bumblebees sold in 2004 consisted of approximately 930,000 colonies of the Eurasian *Bombus terrestris*, approximately 55,000 colonies of the North American *B. impatiens*, and a few thousand colonies each of the Eurasian *B. lucorum*, East Asian *B. ignitus*, and North American *B. occidentalis* (Velthuis and van Doorn, 2006). The large bumblebee *B. terrestris* is naturally distributed in Europe and adjacent territories, including England, most of Scotland, the north coast of Africa, southern Scandinavia, major Mediterranean islands, and some Atlantic islands (Madeira and the Canary Islands) (Estoup *et al.*, 1996; Chittka *et al.*, 2004; Velthuis and van Doorn, 2006). Since 1987, *B. terrestris* has been available commercially in portable boxes for crop pollination (Mitsuhashi, 2000). Colonies of *B. terrestris* have

been imported into many countries, including Korea, Japan, China, Taiwan, Mexico, Chile, Argentina, Uruguay, South Africa, Morocco, and Tunisia (Dafni, 1998).

It has been attempted to substitute Korean native bumblebees for foreign bumblebees. Out of seven Korean native bumblebees tested, *B. ignitus* showed the best results both in artificial multiplication and in pollinating ability, and it was selected as the most reliable native species for crop pollination (Yoon *et al.*, 1999). In this study, we examined the breeding characteristics of *B. ignitus* queens that had been collected from 2000 to 2010 in various locations in Korea under indoor rearing for conservation of native bumblebees.

Materials and Methods

Collection of post-hibernated queens

The post-hibernated queens of the Korean native bumblebee, *Bombus ignitus*, visiting mainly the blossom of *Corydalis speciosa* (Fumariaceae) and *Prunus yedoensis*, were collected in many localities, including Jeong-Sun, Gang-Won do, Korea, during the spring seasons of 2000 to 2010. The number of bumblebees collected in the spring seasons of 2000 to 2010 was 713, 170, 80, 865, 949, 404, 1,147, 713, 407, 752 and 653, respectively. The collected *B. ignitus* queens were weighed before the start of indoor rearing.

Indoor rearing

The collected queens were reared in three types of cardboard (1.5 mm thick) boxes, respectively, for nest initiation (10.5×14.5×6.5 cm), colony foundation (21.0×21.0×15.0 cm), and colony maturation (24.0×27.0×18.0 cm). Each box had a wire net window on its lid for ventilation. The sizes of these windows were 5.5×6.5

cm, 7.0×14.0 cm and 10.0×20.0 cm, respectively. Queens collected in the field were first confined individually in small boxes for colony initiation and remained there until oviposition. When the adults emerged from the first brood, the nest was transferred to a medium box for colony foundation and left there until the number of workers reached 50. The nest was then moved to the large box for further colony development. A 40-50% sugar solution and pollen dough were provided ad libitum. The pollen dough was made from 40-50% sugar solution and fresh pollen collected from an apiary (v:v = 1:1).

Frequency distribution of the body weight of founder *B. ignitus* queens

We investigated the frequency distribution of the body weight of founder *B. ignitus* queens collected from 2000 to 2010. We classified founder queens into seven weight classes: below 0.5 g (below 0.59 g), 0.6 g (0.60-0.69 g), 0.7 g (0.70-0.79 g), 0.8 g (0.80-0.89 g), 0.9 g (0.90-0.99 g), 1.0 g (1.0-1.99 g), and over 1.1 g (over 1.10 g). The number of bumblebees collected was 713 in 2000, 170 in 2001, 80 in 2002, 865 in 2003, 949 in 2004, 404 in 2005, 1,147 in 2006, 713 in 2007, 407 in 2008, 752 in 2009, and 653 in 2010.

Statistical analyses

Statistical analyses were conducted using Tukey's HSD post-hoc test following a one-way ANOVA Welch and chi-square test (MINITAB Release 13 for Windows, 2000). Tukey's pairwise comparison test was used to examine the weight of *B. ignitus* queens collected in 2000 to 2010. The chi-square test was used to compare the rates of colony oviposition, colony foundation, production of progeny-queen, number of progeny-queen per queen, and number of offspring generations.

Results and Discussion

Comparison of body weights of collected founder *B. ignitus* queens

To investigate the correlation between the body weight and developmental characteristics of *B. ignitus*, we weighed *B. ignitus* queens collected in 2000 to 2010 (Fig. 1). The average weight of the 6,852 queens was 0.77 ± 0.44 g. The weight of *B. ignitus* queens collected in 2005 was the greatest, 0.87 ± 0.12 g, which was 1.0–1.3-fold heavier than queens collected in other years. The weight of *B. ignitus* queens collected in 2003 was the lowest, 0.68 ± 0.11 g. There was a significant difference in the weights of queens collected in different years (one-way ANOVA Welch test, Tukey's HSD post-hoc test: $F=162.732$, $df_1=10$, $df_2=1,375.433$, $p=0.0001$).

For the frequency distribution of the collected queens, we classified founder queens into seven weight classes ranging from below 0.5 g to over 1.1 g (Fig. 2). Among these weight classes, the 0.6 g class was the most prevalent in queens collected in 2003, 2004 and 2006 (30.8%-37.2%); the 0.7 g class was the most prevalent in queens collected in 2001 and 2008 (29.5%-32.7%); and the 0.8 g class was the most prevalent in queens collected in 2000, 2005, 2007, 2009 and 2010 (26.2%-32.5%). The below 0.5 g class was more common in 2003 (22.5%) than in any other year, and 2005 had the highest prevalence of the over 1.1 g class (5.0%). The weight of 364 queens of *B. terrestris* collected in another study averaged 0.829 ± 0.141 g and ranged from 0.4 g to 1.2 g (Beekman *et al.*, 2000). Although the shape of the frequency distribution of the weights of those *B. terrestris* queens was similar to that of the 6,852 *B. ignitus* queens collected during the present study in Korea, the post-hibernated queens of *B. ignitus* weighed slightly less than *B. terrestris*.

Body size is one of the most important life-history characteristics of an organism

(Calder, 1984; Schmidt-Nielsen, 1984; Roff, 1992). Body size is one of the dominant features of evolution in many animal lineages, and body weight, as with body size, is considered to be a relevant index for evaluating insect reproduction and development (McShea, 1988). In bumblebee, variations in the sizes of morphological characteristics of pollinators can affect their ability to extract rewards from flowers of different sizes and may affect pollen-collecting behaviors (Dafni and Kevan, 1997; Stout, 2000). Bumblebee pollinators show body size variation at a range of scales: body size varies among species, within the same species and even among individuals within a single colony (Alford, 1978; Cnaani and Hefltz, 1994). In the case of *B. terrestris*, the chance of surviving diapause is strongly determined by the wet weight of the queen at the beginning of hibernation. Queens with a low weight (less than 0.6 g) rarely survive diapause (Beekman *et al.*, 2000).

Colony development of founder *B. ignitus* queens by indoor rearing

The colony development of the collected *B. ignitus* queens was investigated. The average oviposition rate of founder queens was $81.6 \pm 10.7\%$. Queens collected in 2010 had the lowest rate, 59.5%. The oviposition rate of queens collected in 2004 was the highest, at 95.0%. This value corresponded to 1.1-1.6-fold increases over the queens collected in the other years, although there was no statistically significant difference in the oviposition rates among queens collected in 2000 to 2010 (chi-square test: $\chi^2=13.947$, $df = 10$, $p = 0.175$) (Fig. 3). Although egg laying is only one small step in the process of successfully rearing a bumblebee, it is a key factor in the large-scale production of commercial colonies and can reduce production costs (Yoon *et al.*, 2004a).

The rate of colony foundation, which is one of the main criteria for colony quality in commercial rearing, of all queens of this study was $60.9 \pm 11.0\%$ (Fig. 4). The queens collected in 2008 exhibited the best performance, 75.4%, which was 1.0-1.9-fold higher than the other years and was followed by 2007, 2004 and 2009 (73.8%, 69.0% and 68.4%, respectively). Queens collected in 2010 showed the lowest rate of colony foundation, 40.1%. There was a statistically significant difference in the colony foundation rates of queens collected in 2000 to 2010 ($\chi^2=19.377$, $df = 10$, $p = 0.036$) (Fig. 4).

As illustrated in Fig. 5, the rate of average progeny-queen production of all founder queens in this study was $27.0 \pm 9.4\%$ (Fig. 5). The rate in 2001 was 43.2%, which was 1.1–4.7-fold higher than the other years. Queens collected in 2010 had the lowest rate, 9.1%. The progeny-queen production rate of *B. ignitus* queens was significantly affected by collection year ($\chi^2=32.885$, $df = 10$, $p = 0.0001$). The number of queens produced, which is an important factor in the year-round rearing of bumblebees, of all queens in this study averaged $27.6 \pm 10.1\%$ (Fig. 6). Queens in 2005 produced an average of 41.3 queens; this figure corresponded to a 1.1-3.2-fold increase over queens collected in the other years. Queens collected in 2010 had the lowest rate, 12.8. There was a statistically significant difference in the number of progeny-queens produced by queens collected in 2000 to 2010 ($\chi^2=35.649$, $df = 10$, $p = 0.0001$) (Fig. 6).

The average number of generations produced by the founder queens collected during the 11 years was 4.8 ± 2.0 (Fig. 7). Queens in 2000 averaged 9 offspring generations, which was 1.1-3.1-fold higher than the other years and was followed by 2003, 2001 2007, with 8, 6 and 5 generations, respectively. Queens collected in 2008-2010 had the shortest progeny lines, at 3 generations. The number of generations of *B.*

ignitus queens was not significantly affected by collection year ($\chi^2=8.642$, $df = 10$, $p = 0.566$).

Based on the above results, the colony-developmental characteristics of the queens of this study significantly worsened with time. There was no correlation between body weight and the number of queens collected in any year, although body weight was affected by collection year. For example, the body weight of *B. ignitus* queen collected in 2003, which was the lowest in this study (0.68 ± 0.11 g), was 1.3-fold lighter than queens collected in 2005, which were the heaviest (0.87 ± 0.12 g). However, the 8 generations of offspring produced by *B. ignitus* queens collected in 2003 was 2.0-fold longer than the 4 generations produced by queens collected in 2005. Beekman *et al.* (2000) found that *B. terrestris* queens with a wet weight of 1 g or more were no longer produced after several generations when reared under controlled conditions in the laboratory. This finding suggests that the disappearance of heavy queens is caused by a deficiency of scarce nutrients (Beekman *et al.*, 2000). Yoon *et al.* (2004b) reported a positive correlation between the body weight and colony development of founder queens, suggesting that the colony-developmental characteristics of heavier queens were better than those of lighter queens. We think that the reason for the differences between our results and previous results is the collection of queens from different locations. Yoon *et al.* (2001) found a positive correlation between the collection location and the colony development of queens. Queens collected since 2008 in the present study produced only 3 generations each. The reason for this outcome should be investigated through further experimentation. The causes of bumblebee population decreases include climate change, changing agricultural policies and practices, reductions in floral resources, loss of nest sites and hibernation sites and pesticide use (Goulson *et al.*, 2008; William and Osborne, 2009).

The present study examines the breeding of *B. ignitus* queens collected from 2000 to 2010 for conservation of native bumblebees. Altogether, the results indicate that the colony-developmental characteristics of the collected queens was significantly affected by collection year. In addition, there was no correlation between body weight and the number of queens collected in any year during the 11 years of this study, although body weight was affected by collection year. Since 2008, the colony-developmental characteristics of *B. ignitus* queens have decreased.

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the European bumblebee *B. terrestris*. Int. J. Indust. Entomol. 23: 167-174.

Figure legends

Fig. 1. Weights of founder *B. ignitus* queens collected from 2000 to 2010. The number of bumblebees collected in 2000 to 2010 was 713, 170, 80, 865, 949, 404, 1,147, 713, 407, 752 and 653, respectively. There were significant differences in body weight between years ($p < 0.001$ using Tukey's pair-wise comparison test).

Fig. 2. Frequency distribution of the weights of founder *B. ignitus* queens collected in 2000 to 2010. The body weights of queens were classified below 0.5 g (below 0.59 g), 0.6 g (0.60-0.69 g), 0.7 g (0.70-0.79 g), 0.8 g (0.80-0.89 g), 0.9 g (0.90-0.99 g), 1.0 g (1.0-1.99 g), and over 1.1 g (over 1.10 g).

Fig. 3. Oviposition rate of *B. ignitus* queens reared in 2000 to 2010. The number of bumblebees reared in 2000 to 2010 was 412, 170, 80, 844, 822, 404, 1,081, 546, 400, 753 and 827, respectively. There was no significant difference in the oviposition rate between years ($p < 0.05$, chi-square test).

Fig. 4. Rate of colony foundation of *B. ignitus* reared in 2000 to 2010. A statistically significant difference was noted in the rate of colony foundation between years ($p < 0.05$, chi-square test).

Fig. 5. Rate of progeny-queen production of *B. ignitus* reared in 2000 to 2010. There was a statistically significant difference in the rate of progeny-queen production of *B. ignitus* between years ($p < 0.001$, chi-square test).

Fig. 6. The number of progeny-queens produced by *B. ignitus* in 2000 to 2010. A statistically significant difference was noted in the number of progeny-queens produced between years ($p < 0.001$, chi-square test).

Fig. 7. The number of generations of offspring produced by *B. ignitus* queens in 2000 to 2010. There was no significant difference in the number of generations produced by queens of different years ($p < 0.05$, chi-square test).

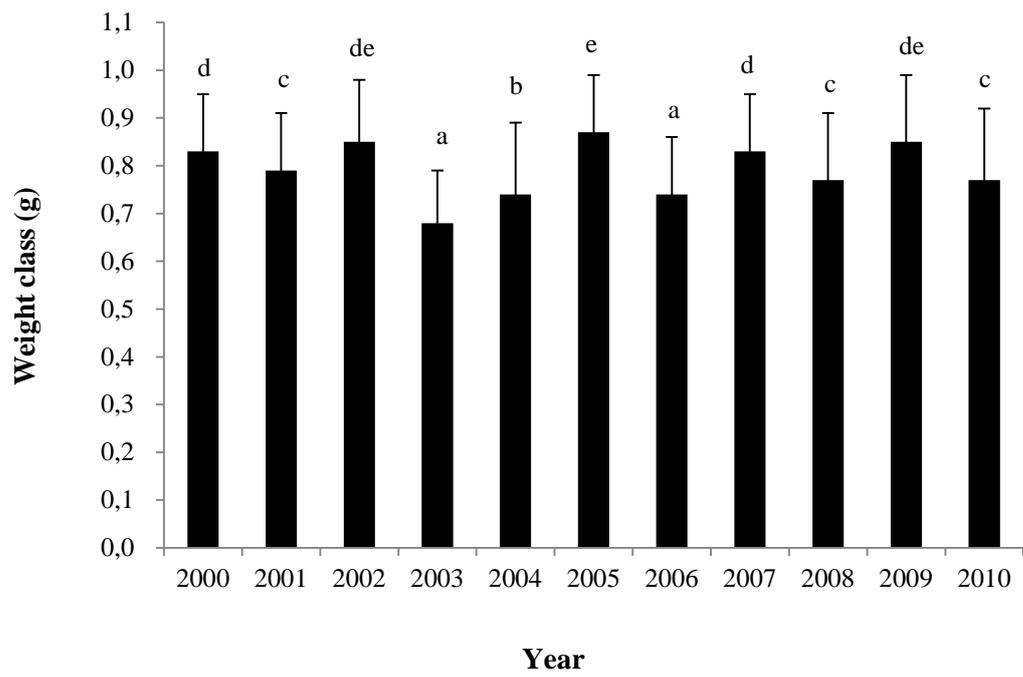


Fig. 1.

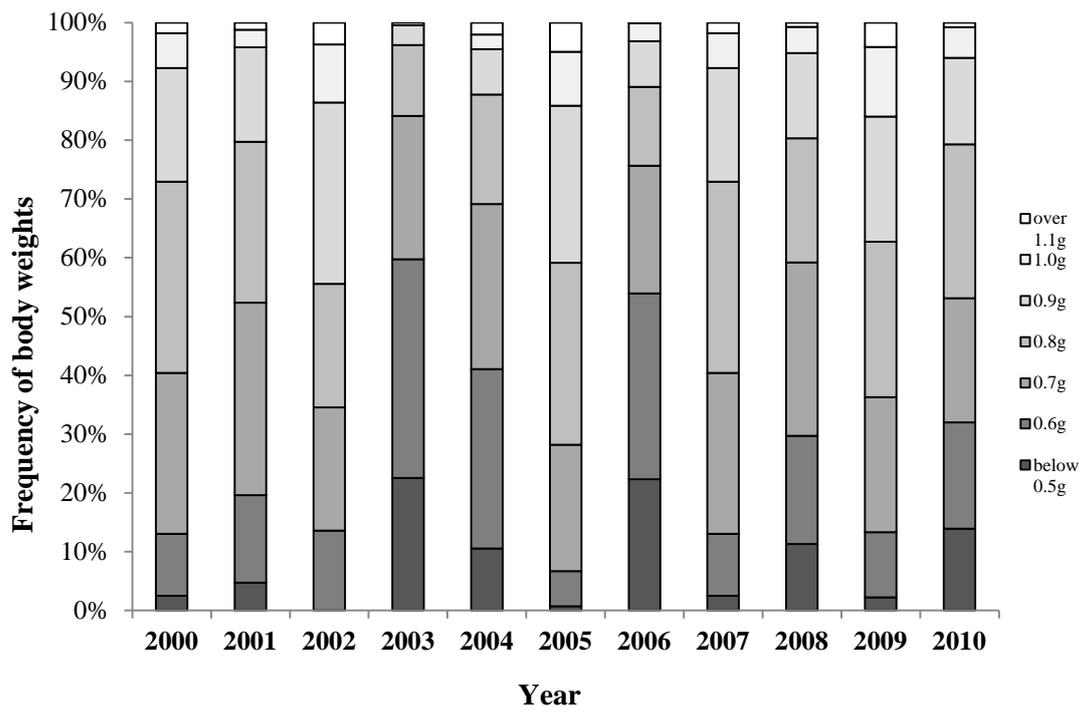


Fig. 2.

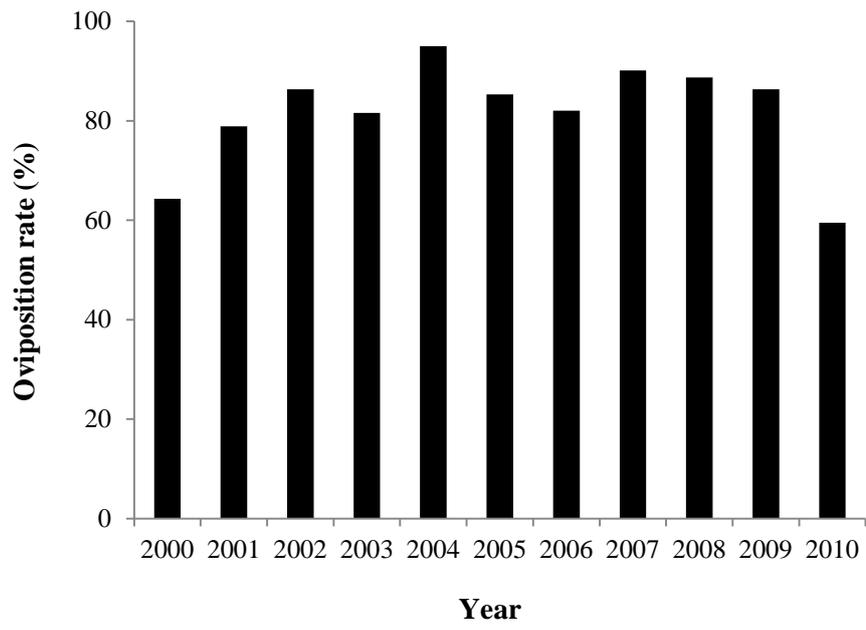


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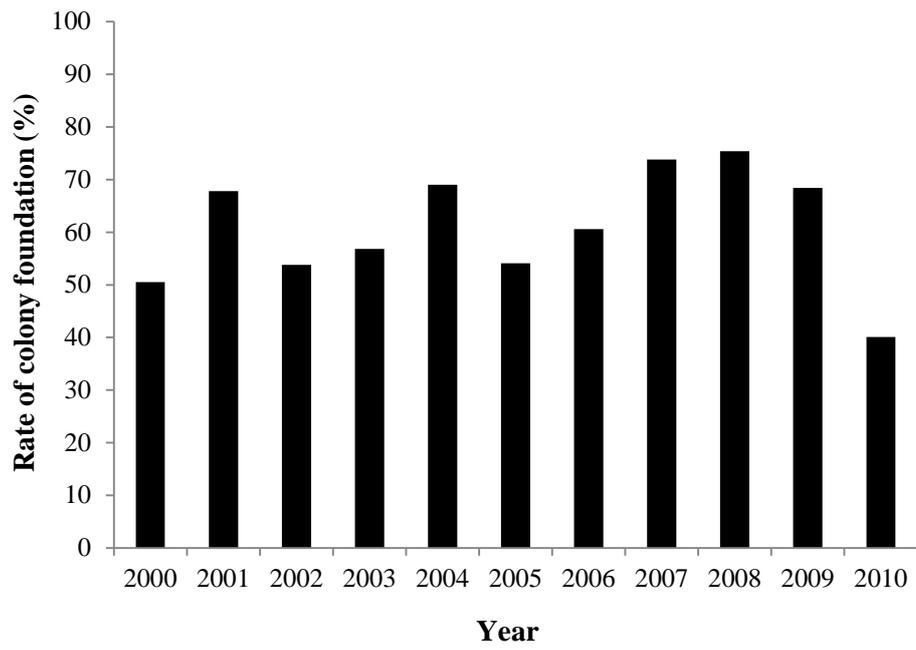


Fig. 4.

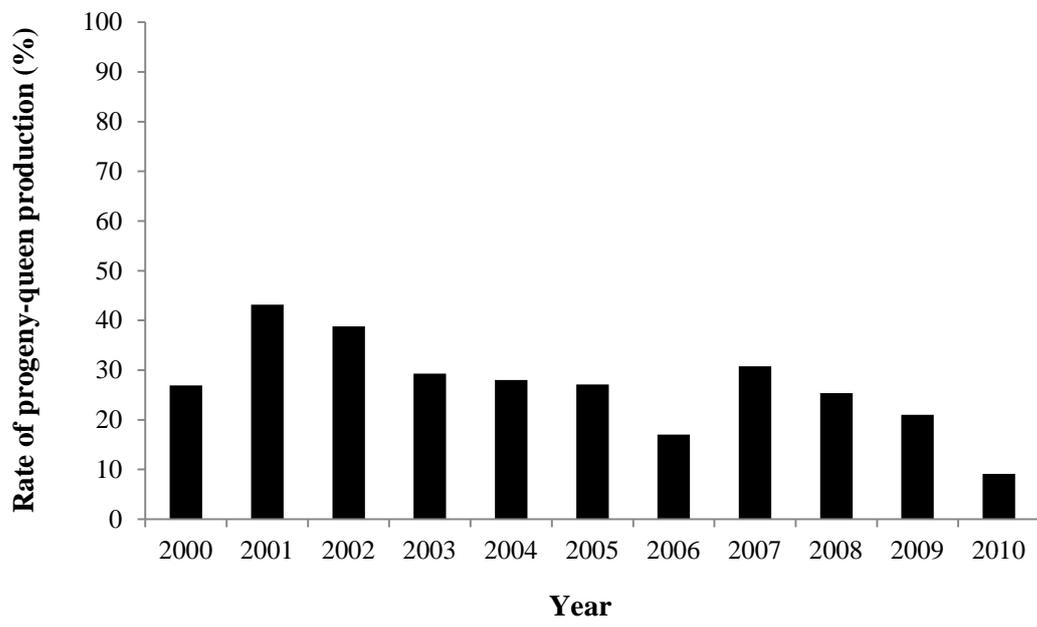


Fig. 5.

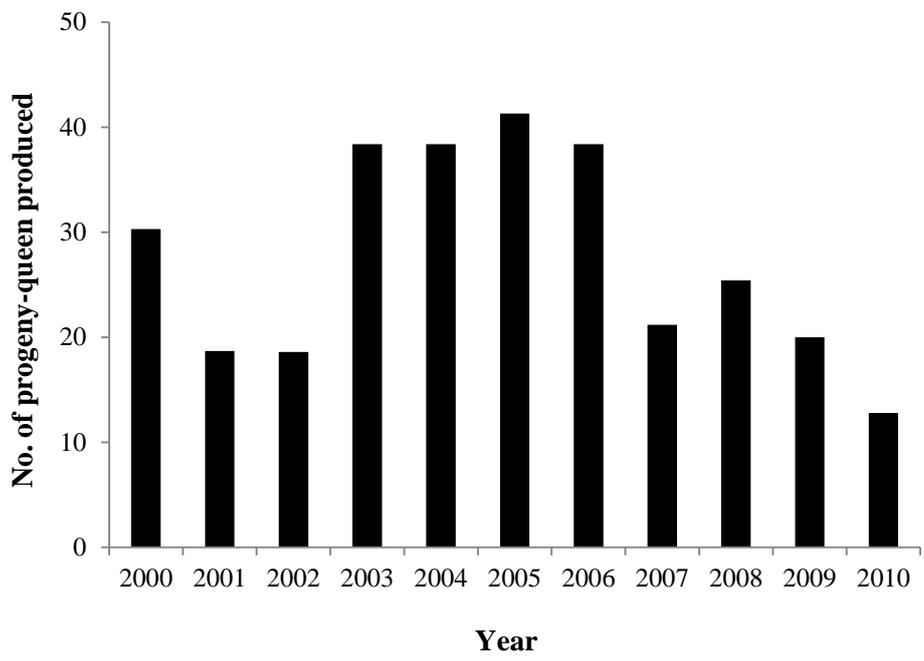


Fig. 6.

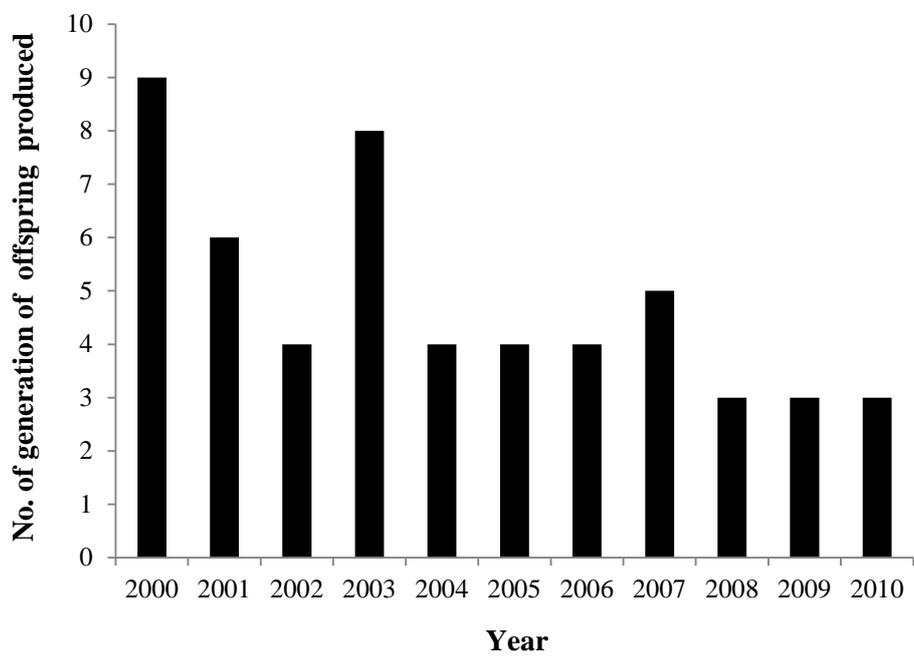


Fig. 7.