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Bigger females, more eggs: the impact of female body weight on egg-laying ability in *Protaetia brevitarsis seulensis* (Kolbe)

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Abstract

One of the necessary conditions for the mass production of the edible insect, the white-spotted flower chafer *Protaetia brevitarsis seulensis* (Kolbe), is to breed a strain with excellent egg-laying ability. To identify external morphological traits related to egg-laying ability, we investigated the effects of the weight, length, and width of female adults on egg production. Correlation and multiple regression analyses revealed a positive correlation between the weight of female adults and egg production. This study suggests that selection of heavier females is a good strategy for breeding strains with superior egg-laying characteristics. The results of this study will serve as important foundational data for future breeding of superior strains.

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Introduction

The edible insect industry has experienced rapid growth in recent years, leading to a surge of interest in developing superior insect strains that can serve as the foundation of this industry (Eriksson *et al.*, 2020; RDA, 2020). Establishing a stable production system requires breeding insects with consistent quality for products and excellent egg-laying capacity for mass production. As of 2020, fourteen insect species have been legally registered as 'livestock' by the Ministry of Agriculture, Food and Rural Affairs (MAFRA, 2020). Additionally, nine insect species, including *Apis mellifera* male pupae, *Baekgangjam* (the silkworm, *Bombyx mori*, became stiffened due to the infection of the fungus *Beauveria bassiana*, causing white muscardine disease), *Bombyx mori* larvae and pupae, *Gryllus bimaculatus*, *Oxya sinuosa*, *Protaetia brevitarsis seulensis* larvae, *Tenebrio molitor* larvae, *Trypoxylus dichotomus* larvae, and *Zophobas atratus* larvae are registered as general food ingredients in the Korea Food Code (MFDS, 2023).

Out of these insect species, *P. b. seulensis* (PBS), commonly known as the white-spotted flower chafer, is currently the most widely cultivated and top-selling species in Korea (Kim *et al.*, 2022; MAFRA, 2022). Notably, PBS larvae have been found to possess various physiological functions and health benefits, including anti-coagulation (Sim *et al.*, 2018), anti-inflammatory effects (Lee *et al.*, 2019), and efficacy in treating liver diseases, making them a popular ingredient in folk remedies and traditional medicine in Korea (Chon *et al.*, 2012; Kang *et al.*, 2000; Lee *et*

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al., 2001). Moreover, the larvae have been approved as a food ingredient in Korea since 2016, after nutritional, toxicological, and stability assessments. They are now considered as a promising alternative protein source to overcome the food crisis caused by climate change and population growth (Chung *et al.*, 2013; Park *et al.*, 2012).

According to a 2020 survey conducted by the National Institute of Agricultural Sciences, PBS account for 67.9 % in the total production of food and medicinal insects (RDA, 2020). As of 2021, there are 1,208 domestic farms in Korea that raise white-spotted flower chafer beetles, representing about 45 % of all insect farms in Korea, with sales reaching 16.6 billion won (MAFRA, 2022). With the industrial growth, insect farmers increasingly demand superior insect strains for consistent quality and stable production, similar to other livestock (RDA, 2020). However, the development of such strains for this species has not yet been fully achieved, and dissemination to farms remains limited.

To overcome this demand, it is necessary to identify external traits that are indicative of excellent developmental characteristics, as these can serve as important target for the development of superior strains. One of such targets could be larger body size, which generally promotes greater fecundity (Honěk, 1993; Jabłoński, 1996). This relationship has been reported in literature surveys of many insect species (Bond *et al.*, 2022; Sokolovska *et al.*, 2000; Stewart *et al.*, 1991). When larger adults are competitively superior, they are likely to acquire more resources and have greater reproductive success (Wall and Begon, 1987).

In this study, we investigated the relationship between egg-laying capacity and body size (weight, length, and width) of female PBS for breeding purposes. Our aim was to determine whether there is a correlation between body size and egg-laying capacity that could guide the selection of breeding stock.

Materials and methods

All adults of *Protaetia brevitarsis seulensis* (Kolbe) used in the study were reared in laboratory conditions at the Department of Agricultural Biology, National Institute of Agricultural Sciences. The rearing conditions included a constant temperature of 25 °C, a light-dark cycle of 16:8 h, and a relative humidity of 60 %. We measured the morphological characteristics of the emerged adults within 24 hours. Firstly, we separated the beetles by gender and measured their weight, body length, and width. Weight was measured using a precision balance (ML204T / 00, Mettler Toledo, USA), body length was measured from the head to the tip of the abdomen, and body width was measured across the pronotum using digital calipers (CD-AX / C, Mitutoyo, Japan).

The female beetles were housed in 3 L plastic cages filled with fermented sawdust and provided with insect jelly as a food source. The adults were selected across a range of sizes, with males weighing 865 ± 139 (range: 465–1079) mg and females weighing 815 ± 135 (range: 573–1067) mg. A total of thirty pairs of males and females were used in the experiment.

Unmated female beetles were transferred to plastic breeding trays containing 5 cm of fermented sawdust, insect jelly, and water, along with mature males. After allowing the females to receive the eggs for seven days, both males and females were transferred to new breeding trays. This process was repeated a total of six times over a period of six weeks. The number of eggs laid in each tray was counted on a weekly basis.

The relationship between female body size (width, length, and weight) and fecundity was evaluated using multiple regression analysis. Pearson's coefficient of correlation was calculated to determine the relationships between the number of eggs, weight, body length, and width of adult females using Jamovi v.2.3.21 (Jamovi, 2022). The stepwise multiple regression analysis was conducted using SPSS 27.0 statistical software (SPSS Inc., Chicago, IL, USA).

Results

In this study, we investigated the weight, body length, and width of adult female industrial insects, as well as their fecundity over a period of six weeks. The average weight, body length, and width of adult females were found to be 814.8 ± 134.6 (573–1067) mg, 20.7 ± 0.9 (18.9–22.1) mm, and 11.7 ± 0.5 (10.6–12.5) mm, respectively (Table 1). The average fecundity was 74.2 ± 23.4 (30–144) eggs (Table 1).

Table 2 presents the results of the multiple regression analysis, which used weight, length, and width as independent variables, and eggs as the dependent variable. The regression model showed an explanatory power of 45.8 % (R2), and a significant fit ($p < 0.01$). Further analysis of the independent variables revealed that only weight (β = 0.620) had a significant positive effect on fecundity ($p < 0.05$). Although length and width also showed positive and negative correlations, respectively, with

Table 1. Mean fecundity and adult female characteristics. Data are shown means ± standard deviation.

Fecundity (*N*=30)		Weight (mg, *N*=30)	Body length (mm, *N*=30)	Body width (mm, *N*=30)
Week	The number of eggs			
1	11.4±10.8	814.8±134.6	20.7±0.9	11.7±0.5
2	18.0±6.4			
3	10.8±6.3			
4	14.6±5.3			
5	9.5±3.4			
6	9.9±4.7			

fecundity, these were not statistically significant.

To gain further insight into the relationship between weight, length, width, and eggs, we also conducted a correlation heatmap analysis, as shown in Figure 1. This analysis revealed that weight had a significant positive correlation with length (r = 0.77), width (r = 0.84), and eggs (r = 0.62) ($p < 0.01$). Similarly, length was found to be significantly correlated with width (r = 0.71) and eggs (r = 0.60) in a positive direction ($p < 0.01$). Finally, eggs and width also showed a significant positive correlation ($p < 0.05$), respectively.

Discussion

With the growth of the edible insect industry, there is increasing interest in developing superior insect strains that can serve as the foundation of this industry. To establish a stable production system, it is necessary to breed insects with consistent quality. Additionally, for mass production, excellent egg-laying capacity is a prerequisite. In this study, we investigated the relationship between egg-laying capacity and body size (weight, length, and width) of female PBS for breeding purposes. Our aim was to determine whether there is a correlation between body size and egg-laying capacity that could guide the selection of breeding stock.

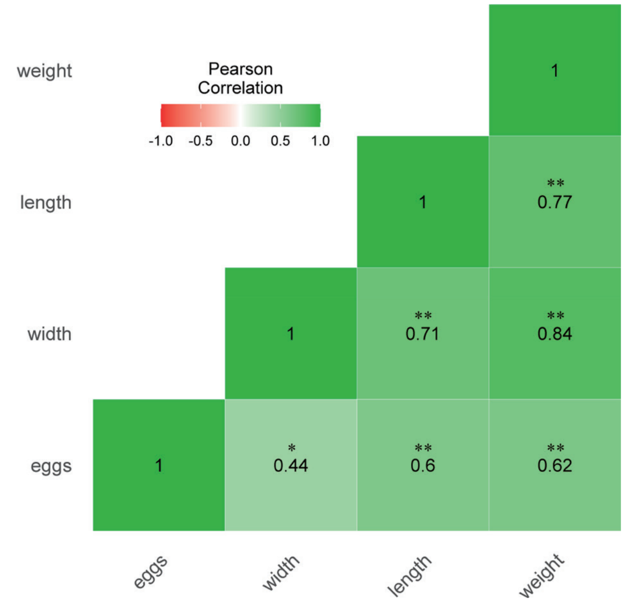

Fig. 1. Pearson correlation matrix between the number of eggs, body width, length, and weight of adult females of *Protaetia brevitarsis seulensis*. The colored gradient legends represent coefficients of correlation r-values from +1.0 (dark green) to -1.0 (dark red). The asterisk denotes significant difference (*$p < 0.05$, ** $p < 0.01$).

We found a positive correlation between the weight of female adults and egg production, as revealed by correlation and multiple regression analyses (Table 2, Fig. 1). This finding supports Darwin's hypothesis that larger females have an advantage in producing more eggs than smaller ones (Darwin, 1874). Similar positive correlations between female body size

Table 2. Multiple regression analysis of factors affecting female egg-laying ability.

Model		Unstandardized Coefficients		Standardized Coefficients	t-value	Collinearity Statistics	
		B	SE	β		Tolerance	VIF
Dependent variable: eggs	(Constant)	-39.903	136.732	-	-0.292		
	weight	0.108	0.052	0.620	2.093*	0.238	4.29
	length	9.785	6.151	0.366	1.591	0.393	2.54
	width	-15.081	12.125	-.335	-1.244	0.288	3.48

$Y = 0.108X_1 + 9.785X_2 - 15.081X_3 - 39.903$, $R^2 = 0.458$, F-value = 7.317**, *$p < 0.05$, **$p < 0.01$

and egg-laying capacity have been reported in other insect species (Berger *et al.*, 2008; Omkar and Afaq, 2013; Sagarra *et al.*, 2001; Salavert *et al.*, 2011). It can be explained that as body size increases, there is more space available for storing eggs (Preziosi *et al.*, 1996; Reiss, 1989). Furthermore, a recent study has shown a positive correlation between egg size and female body size (Kojima, 2015).

Our study suggests that selecting heavier females for breeding can potentially increase egg-laying capacity. However, the egg-laying capacity of the offspring resulting from the selected females has not been evaluated yet. Therefore, further research is necessary to confirm whether the egg-laying capacity of the offspring from the selected females is maintained in subsequent generations. This information is critical for developing an effective breeding program to improve the productivity of the insect industry.

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Conflicts of Interest

The authors declare no conflict of interest.

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