Elytra, *Tokyo*, New Series, **9** (2): 317–323
December 25, 2019

Meat-eating by Larvae Changes the Life History Strategy of *Protaetia brevitarsis* (Lewis, 1879) (Coleoptera, Scarabaeidae, Cetoniinae), which Breeds in Bird Nests

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Abstract The larval development of *Protaetia brevitarsis* (Lewis, 1879), the larvae of which have found in both carnivorous bird's nests and decayed plant matter in nature, was investigated by feeding of humus soil with or without chicken meat in the laboratory. The larval developments of *Rhomborrhina polita* Waterhouse, 1875 and *Pseudotorynorrhina japonica* (Hope, 1841), neither of which has been reported to be found in bird's nests, were also observed using the same method for interspecific comparison. The survival rate of *R. polita* larvae was low under both feeding conditions, although meat-eating had a slightly positive effect on their survival rate. In contrast, the survival rates of *Ps. japonica* larvae were equally high under both feeding conditions, and meat-eating had a positive effect on growth rate or weight increase. The result in *Pr. brevitarsis* was distinctly different from *R. polita* and *Ps. japonica*. Although the non-meat-eating larvae took more than 200 days to pupate (assumed to hibernate as larvae in nature), meat-eating larvae had two alternative tactics of development: pupate around the 50th day with a relatively small body size but as large as non-meat-eating larvae (assumed to hibernate as adults) or pupate around the 175th day with a relatively large body size (assumed to hibernate as larvae). Although the adaptive advantage of earlier pupating or larger body size has not revealed in *Pr. brevitarsis*, adults emerge before winter and carry out some reproductive activity in fall or start it early in the following spring may have greater fitness than adults emerge after winter. Further, larger adults may have higher benefits in fertility, mating success, and strength in competing for feeding sites than smaller adults. Therefore, a nest of carnivorous birds is considered to be a valuable resource for larvae of *Pr. brevitarsis*, and female adults may favor it as an oviposition site.
Key words: *Anthracophora rusticola*, carnivore, habitat, *Strix uralensis*, *Ciconia boyciana*.

Introduction

Larvae of the Cetoniinae beetle *Protaetia brevitarsis* (Lewis, 1879) (Coleoptera, Scarabaeidae) are sometimes found in bird's nests, including artificial nest boxes of the Ural owl *Strix uralensis* Pallas, 1771 (Nasu *et al*., 2011) and in natural nests of the Oriental stork *Ciconia boyciana* Swinhoe, 1873 (Nasu *et al*., 2010). They are also found in other places, such as under litter composed mainly of grass (Iijima, 2017), in the tree hollows of willows, and in the horticulture soil in plastic planters on balconies (Koshiyama *et al*., 2019).

Another cetoniine beetle, *Anthracophora rusticola* Burmeister, 1842, has similar ecological habits. The larvae and/or pupae are found in decayed plant matter such as compost and straw-thatched roofs (Murayama, 1952) and the nests of carnivorous birds such as several Falconiformes species (Makihara *et al*., 2004; Yamada *et al*., 2007; Choi *et al*., 2008, 2017; Nonaka *et al*., 2010; Koshiyama, 2012; Koshiyama *et al*., 2012 b; Osafune & Koshiyama, 2014), the great cormorant *Phalacrocorax carbo* (Linnaeus, 1758) (Yamamoto, 2010), the Oriental stork (Nasu *et al*., 2010, 2012), and the omnivorous carrion crow *Corvus corone* Linnaeus, 1758 (Nagahata *et al*., 2013).

Our previous laboratory study (KOSHIYAMA *et al*., 2012 a) clearly showed that *A*. *rusticola* larvae pupated faster, survived at higher rate, and gained more weight when they were fed humus soil with dried chicken meat than without dried chicken meat, indicating that meat-eating enhances larval development of this beetle. Therefore, larvae of *A*. *rusticola* living in the bird's nests may benefit from the continuous availability of prey meat supplied for chicks by the bird parents.

Because both the Ural owl and the Oriental stork are carnivorous, there is a possibility that *Pr*. *brevitarsis* larvae living in the bird's nests gain some benefits by eating meat as well as *A*. *rusticola* larvae. We here compared the larval development of *Pr*. *brevitarsis* under feeding conditions of humus soil with and without dried chicken meat in the laboratory. For interspecific comparison, we also observed the larval development of two other cetoniine beetles, *Rhomborrhina polita* WATERHOUSE, 1875 and *Pseudotorynorrhina japonica* HOPE, 1841, neither of which has been reported to be found in bird's nests.

Material and Methods

Parental insects

Four *Rhomborrhina polita* adults were collected in Yawatahama City, Ehime Prefecture in August 2006. Seven *Pseudotorynorrhina japonica* adults and eight *Protaetia brevitarsis* adults were collected in Okayama City, Okayama Prefecture in June and May 2007, respectively. Although the life history including larval habitat of *R*. *polita* have not been discovered in detail, larvae of *Ps*. *japonica* could develop in general humus resources in nature, as were reported by SUZUKI (2011) to grow in humus soil under a kudzu-vine community, because it is a most common cetoniine beetle in Japan (SAKAI & FUJIOKA, 2007). On the other hand, the larval habitat of *R*. *polita* could be unique because it is uncommon (IGA, 1956) and the type of development is semi-voltine with long larval period (SAKAMOTO, 2000).

Each species was reared separately in a plastic case (20 cm long, 12 cm wide, 14 cm high), which was half-filled with humus soil made from broad-leaved tree leaves, Amigo Original Insect Mat (Lic, Okayama, Japan). Sliced apple and banana and a commercial food for pet beetles, Amigo Original Insect Jelly (Lic), which contains brown cane sugar, were introduced into the cases as the adults' food. All of the experiments were conducted at 25℃ under a LD 16 : 8 photoperiod.

Effect of meat-eating on larval development

Eggs laid by the adults of *R*. *polita* were collected from the humus soil on 8 September 2006, those of *Ps*. *japonica* on 13 July 2007, and those of *Pr*. *brevitarsis* on 20, 23, and 29 May 2007. Each egg was placed in a plastic cup (6.5 cm diameter, 4 cm high) filled with the humus soil. The hatched larvae were fed with (treatment group) or without (control group) a piece of chicken meat (about 200 mg) that had been dried for three days by a window under room temperature in the cups on the day of hatching. Thirteen *R*. *polita* and nine *Ps*. *japonica* larvae for each group, and twelve and 13 *Pr*. *brevitarsis* larvae for control and treatment groups, respectively, were reared at 25℃ under a LD 16 : 8 photoperiod. Each larva was weighed using an electrobalance, FX-300N (Seiken, Ibaraki, Japan) at two-day intervals during the initial 114 days for *R*. *polita*, four-day intervals during the initial 160 days for *Ps*. *japonica*, and two- to four-day intervals during the initial 210 days for *Pr*. *brevitarsis*. When a larva was just after hatching or molting, it was not weighed to avoid injuring it. The chicken meat and humus soil were renewed every two and six to eight days, respectively. When formation of a cocoon was observed, we regarded it as pupating. Survival rate of larvae was calculated as the proportion of pupated individuals during rearing and living larvae on the final day of rearing.

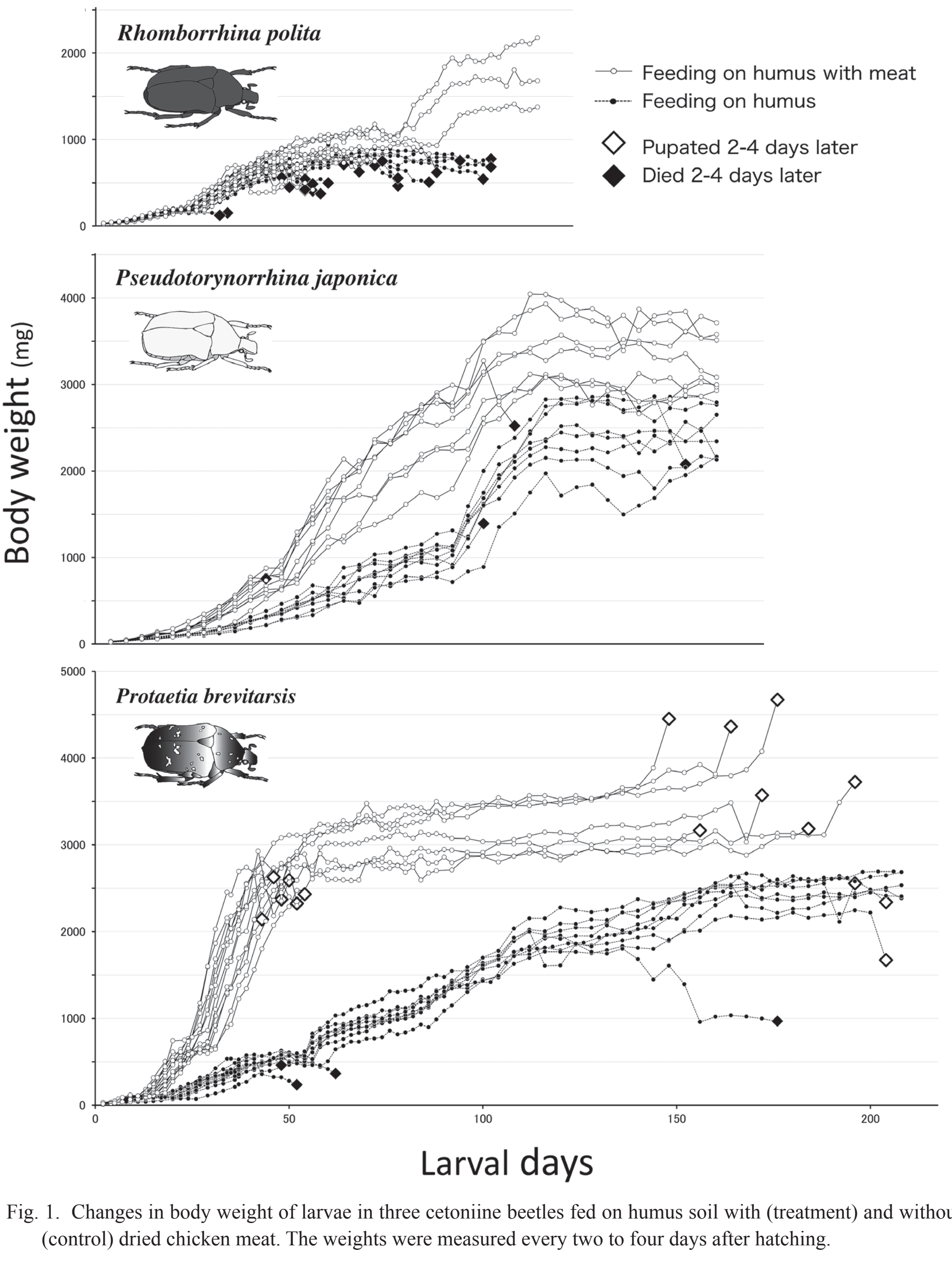

Fig. 1. Changes in body weight of larvae in three cetoniine beetles fed on humus soil with (treatment) and without (control) dried chicken meat. The weights were measured every two to four days after hatching.

Statistical analysis

To analyze the effect of meat-eating on the survival rate, we used generalized linear model (GLM) with binomial distribution, logit link function, and treatment as an explanatory variable. In analyses for data of the body weight, first, we used the O'Brien test for checking homogeneity of variance. For *Ps. japonica*, we used the t-test because the two sets of data did not have different variances ($F = 0.33$, $p = 0.5791$). While for *Pr. brevitarsis*, we used the Kruskal-Wallis test and the Wilcoxon test as a post hoc analysis, because the three sets of data had different variances ($F = 6.22$, $p = 0.0140$). The statistical package JMP version 12.2 for the Windows (SAS Institute Inc., 2015) was used for all analyses.

Results

Rhomborrhina polita

The body weights of both control and treatment larvae increased slowly, and they died one after another (Fig. 1). Only three individuals of the treatment group (n =13) and one individual of the control group (n = 13) reached the 3rd (last) instar. The survival rate at the end of the observation, the 114th larval day, was significantly higher in the treatment larvae (23.1%, no. of alive individuals = 3, n = 13) than in the control larvae (0%, no. of alive individuals = 0, n = 13) (GLM, *d.f.* = 1, $\chi^2 = 4.55$, $p = 0.0329$).

Pseudotorynorrhina japonica

The increase of body weight was faster in the treatment group than in the control group (Fig. 1). The body weight at the end of the observation, the 160th larval day, was significantly higher in the treatment group (mean ± SE = 3281.57 ± 120.14 mg, n = 7) than in the control group (2450.14 ± 120.14 mg, n = 7) (t-test, *d.f.* = 1, $t = 4.89$, $p = 0.0004$). The survival rates of treatment and control groups at the end of the observation were the same (77.8%, no. of alive individuals = 7, n = 9, for each group). No pupated individuals were observed, and all of the surviving larvae were in the 3rd (last) instar at the end of observation.

Protaetia brevitarsis

The larval growths of treatment and control groups were qualitatively quite different (Fig. 1). All 13 larvae that ate meat grew promptly from about the 20th larval day, and six of them pupated (formed cocoons) early (mean ± SE = 51.0 ± 1.53 days, range 46–56 days, date 18–28 July 2007) (early-pupating individuals of treatment). The remaining seven larvae grew slowly and all pupated late (174.6 ± 6.18 days, 152–200 days, 1–27 November 2007) (late-pupating individuals of treatment). On the other hand, the body weight of control group, which did not eat meat, increased linearly and three individuals pupated (204.7 ± 2.40 days, 200–208 days, 17–25 December 2007). Five other individuals were still in the 3rd instar at the end of observation, the 210th larval day. Assuming that the pupated individuals were alive at the end of observation, the survival rate was significantly higher in treatment group (100%, no. of alive individuals = 13, n = 13) than in control one (66.7%, no. of alive individuals = 8, n = 12) (GLM, *d.f.* = 1, $\chi^2 = 6.71$, $p = 0.0096$).

The larval weights on the measurement day just before pupation (two to four days before pupation) were significantly different among three groups (i.e., control, early-pupating individuals of treatment and late-pupating individuals of treatment) (Kruskal-Wallis test, *d.f.* = 2, $\chi^2 = 10.35$, $p < 0.0057$) (Table 1). The body weight was significantly heavier in the late-pupating individuals of treatment than the other two groups at the 5% level based on Wilcoxon test (Table 1).

Table 1. Body weight of just-before-pupation larvae of *Protaetia brevitarsis* reared on humus soil with (treatment) and without (control) dried chicken meat.

Group of larvae	Body weight (mg)
Control	2188.33 ± 271.95 (n=3) [a]
Treatment: early-pupating individuals	2413.17 ± 192.30 (n=6) [a]
Treatment: late-pupating individuals	3875.71 ± 178.03 (n=7) [b]

Values are mean ± SE. Means followed by the same letters are not significantly different from each other ($p > 0.05$) based on Wilcoxon test.

Discussion

In all three beetle species, meat-eating by larva had a positive effect on larval development. However, the efficacies were different. In *Rhomborrhina polita*, the survival rate was very low in both food conditions, although it was slightly higher in larvae fed on the humus soil with than without chicken meat. Larvae of *R. polita* may need some other nutrient sources to complete their development. In contrast, the survival rates of *Pseudotorynorrhina japonica* were high in both food conditions. Larvae of *Ps. japonica* are considered to be able to complete their normal development without meat, but meat-eating has a positive effect on the rate of growth or weight increase.

On the other hand, the effects of meat-eating on larval development in *Protaetia brevitarsis* were distinctly different from the two species described above. All *Pr. brevitarsis* larvae that fed on the humus soil with chicken meat grew rapidly, and half of the larvae required only about 50 days until pupation, although more than 200 days were required for larvae fed only on the humus soil. In spite of the difference in their growth rates, their body weights just before pupation were not different from each other (Table 1). These results indicate that one effect of meat-eating on *Pr. brevitarsis* was drastic acceleration of development. Furthermore, another half of the larvae fed on the humus soil with chicken meat grew larger in body size and pupated at around 175 days. Their body weight just before pupation was significantly heavier than that of larvae fed on only the humus soil (Table 1). These data indicate that another effect of meat-eating on *Pr. brevitarsis* was an increase in body size. These two effects mean that *Pr. brevitarsis* larvae possess two alternative tactics of development due to meat-eating: emerging early with small body size (but the same size as individuals that did not eat meat) or emerging late with large body size.

What is the benefit for *Pr. brevitarsis* of emerging early? The early pupation of meat-eating larvae in this study occurred in July, suggesting their emergence before hibernation in nature. The larvae fed only on the humus soil pupated in December under the laboratory conditions, suggesting their hibernation as larvae in nature. If the adults emerge before winter and carry out some reproductive activity in fall or start it early in the following spring, they may have greater fitness than the wintering larvae that fed only on the humus soil. Further, the meat-eating individuals with large body size and late pupation may have higher benefits in fertility, mating success, and strength in competing for feeding sites than the non-meat-eating individuals with small body size. Therefore, nests of carnivorous birds, such as the Ural owl and the Oriental stork, are valuable resource for larvae, and female adults may favor them as oviposition sites, if available.

Iijima (2017) concluded that a bird nest is not necessary for *Pr. brevitarsis* to reproduce, and its breeding place is grassland, based on the observation of habitat and behavior of outbreaking *Pr. brevitarsis* at Oi Central Seaside Park in Ota-ku, Tokyo. This conclusion seems not to conflict with the present results of our laboratory experiment. However, Suda and Horiguchi (2019) reported that the morphological features of *Pr. brevitarsis* adults collected at the park are quite different from those of other populations in mainland Japan and that they are considered to be an introduced population. Because the introduced *Pr*. *brevitarsis* could be different from native *Pr*. *brevitarsis* not only in morphological features but also in ecological traits, it is worth clarifying whether the effect of meat-eating on larval development of the introduced *Pr*. *brevitarsis* is the same as that of the native *Pr*. *brevitarsis* examined in this study.

Finally, we mention the similarity of larval development of two cetoniine beetles, *Pr*. *brevitarsis* and *Anthracophora rusticola*, both of which grow in bird's nests. Our previous laboratory experiment with *A*. *rusticola* conducted by the same method as this study, showed that *A*. *rusticola* larvae fed on the humus soil with meat grew rapidly (pupated within 36 days after hatching) like the early pupation group of *Pr*. *brevitarsis* in this study, and those fed only on the humus soil grew slowly (pupated at 76 or 80 days after hatching) like the *Pr*. *brevitarsis* of the control in this study (see Figure 1(B) of KOSHIYAMA *et al*., 2012 a). Such a distinct difference in larval development depending on consuming meat or not was not observed in *R*. *polita* and *Ps*. *japonica* in this study, neither of which has been reported to be found in bird's nests. Laboratory experiments, as shown in this study, can help to screen which beetle species breeds in bird nests in nature.

Acknowledgements

We thank Mr. M. SHIMONO (Kankyo Kagaku Osaka Co., Ltd) for helpful information about *Protaetia brevitarsis*. A part of this work was supported by a grant from Japan Society for the Promotion of Science (KAKENHI) 18H02510 to TM.

要　約

越山洋三・宮田　亮・松村健太郎・宮竹貴久：鳥の巣で繁殖するシラホシハナムグリ（鞘翅目コガネムシ科ハナムグリ亜科）は，幼虫が肉食すると生活史戦略が変化する．――――幼虫が肉食性の鳥の巣と腐植質の両方から見つかるシラホシハナムグリについて，幼虫に餌として腐植土だけを与えた場合と腐植土に加え鶏肉を与えた場合とで，発育にどのような差が生じるかを室内実験によって調べた．また，鳥の巣から見つかっていないクロカナブンとカナブンについても同様の実験を行い，3 種間で比較した．その結果，クロカナブンでは餌条件に関わらず生存率が低く，肉食はわずかに生存率を上げる効果しかなかった．対照的に，カナブンでは餌条件に関わらず生存率が高く，肉食によって成長速度や体重が増加した．シラホシハナムグリの結果は前記 2 種とは 明らかに異なっていた．肉食をしなかった幼虫は蛹化するのに 200 日以上かかったが（自然状態では幼虫越冬と推測された），肉食をした幼虫は，肉食をしなかった場合と同じ体サイズで 50 日程度を経て蛹化するか（成虫越冬と推測），もっと大きい体サイズで 175 日程度を経て蛹化するか（幼虫越冬と推測）の発育における二者択一の戦術が見られた．より速やかに，あるいは，より大きい体サイズで蛹化することの適応度上の利点はシラホシハナムグリにおいて明らかにされていないが，越冬前に羽化し秋や翌早春から繁殖活動を始めた成虫は，越冬後に羽化した成虫より適応度が高いと思われる．さらに，体サイズの大きい成虫は小さいものに比べ蔵卵数，交尾成功度，あるいは餌場をめぐる争いに有利であると思われる．したがって，シラホシハナムグリ幼虫にとって肉食性の鳥の巣は発育場所として価値が高いと考えられ，メス成虫は産卵場所として鳥の巣を選好するものと思われる．

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Manuscript received 8 March 2019;
revised and accepted 20 November 2019.