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ORIGINAL ARTICLE

Meat-eating enhances larval development of *Anthracophora rusticola* Burmeister (Coleoptera: Scarabaeidae), which breeds in bird nests

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Abstract

The scarab beetle *Anthracophora rusticola* Burmeister breeds in the nests of carnivorous birds including the honey buzzard *Pernis apivorus* (Linnaeus), Chinese goshawk *Accipiter soloensis* (Horsfield), oriental stork *Ciconia boyciana* Swinhoe and great cormorant *Phalacrocorax carbo* (Linnaeus). Although larvae of the beetle probably feed on animal matter (e.g. remnants of birds' food) in the nests of these birds, the effect of eating flesh on the growth of larvae has not been investigated. Laboratory experiments clearly showed that the development was faster, survival rate higher and body heavier just before pupation in larvae fed dried chicken meat with humus soil than in those fed only humus soil. We also investigated feeding behaviors of larvae of the beetle. A piece of chicken meat placed on the surface of humus soil was pulled under the soil by the larva, mostly at night. Video recordings made during the night showed that larvae came to the humus soil surface and ate the dried chicken meat or tried to take the meat under the soil. Our laboratory observations suggest that larvae in nature stay under the humus accumulated in bird nests during the daytime, and then come up to the surface mostly at night to actively seek and eat remnants of the birds' food.

Key words: *Accipiter soloensis*, carnivore, *Ciconia boyciana*, feeding behavior, larval development, *Pernis apivorus*, *Phalacrocorax carbo*.

INTRODUCTION

Anthracophora rusticola Burmeister is a member of the Scarabaeidae (subfamily Cetoniinae), which was a common species in thickets in the agricultural fields of Japan more than 40 years ago (Makihara *et al.* 2004). However, the beetle has declined in numbers in recent years and is considered to be threatened with extinction; it is listed in more than twenty Red Data Books published by local governments of Japan (e.g. Investigative Commission for Review of Red Data Book in Hiroshima 2003; Investigative Commission for Protective Measure of Rare Wildlife in Kagawa 2004; Okayama Prefectural Government 2009).

Anthracophora rusticola adults are well known to feed on tree sap and flower honey (Kurosawa 1985), but the biology of the larvae was not clearly known and they were presumed to feed on humus soil in the manner of other Cetoniinae (Okayama Prefectural Government & Okayama Prefectural Environmental Conservation Corporation Foundation 2003). Ishikawa and Arai (2004), Makihara *et al.* (2004) and Choi *et al.* (2008) reported finding the beetle in the nests of raptors, including the honey buzzard *Pernis apivorus* (Linnaeus) and Chinese goshawk *Accipiter soloensis* (Horsfield). Choi *et al.* (2008) observed *A. rusticola* larvae feeding on a dead nestling of the Chinese goshawk in its nest, and they proposed that the larvae feed on animal debris and prey remains in raptor nests. However, the effects of eating flesh on growth of larvae have not been investigated.

In this study, we compared the larval development of *A. rusticola* under feeding condition of humus soil with

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dried chicken meat with that of only humus soil in the laboratory to investigate the effect of meat eating in bird nests. We also observed the feeding behavior of larvae in the laboratory and discuss the feeding traits of this species in nature.

MATERIALS AND METHODS

Treatments of parental insects

Larvae of *A. rusticola* were collected in Mimasaka, Okayama Prefecture in August 2005 (Koshiyama in press). They were reared in a plastic case (28 cm long, 16 cm wide, 18 cm high) filled with humus soil made from broad-leaved tree leaves (Amigo Original insect Mat; Lic Co. Ltd, Okayama, Japan) at room temperature (25–27°C) and a natural photoperiod. After adults emerged, apple and banana slices were placed on the surface of the soil.

Adult beetles were moved outdoors for hibernation in October 2005. To prevent death of all adults during winter, adults were divided between two plastic cases: one case was kept in the open air in a field (8 individuals) and the other inside a greenhouse (11 individuals) at the campus of Okayama University. The cases were buried at ground level and a mesh container was used as a cover to protect the insects against heavy rain and/or warm sunlight. No food was given during winter. The cases were brought to a laboratory kept at 25°C and 60% relative humidity (RH) under a 16 h light : 8 h dark (LD 16:8) photoperiod in April 2006. Sliced apple and banana and a commercial food for pet beetles (Amigo Original Insect Jelly; Lic Co. Ltd, Okayama, Japan), which contains brown cane sugar, were introduced into the cases.

Because the number of eggs laid just after hibernation was not sufficient for experiments, we applied vernalization in the laboratory to force the adults to lay eggs again. This treatment comprised exposing the plastic cases containing adults to a series of consecutive conditions: at 25°C under LD 12:12 for 7 days, 20°C under LD 12:12 for 7 days, 15°C under LD 12:12 for 7 days, 10°C under LD 12:12 for 31 days, 15°C under LD 16:8 for 7 days and 20°C under LD 16:8 for 7 days. The vernalization treatment of the adults that had hibernated in the open-air field was conducted twice, terminating in November 2006 and August 2007. Vernalization of the adults that had hibernated in the greenhouse was conducted once and terminated in April 2007. Eggs were obtained after each treatment and used for experiments below.

Effect of meat-eating on larval development

Each egg was placed in a plastic cup (6.5 cm diameter, 4 cm high) filled with the humus soil described above. A piece of chicken meat (about 200 mg) that had been dried for 3 days by the window was added to the cup on the day the first instar larva hatched. Control larvae were not given the meat. The body weight of each larva was measured using an electrobalance (FX-300N; Seiken Co. Ltd, Ibaraki, Japan), and the larva's instar was recorded at 48 h intervals until pupation or death. The meat and humus soil were replaced every 2 and 7 days, respectively. Insects were incubated at 25°C and 60% RH under a LD 16:8 photoperiod. This experiment was conducted twice. Eggs obtained in November 2006 (20 individuals) and in August 2007 (10 individuals) were used for replicates I and II, respectively.

To analyze the effect of meat-eating on the rate of pupation (i.e. success in larval development), a logistic regression was conducted using JMP v6.0 software (SAS Institute, Cary, NC, USA).

Feeding behavior

During the rearing experiment, a piece of chicken meat was often found under the humus soil although we had placed it on the surface, suggesting the larva dragged the meat into the soil. To confirm this, we first investigated the time of day the behavior occurred. A third (i.e. last) instar larva was introduced into a tall plastic tumbler (6 cm diameter, 17 cm high) with humus soil 10 cm deep and a piece of dried chicken meat on top. Two days later, the chicken meat was removed, and a new piece attached to a thread 10 cm long was placed on the soil surface. The length of thread remaining on the soil surface was measured at 8 h intervals for 48 h. Next, we recorded the feeding behavior using an infrared video camera (Handycam DCR-H90 NTSC; Sony, Tokyo, Japan). A third instar larva was placed in a glass cup (5 cm diameter, 9 cm high) with humus soil. A piece of dried chicken meat was hung 1 cm above soil surface by a kite string. Video recording started at the beginning of the dark period and lasted 80 min.

Experiments were conducted in a laboratory kept at 25°C and 60% RH under a LD 16:8 photoperiod. Eggs obtained in April 2007 were reared until the third instar larva, as described above, and used for the experiments.

RESULTS

Effects of meat-eating on larval development

In replicate I, six of ten larvae given chicken meat pupated within 56 days after hatching. The remaining four larvae died in the first instar (1 individual), second

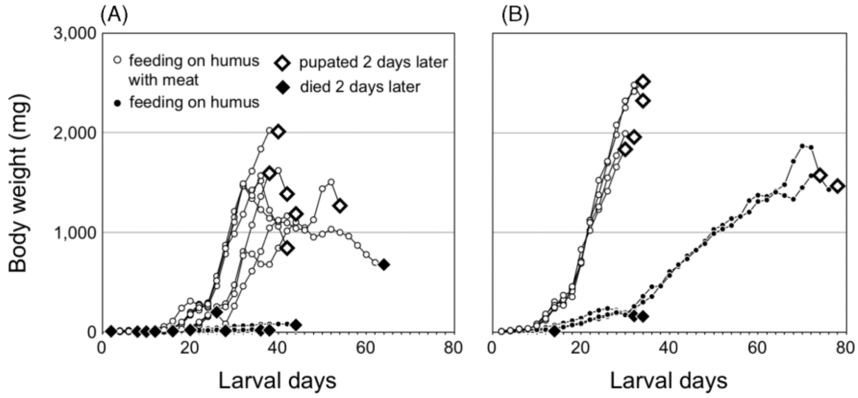

Figure 1 Changes in body weight of *Anthracophora rusticola* larvae fed humus soil with dried chicken meat or only humus soil. The weights were measured every two days after hatching until pupation or death. (a) Replicate I; (b) Replicate II.

Table 1 Logistic regression of pupation rate of *Anthracophora rusticola*

Factor	d.f.	χ^2	*P*
Treatment (presence of meat)	1	10.024	0.0015
Replicate	1	5.257	0.0219
Treatment × replicate	1	0.142	0.7067

instar (1 individual) or third instar (2 individuals). In contrast, larvae without chicken meat did not pupate at all, and their body weight increased little until death. The stage at the death of was the first (9 individuals) or second instar (1 individual) (Fig. 1a).

In replicate II, four of five larvae with chicken meat pupated within 36 days of hatching. The remaining larva, which died of an injury to the soft body surface just after molting and which was considered to have been caused by our handling, was removed from the analysis. Three of five larvae without chicken meat died at the first, second or third instars. The remaining two larvae pupated at 76 or 80 days after hatching. The average body weights of larvae with and without meat at 2 days before pupation were 2158 mg ($n = 4$) and 1521 mg ($n = 2$), respectively (Fig. 1b).

The rates of pupation were 60% with meat and 0% without meat in replicate I and 100% with meat and 40% without meat in replicate II. The logistic regression showed a significant difference in pupation rate between larvae with and without meat ($P < 0.01$), indicating that the chicken meat had a positive effect on larval development. The effect of replication on pupation rate was also significant ($P < 0.05$) (Table 1).

Feeding behavior

Four of six larvae pulled a piece of chicken meat attached to a thread into the humus soil within 48 h. The length of the thread remaining on the soil surface at the end of the experiment was 0 mm (2 individuals), 10 mm (2 individuals), 71 mm (1 individual) and 87 mm (1 individual). Most pulling of the meat with a thread into the soil occurred during the dark period (Fig. 2).

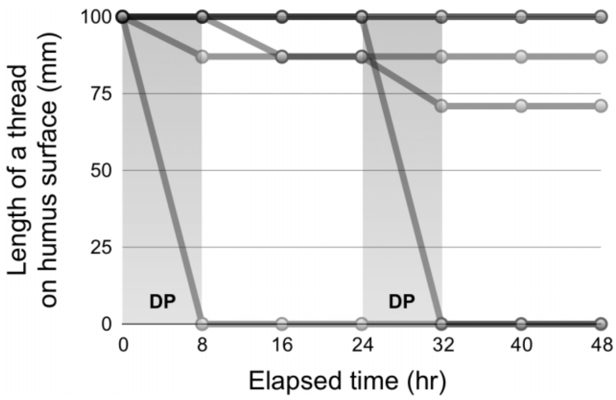

Figure 2 Length of thread remaining on the surface at 10 cm depth of humus soil in a tumbler with an *Anthracophora rusticola* larva under a LD 16:8 photoperiod. A piece of dried chicken meat was attached to the thread, and the length of the thread was measured every 8 h. The results for six individuals are shown. DP, dark period.

The video showed the behavior of one larva, which moved actively just under the soil surface and sometimes exposed its cephalothorax vertically in the open air, suggesting it was searching for food. Once the larva reached the piece of chicken meat attached to a string, it started to eat the meat. Another larva grasped the meat with its mandibles and tried to carry the meat backwards into the soil against the tension of the string. Thereafter, the larva emerged in its entirety on the soil surface and fixed the meat using its thoracic legs against the swinging of the string, and ate the meat (Appendix S1 in Supporting Information).

DISCUSSION

Results of our rearing experiments clearly showed that consuming chicken meat enhanced the development of *A. rusticola* larvae (Fig. 1, Table 1). However, chicken meat was not essential for larvae to pupate, although the speed of development was slower, survival rate lower and body weight just before pupation smaller in larvae fed only humus soil (Fig. 1b). Field observations of larva found both in the nests of carnivorous birds where animal matter is available (Makihara *et al.* 2004; Choi *et al.* 2008; Nasu *et al.* 2010), and in decaying plant matter including compost and a straw-thatched roof (Iga 1955), do not contradict our conclusion.

The logistic regression indicated significant effects not only of the presence of chicken meat, but also which of two replicates, on the pupation rate (Table 1). The different times and/or timing of vernalization of parental adults of each replicate might have caused a physiological difference in offspring. Alternatively, because eight parental adults were reared in one plastic case, different adult(s) might have produced offspring in each replicate, causing a genetic difference between them. Another possibility is a difference in the content of humus soil used in each replicate. Because the soil was a commercial product, we could not control its quality.

Our observation of feeding behavior in the laboratory suggests that larvae stay under the humus accumulated in a bird nest during the daytime, and then come up to the surface mostly at night to actively seek and eat regurgitated meat intended for the chicks. This feeding behavior is nocturnal, probably to avoid not only accidental injury by a bird's talons but also predation by birds: a gray-faced buzzard *Butastur indicus* (Gmelin) was observed to pick up a Scarabaeoidea beetle larva from inside the nest in its bill and eat it (Watanabe & Koshiyama in press), although breeding of *A. rusticola* in a nest of gray-faced buzzards has not yet been documented.

Breeding of *A. rusticola* in bird nests was first discovered by Ishikawa and Arai (2004) and Makihara *et al.* (2004). The former reported "a raptor" as the host bird and the latter the honey buzzard, a threatened bird of Japan (Ministry of the Environment Government of Japan 2006). Sato *et al.* (2006) considered that the beetle breeds specifically in the raptor's nest and that the decline of the beetle might be due to the reduction of breeding populations of raptors in Japan. Nasu *et al.* (2010) discovered the beetle breeding in nests of the oriental stork *Ciconia boyciana* Swinhoe, a critically endangered bird in Japan (Ministry of the Environment Government of Japan 2006), and mentioned the possibility that the beetle could be found in the nests of other carnivorous or omnivorous birds, and also in manure made from domestic animal excreta. They argued that the decline in the beetle population was caused by not only a decline in raptor populations but also in a change in the traditional agricultural environment in Japan, including a decrease in such birds and open-air storage of manure.

Interestingly, the beetle was recently found in nests of the great cormorant *Phalacrocorax carbo* (Linnaeus) (Yamamoto 2010). Before 1920, cormorants were widely distributed throughout Japan. However, their population has decreased due to human activities, resulting fewer than 3000 cormorants in 1971. From the late 1970s onwards, the number of cormorants began to increase and there were an estimated 50 000–60 000 great cormorants in 2002 in Japan (Fukuda *et al.* 2002). Future studies should pay attention to future trends in *A. rusticola* populations and living environments.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Feeding behavior in *Anthracophora rusticola*.

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