Research Article

Effect of Carcass Size on Feeding Modes of Larvae of *Nicrophorus quadripunctatus* Kraatz (Coleoptera: Silphidae)

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In the parental care of burying beetles of *Nicrophorus*, the role of males has not been clearly elucidated. To test our hypothesis that the investment in resource manipulation by males influences the feeding of larvae by males, we investigated parental efforts of *N. quadripunctatus*. On the small carcasses, the time spent on resource manipulation by males was short, and the males left the carcasses without feeding the larvae (maternal feeding). On the large carcasses, the males spent a long time on resource manipulation, and the male participated in the feeding of larvae (biparental feeding). This suggests that one of the reproductive roles of males in the absence of predators and/or competitors is resource manipulation, and the paternal efforts change depending on carcass size. A longer time spent on resource manipulation by males may be a trigger for the males to participate in the feeding of larvae on large carcasses.

1. Introduction

Burying beetles of *Nicrophorus* (Coleoptera: Silphidae) are subsocial insects exhibiting an elaborate system of biparental care. The parents prepare a small vertebrate carcass as a food resource for their offspring and defend the carcass from predators and/or competitors. The parents enter the carcass, remove its hair or feathers, shape the carcass into a brood ball, and deposit anal or oral secretions around it. This series of behaviors is known as resource manipulation. Additionally, the parents feed their larvae by regurgitating food and repair the crypt as needed [1–3].

The breeding schedule after pair formation is divided into the resource manipulation period and the feeding period. Although previous studies have focused on feeding behaviors to the brood, resource manipulation prior to feeding has been little examined to date.

It was thought that one important role of males in this biparental care was their defense against predators and/or competitors, supported by the fact that the presence of both parents on a carcass decreases the risk of a takeover by other beetles (*N. orbicollis*: [4, 5]; *N. defodiens*: [6]). However, many

studies have found that in the absence of competitors under benign conditions in the laboratory, there is no evidence that the participation of a male in the feeding of larvae confers any advantages on the survival or growth of the brood [1, 7–10]. Some studies reported that female burying beetles attacked males that remained during the feeding period [3, 11]. Thus, the adaptive significance of the presence of a male and the contribution of feeding by a male to larval development and growth have not yet to be fully elucidated.

Xu [11] reported that resource manipulation in *N. quadripunctatus* was carried out by both females and males but that thereafter, there appeared to be two modes of feeding: biparental feeding, in which larvae were fed by both a female and a male, and the uniparental (maternal) feeding, in which larvae were fed by only a female. To elucidate how these two modes of feeding occur, it is necessary to analyze male and female behaviors during the resource manipulation period prior to the feeding of larvae. We hypothesized that the investment in resource manipulation by males influences the feeding of larvae by males.

In the present study, to test our hypothesis, we analyzed the behaviors of *N. quadripunctatus* males and females in

the resource manipulation period under different amounts of resources, and the following questions in particular were addressed. (1) Do differences in the amount of resources influence the resource manipulation of males and females? (2) Do differences in resource manipulation behaviors influence a feeding mode? (3) Do female attacks against males influence the participation of a male in the feeding of larvae?

2. Materials and Methods

Bait trap surveys by Kouge [12] showed two peaks in the appearance of *N. quadripunctatus* adults in northern Kyushu, Japan, in April to May and September to October. Thus, the beetles were collected using traps baited with chicken at Mt. Hinokuma, Kanzaki, Saga (33°20'N, 130°21'E) and at Mt. Kinryu, Saga (33°20'N, 130°18'E), in May and September, 2004, 2005, 2006, and 2008. The collected beetles were reared individually in plastic cups (10 cm in diameter and 4 cm in depth) with a little soil in a laboratory at 20°C under a 12 hours light and 12 hours dark (12 L12D) photoperiod condition. Each beetle was supplied with approximately 0.2 g of chicken every 3 days as food.

All experiments were carried out in a laboratory at 20°C under a 12L12D photoperiod condition. We used chicken pieces because it was impossible to prepare exact amounts of mouse carcasses. The chicken pieces were wrapped in tissue paper in imitation of the skin of carcasses.

Eleven, 26, and 10 male-female pairs were individually released into 11, 26, and 10 containers (7 cm in diameter and 14 cm in depth, with soil about 5 cm in depth) with large (25 g), medium (10 g), and small (5 g) chicken pieces as carcasses, respectively. The reproductive behaviors of the males and females were examined using a video camera (SONY, CCD-TRV80) for one hour (09:00 to 10:00) every day from the day when the male-female pairs were released on the carcasses (Day 1) to the day when hatchlings first appeared on the carcasses.

In all cases in which a male did not appear on the video for one hour, the male did not later reappear on the video and was considered to have left the carcass. The period from the day when a male was released on the carcass (Day 1) to the day when the male disappeared from the carcass was regarded as the residence time of the male on the carcass.

Although the parent beetles manipulated the resource by conducting simultaneously both anal secretion and oral one, it was difficult to examine quantitatively both behaviors of anal secretion and oral one at the same time. Thus, we examined the depositing oral secretion as resource manipulation. Each pair copulated many times during resource manipulation. Furthermore, the females often showed aggressive behaviors in which they dashed up to males and bit the male's legs. We examined the time spent on resource manipulation by each male and each female, the frequency of copulations, and the frequency of aggressive behaviors by a female against a male for 1 hour every day.

Each carcass was manipulated by both a female and a male, but thereafter, there were two modes of feeding: biparental and maternal.

If the male was present when the hatchlings arrived at the carcass, we noted whether the male fed the larvae (biparental feeding) or not (maternal feeding).

The number and body weight of larvae were measured on the day when they dispersed from the carcasses for pupation.

2.1. Statistical Analysis. The arrival time of hatchlings on the carcass, the time spent of resource manipulation by males and females, the frequency of copulations, and the number and biomass of larvae dispersed from the carcasses were analyzed using the generalized linear model with a Gaussian distribution and an identity link function, following square-root transformed. Carcass size and feeding mode were included as covariates. We used Steel-Dwass test for multiple tests. The days elapsed after the commencement of the experiments were included as a covariate. The proportion of biparental feedings was compared among the carcass sizes, using Fisher's exact probability test. Change in the proportion of males remained on the small and medium carcasses where maternal feeding occurred was analyzed using Log-Lank-test. Correlation of the frequency of aggressive behaviors by a female against a male and the residence time of the male on the carcass was analyzed using Spearman's rank correlation. Using generalized linear models, we conducted analysis of deviance with a binomial error distribution and a logit link function to model a trigger for the males to participate in the feeding of larvae on the medium carcasses. We used five independent variables in the analysis: frequency of copulations, arrival time of hatchlings on the carcass, frequency of aggressive behaviors by a female against a male, time spent on resource manipulation by males, and time spent on resource manipulation by females.

3. Results

3.1. Frequency of Copulation. The frequency of copulations did not differ among carcass sizes ($P = .0503$) and feeding mode ($P = .2262$), but differed significantly among the first ($n = 46$, 0.25 ± 0.08 (mean $\pm$ SE)), second ($n = 39$, 0.08 ± 0.04), and third days ($n = 27$, 0.00 ± 0.00) (Friedman test, $P = .0018$).

3.2. Proportion of Biparental Feedings. On the large carcasses, maternal feeding was observed on only one carcass, which was then excluded from our analysis because parental behaviors were abnormal in this case. The parents spent an extremely short time on resource manipulation (about 17% of the time spent on resource manipulation by normal beetles), and the male was frail and died immediately following the experiments.

The proportions of biparental feedings on the small, medium and large carcasses were 0%, 31%, and 100%, respectively (Fisher's exact probability test, $P < .0001$).

3.3. Arrival Time of Hatchlings on the Carcass. The arrival time of the hatchlings on the carcass differed among carcass sizes ($P = .0487$) but did not differ among feeding modes ($P = .7106$) (small carcass: $n = 10$, 3.9 ± 0.3 days (mean $\pm$ SE), medium carcass: $n = 26$, 4.0 ± 0.1 days, and large

FIGURE 1: The proportion males of *N. quadripunctatus* remained on the small (solid circle) and medium carcasses (open circle) where the maternal feeding occurred.

carcass: $n = 10$, 4.5 ± 0.2 days). The arrival time of hatchlings on the medium carcasses was significantly earlier than that on the large carcasses, on which all feedings were biparental (Steel-Dwass test, $P = .0193$).

3.4. Leaving Time of Males from the Carcass. Changes in the proportion of males remained on the small and medium carcasses where maternal feeding occurred are shown in Figure 1. The males left small carcasses earlier than medium ones (Log-Lank-test, $P = .0106$). On the small and medium carcasses, 90% and 50% of males, respectively, left the carcasses before the hatchlings arrived. On the medium carcasses, furthermore, 38.5% of the males that were present when the hatchlings arrived left the carcasses without feeding the larvae.

3.5. Aggressive Behavior by a Female against a Male. The frequency of aggressive behaviors by a female against a male did not differ significantly among carcass sizes ($P = .3898$) and between feeding modes ($P = .1646$), and it did not influence the residence time of the male on the carcass (Spearman's rank correlation, $P = .5148$).

3.6. Time Spent on Resource Manipulation by Males. The time spent on resource manipulation by males depended significantly on carcass size ($P = .0051$) and feeding mode ($P < .0001$, Figure 2(a)). Although the time spent on resource manipulation by males did not differ significantly between maternal feedings on the small and medium carcasses (Steel-Dwass test: $P = .2387$) and between biparental feedings on the medium and large carcasses (Steel-Dwass test: $P = .0772$, Figure 2(a)), it differed significantly between maternal feedings on small carcasses and biparental feedings on medium (Steel-Dwass test: $P = .0099$) and large ones

FIGURE 2: Time spent on resource manipulation by males (a) and females (b). Bars show SE. Different letters indicate a significant difference (Steel-Dwass test: $P < .05$). MF: Maternal feeding, BF: Biparental feeding.

(Steel-Dwass test: $P = .0012$), and between maternal feedings on medium carcasses and biparental feedings on medium (Steel-Dwass test: $P = .0278$) and large ones (Steel-Dwass test: $P < .0001$, Figure 2(a)).

3.7. Time Spent on Resource Manipulation by Females. The time spent on resource manipulation by females was found to depend significantly on carcass size ($P = .0268$) and feeding mode ($P = .0332$, Figure 2(b)), and it differed significantly between maternal feedings on small carcasses and biparental feedings on large carcasses (Steel-Dwass test: $P = .0133$, Figure 2(b)).

TABLE 1: Analysis of deviance from generalized linear model for the males to participate in the feeding of larvae.

Independent variable	df	Deviance	Resid. df	Resid. Dev	P
Frequency of aggressive behaviors by a female against a male	1	0.002	22	29.104	.968
Arrival time of hatchlings on the carcass	1	2.739	23	29.105	.098
Frequency of copulations	1	0.252	24	31.844	.615
Time spent on resource manipulation by females	1	0.003	21	29.1	.954
Time spent on resource manipulation by males	1	15.58	20	13.52	<.0001

3.8. Number and Biomass of Larvae Dispersed from the Carcass. The number of larvae dispersed from the carcasses depended significantly on carcass size ($P = .0007$) and feeding mode ($P = .0031$), and it differed significantly between the large and the medium carcasses (Steel-Dwass test: maternal feeding, $P = .0128$, biparental feeding, $P = .0127$) and between the large and the small ones (Steel-Dwass test: $P < .0001$, Figure 3(a)). The biomass of larvae dispersed from the carcasses differed significantly among carcass sizes ($P = .0250$) and between feeding modes ($P = .0425$, Figure 3(b)).

3.9. Male's Decision to Participation in the Feeding. The significant effect on the male's decision to participation in the feeding was found only in the time spent of resource manipulation by males (Table 1).

4. Discussion

The frequency of aggressive behaviors by a female against a male did not affect the residence time of the male on the carcass. Therefore, we consider that the male is not ejected from the carcass by the female, but rather chooses the time to leave the carcass for himself. Müller et al. [13] reported that females of *Nicrophorus vespilloides* Herbst were able to recognize their male partners. Then, when a male and a female collaborate in a breeding attempt, they usually do not exhibit aggressive behavior toward each other, but do attack newly intruding conspecifics that attempt to usurp the carcass [13]. In this study, however, female's aggressive behavior was observed. Although the reason why the females attack the males is unknown, their recognition to the partner may be incomplete in *N. quadripunctatus*.

This study showed short time spent on resource manipulation and no feeding of larvae by males on the small carcasses. On the medium carcasses, on the other hand, the time spent was longer on the carcasses with biparental feeding than on those with maternal feeding (Figure 2(a)). Furthermore, time spent on resource manipulation by males had a significant effect on male's decision to participation in the feeding (Table 1). This suggests that the male determines the time to be spent on resource manipulation and his residence time in response to carcass size.

As Smiseth and Moore [14] suggested, there is a possibility that on the large carcasses, the residence time of males elongates and males encounter the larvae that beg a feeding from the males, and then they participate in the feeding of

larvae. However, on the medium carcasses, approximately 40% of the males that were present when the hatchlings arrived left the carcasses without feeding the larvae, though the reason was unknown. These results may show that the presence of larvae on the carcasses and/or begging behavior by the larvae [15, 16] do not necessarily lead for males to participate in the feeding of larvae.

The present results indicate that one of the reproductive roles of *N. quadripunctatus* males in the absence of predators and/or competitors is resource manipulation, and that paternal efforts change depending on carcass size. Only the time spent of resource manipulation by males significantly affected on the male's decision to participation in the feeding (Table 1). A longer time spent on resource manipulation by males may be a trigger for the males to then participate in the feeding of larvae on large carcasses.

Although on the medium carcasses, the time spent on resource manipulation by males was shorter on the carcasses with maternal feeding than on those with biparental feeding, the time spent on resource manipulation by the female did not differ significantly between maternal feeding and biparental feeding. Rauter and Moore [17] reported that widowed males increased their effort on the feeding of larvae, whereas widowed females showing no change in their effort, and that the time spent of parental care by females increased slightly with increasing brood size. These suggest that although females are working at their maximum for parental activities, males may be able to adjust their care.

Therefore, we may consider that males are influenced more strongly than females by an immediate benefit (the number of offspring). If the benefit is great enough (enough offspring), the males stay; if not, they abandon the larvae and seek further mating opportunities [17]. We consider that on the large and small carcasses, the males can assess the number of larvae which correlates to the amount of resource, according to the information for the time spent on resource manipulation. On the medium carcasses, however, it may be hard to make a decision whether the males participate feeding larvae or do not. This suggests that males may be able to tune their reproductive efforts more finely than females based on carcass size.

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FIGURE 3: The number (a) and biomass (b) of larvae dispersed from the carcasses in relation to the carcass size and feeding mode. Bars show SE. Different letters indicate a significant difference (Steel-Dwass test: $P < .05$). MF: Maternal feeding, BF: Biparental feeding.

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