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Asynchronous hatching and brood reduction by filial cannibalism in the burying beetle *Nicrophorus quadripunctatus*

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Running title

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Abstract

Despite decades of intensive research, there is much debate about the adaptive significance of asynchronous hatching. A major obstacle in understanding the significance of this process is the difficulty in separating the hypotheses that explain asynchronous hatching as an adaptive trait from those that explain it as a by-product of physiological constraints on hatching or egg laying patterns. We investigated the burying beetle *Nicrophorus quadripunctatus*, a species in which the parent can eliminate less-adaptive offspring (e.g. slower-growing offspring) by filial cannibalism and adjust the age structure of offspring to adaptive pattern. The main aim of this study was to determine the age composition of offspring that survived and to determine the effect of larval growth on filial cannibalism. We investigated how the point in time at which each group of larvae hatched affects the timing of filial cannibalism by the female parent. We found that *N. quadripunctatus* exhibited asynchronous hatching, and reared larvae of different ages. We also found that larvae hatching at latter intervals had lower survival and growth rates; therefore, filial cannibalism plays a role in eliminating later-arriving, slower-growing, and hence less-adaptive offspring.

Keywords: *Nicrophorus*; burying beetle; asynchronous hatching; filial cannibalism; sibling competition

Introduction

Asynchronous hatching refers to the time span across which a clutch hatches, from the hatching of the first egg to the hatching of the last egg (Stenning 1996). This process usually establishes competitive asymmetries within the brood, with the younger siblings facing higher risk of mortality from starvation and showing slower growth, because they typically obtain less food. Many hypotheses have been proposed to explain how selection might favour asynchronous hatching in spite of the higher mortality risk of the youngest siblings (Magrath 1990; Stoleson and Beissinger 1995; Stenning 1996). There are 2 main groups of hypotheses. One group of hypotheses explains that asynchronous hatching provides a mechanism to increase fitness (e.g. peak-load–reduction hypothesis (Hussell 1972), brood-reduction hypothesis (Lack 1954), sibling-rivalry hypothesis (Hahn 1981), insurance hypothesis (Stinson 1979) and sex-ratio–manipulation hypothesis (Slagsvold and Lifjeld 1989)). The other group of hypotheses explains that asynchronous hatching is a by-product of physiological constraints on egg laying patterns and selection for the early onset of incubation (nest-failure hypothesis (Clark and Wilson 1981), limited-breeding–opportunity hypothesis (Beissinger and Waltman 1991) and egg-viability hypothesis (Arnold et al. 1987)). Despite decades of intensive research on asynchronous hatching in birds, none of the hypotheses has gained overall support, and the reason why asynchronous hatching has evolved in altricial birds is still unclear (Magrath 1990; Stoleson and Beissinger 1995; Stenning 1996). A major reason for the failure to verify the reason is the close relationship between the onset of incubation and asynchronous hatching in birds. Because of this physiological constraint, separating the hypotheses explaining asynchronous hatching as an adaptive trait and those explaining it as a by-product of selection for the early onset of incubation is difficult in altricial birds (Stenning 1996). However, asynchronous hatching is not restricted to altricial birds, but is known over a range of taxa, e.g. in the White's skink *Egernia whitii* (While et al. 2007; While and Wapstra 2008), in the woodroach *Cryptocercus punctulatus* (Nalepa 1988) and in the burying beetle *Nicrophorus vespilloides* (Müller 1987; Müller and Eggert 1990; Smiseth et al. 2006). Using a non-avian species allowed us to test each hypothesis separately from the onset of incubation.

The burying beetles, *Nicrophorus* spp., provides a particularly valuable system because, as in altricial birds, both parents provide elaborate care to their offspring, including food provisioning (Eggert et al. 1998). *N. quadripunctatus*, alike other species of the same genus, uses the carcass of small vertebrates (e.g. bird chick and small mouse) as a food resource for their larvae. Females lay eggs in the soil near the carcass. In *N. vespilloides*, the larvae hatch asynchronously over a mean period of 27 h (range 8–56 h) on a 10-g carcass (Müller and Eggert 1990) and a mean period of 30 h (range 8–56 h) on a 25-g carcass (Smiseth et al. 2006). After hatching, the larvae crawl to the carcass and obtain some food by begging for pre-digested carrion from their parents and some by self-feeding on the carcass. *Nicrophorus* spp. can directly regulate the number of offspring by filial cannibalism (Bartlett 1987); therefore, the parent can eliminate less-adaptive offspring (e.g. slower-growing offspring) from an asynchronously hatching brood and adjust the age structure of offspring to adaptive pattern. To address whether asynchronous hatching in *Nicrophorus* is an adaptive trait or a by-product of physiological constraints on egg laying patterns, it is important to demonstrate how the point in time at which each larva hatches affects filial cannibalism by the parent; however, there is currently no information on these effects. We predict that if asynchronous hatching is a by-product of physiological constraints on egg laying patterns and incur a fitness cost to parents, parents tend to kill later hatching offspring and synchronize the age structure of offspring. Conversely, if asynchronous hatching in *Nicrophorus* has been evolved as an adaptive trait, we predict that parents maintain the age structure of offspring.

The purposes of this study were to (1) determine the age composition of larvae that survive until they grow to the stage at which no parental care is required, and (2) determine the effect of larval growth on filial cannibalism in *N. quadripunctatus*. To our knowledge, this is the first report that documents the consequence of direct parental regulation on asynchronous broods.

Materials and methods

(a) Collection and maintenance of the beetles

We collected 60 adult *N. quadripunctatus* Kraatz in baited pitfall traps in Chiba, Japan, and reared first-generation offspring in the laboratory. The beetles were maintained individually in small transparent plastic cups (height 4 cm, diameter 6 cm) at 20 ± 1 °C under a 14:10 h light:dark cycle. They were fed small pieces of chicken meat twice a week. All males and females used in this experiment were sexually mature and ranged between 21 and 35 days of age.

(b) General experimental procedure

Twenty-three pairs (10 pairs for behavioural observation, 13 pairs for measuring survival rate and growth) of randomly selected, non-sibling, virgin male and female beetles were each placed in a plastic cup (height 8 cm, diameter 15 cm) filled with 2 cm of moist peat and were provided with 15 ± 0.5 g of chicken meat (15 g of meat is an appropriate amount for rearing larvae (Suzuki and Nagano

2007)). We placed them in a dark incubator at 20 ± 1 °C. After 93 h, the female and the meat were transferred to a new plastic cup filled with 2 cm of moist peat. The male was removed from the old plastic cup at this stage because male care has no effect on larval growth or survival under laboratory conditions (Takata, unpublished study). The eggs were left to hatch.

We recorded parent and offspring behaviour under infrared light to investigate cause of larval death. First, we checked for hatching at 8-h intervals and transferred newly hatching offspring to the cup containing their mother. Second, parent and offspring behaviour were recorded using a video camera (HOGA, HCIR-41F690) under infrared light until the age at which the larvae dispersed from the carcass. We noted the number of hatching and surviving larvae at 8-h intervals. If some larvae disappeared from the brood, we checked the video and investigated the cause of the larvae's death. We defined a larva that was bitten by the parent as one that died because of filial cannibalism. We defined a larva that appeared shrunken and that died near the carcass as one that died because of hunger or infection. Additionally, we weighed larval body mass at 8-h intervals until dispersal, to obtain general information on larval growth. The age of dispersal is defined as the day at which the larvae left the crypt surrounding the carcass. Dispersal from the carcass is synchronous and occurs normally when the earliest hatched larvae are 144 h old.

To investigate the hatching pattern and measure the growth and survival rate of different-aged larvae, we noted the number of hatching and surviving larvae at 4-h intervals and measured their body mass. First, we checked for hatching larvae at 4-h intervals and transferred newly hatching offspring to the cup containing their mother. Then, we used the hatching time of each larva from the onset of hatching as the time of hatching in the subsequent analysis. Second, to measure the growth of each group of larvae that hatched at the same time interval, we individually weighed living larvae that were on the carcass at 6-h intervals until the age at which they dispersed from the carcass. Because of their rapid growth, different-aged larval body mass was determinably different from each other; therefore, we could identify the hatching time of each larva in each measurement of body mass. We confirmed in the pilot study that no larvae caught up to the body weight of earlier hatching larvae, therefore we could identify the time of hatching of each larva and growth of each group of larvae using this method. But, we could not identify the body mass of individual larva, because the larvae which hatched at the same time interval exhibited similar growth, we calculated mean body mass of each group of larvae and used it to calculate the growth rate of each group of larvae in the subsequent analysis. We used the number of each group of larvae in each brood that had survived to the age at which the larvae dispersed from the carcass in the subsequent analysis for survival. We confirmed in the pilot study that our experimental manipulation did not affect larval survival.

(c) Statistical analysis

First, the effects of the point in time at which each group of larvae hatched and clutch size on offspring survival were analysed using a generalized linear mixed model (GLMM) with the lme4 package (Bates and Maechler 2010). Survival rate was treated as a response variable assuming a binomial distribution, hatching time and clutch size as an explanatory variable and brood identity as a random factor. Second, to test the effect of clutch size on hatching pattern, we used two different indices for asynchronous hatching patterns, hatching spread and hatching skew, in the following analysis. Hatching spread is the time between hatching of the first and that of the last larva from each brood (Smiseth et al. 2006). Hatching skew is an index of the degree to which hatching was skewed towards the part of hatching period. Hatching skew index, V(t), was calculated as follows:

$V(t) = \sum(T_i - T_m)/T_m \times P_i$

where T_i refers to a particular time interval of the hatching period ranging between 0 and n, T_0 refers to the first time interval of the hatching period, T_n refers to the last time interval of the hatching period, T_m refers to the midst time interval of hatching period ($T_m = (T_n - T_0)/2$), and P_i refers to the proportion of the larvae that hatched a particular time interval of the hatching period. A hatching skew index approaching a value of -1 indicate that hatching was skewed toward the earlier hatching period, and it approaching a value of 1 indicate that hatching was skewed toward the later hatching period. More detailed information is presented on Smiseth et al. (2008). The effects of clutch size on hatching spread and hatching skew were analysed using a generalized linear model (GLM), hatching spread and hatching skew was treated as a response variables assuming a binomial distribution, clutch size as an explanatory variable. Third, correlation between the hatching spread and hatching skew was analyzed using a GLM. Hatching skew was treated as response variables assuming a Gaussian distribution, hatching spread as an explanatory variable. Finally, the effects of the point in time at which each group of larvae hatched on the growth rates of each group of larvae were analysed using a GLMM. Growth rate was treated as a response variable assuming a Gaussian distribution, hatching time as an explanatory variable and brood identity as a random factor. Methods for calculation of the growth rates were described below. In this study, we could not identify the body mass of each larva which hatched at the same time interval. So, we calculated mean body mass of each group of larvae and used it to calculate the growth rate of each group of larvae. The body mass of *N. quadripunctatus* larvae increased exponentially within the first few days. We transformed the first 24 h of larval body masses which were weighed 6 h intervals into logarithmic values to calculate growth rate; therefore there were 4 data points for each group of larvae. Then, we calculated slopes of the regression line of log (body mass) on time as growth rates. The slope of the regression line for each group of larvae was calculated as follows:

$\sum(X_i - X_m)(\log Y_i - \log Y_m)/(X_i - X_m)^2 \times 100$

where X_i is the time after hatching, Y_i is mean body mass (mg) for each group of larvae at time X_i, and X_m and Y_m are the mean values of X and Y. All correlation coefficient values were >0.97. We performed all analyses using R 2.12.1 GUI 1.35 (http://cran.r-project.org). Data are expressed as mean ± SD.

Results

(a) The cause of larval death and general information on larval body mass

We observed 156 larvae hatched from 10 clutches. Sixteen out of 156 larvae had died before dispersing from the carcass. Our video analysis revealed that 14 larvae (1.4 ± 1.7 per clutch) were killed by their female parent and 1 larva died as a result of hunger or infection. We could not determine the cause of death of the remaining dead larva.

The mean larval body mass at hatching was 1.9 ± 0.3 mg and larvae grew up to 13.1 ± 3.0 mg by age 24 h, 38.5 ± 14.0 mg by age 48 h, 103.8 ± 38.2 mg by age 72 h and 172.7 ± 53.5 mg by age 96 h. The mean larval weight at the dispersal stage (at age 120 h) was 193.1 ± 57.9 mg.

(b) Number of hatching larvae and larvae that survived

N. quadripunctatus exhibits asynchronous hatching (Fig. 1, sample size: 13 clutches). The number of hatching larvae was skewed towards earlier hatching periods. Hatching spread ranged from 4 to 56 h (25 ± 12 h). The mean number of hatching larvae was 22 ± 8. Hatching skew ranged from -0.52 to 0.06 (-0.15 ± 0.27). Clutch size did not have a significant effect on hatching spread (GLM: estimate = 0.368, $t = 0.942$, $p = 0.365$) and hatching skew (GLM: estimate = -0.006, $t = -0.372$, $p = 0.719$). There was no significant correlation between hatching spread and hatching skew (GLM: estimate = -0.004, $t = -0.900$, $p = 0.378$).

Larvae of different ages survived until dispersal (Fig. 1). The hatching spread across larvae that survived (17 ± 9 h) was lower than the hatching spread across the entire brood. Larvae hatching at latter intervals had lower survival (GLMM: estimate = -0.098, $z = -4.380$, $p < 0.001$, sample size:

219 larvae from 13 clutches). The mean number of larvae that survived was 11 ± 4. Clutch size did not have a significant effect on offspring survival (GLMM: estimate = -0.036, $z = -0.858$, $p = 0.391$).

(c) Effect of hatching time on growth rate

The point in time at which each group of larvae hatched had a significant negative effect on the growth rate (Fig. 2, GLMM: estimate = -0.001, $F_{1, 40} = 4.435$, $p = 0.042$).

Discussion

We found that *N. quadripunctatus* exhibited asynchronous hatching, and the number of hatching larvae was skewed towards earlier hatching periods. Our study demonstrated that the female parent decreases hatching spread by filial cannibalism, but still rears larvae of different ages. The point in time at which each group of larvae hatched had a significant negative effect on the growth rate. Additionally, we found that later hatching offspring faced higher risk of mortality from filial cannibalism by the female parent; therefore, filial cannibalism plays a role in eliminating later-arriving, slower-growing, and hence less-adaptive offspring. To our knowledge, this is the first demonstration of how the point in time at which each group of larvae hatched influences larval growth and filial cannibalism by the female parent in an asynchronous hatching brood.

The point in time at which each group of larvae hatched had a significant negative effect on the growth rate, suggesting that larvae hatching at latter intervals had lower growth rate. In this study, we calculated mean body mass of each group of larvae and used it to calculate the growth rate of each group of larvae. Since the number of larvae that hatched was not the same in each interval, the mean body mass used to calculate the growth rate is based on inevitably unbalanced group size. The result could be biased due to the unbalanced group size. However, our results are corresponding to the results reported in the recent study on other species of burying beetle *N. vespilloides* (Smiseth et al. 2007). Smiseth et al. (2007) used an experimentally established brood of *N. vespilloides* and found that later hatching larvae grew less than earlier hatching larvae when the female parent provided care for them. These findings suggest that there is age-based asymmetric sibling competition. Interestingly, asynchronous hatching also forms competitive asymmetries among siblings in many altricial birds (Magrath 1990; Stoleson and Beissinger 1995; Mock and Paker 1997). The brood-reduction hypothesis explains that asynchronous hatching provides a mechanism by which asymmetric sibling competition can reduce broods when resources are limited (Lack 1954). It predicts that competitively disadvantaged offspring have a higher mortality risk resulting from sibling competition when resources are limited. Coincidently, our results show that later hatching offspring faced higher mortality risk; however, the burying beetle directly reduces its brood by filial cannibalism (Bartlett 1987). It is therefore unlikely that the mechanism for brood reduction promotes the evolution of asynchronous hatching in the burying beetle.

Larvae hatching at latter intervals had lower survival. Our video analysis revealed that the major cause of larval death in this species was from filial cannibalism by the female parent; therefore, the negative effect of hatching time on survival rate suggests that later hatching offspring face a higher risk of mortality from filial cannibalism. Furthermore, larvae hatching at latter intervals had lower survival. These findings suggest that the female parent is more likely to kill the offspring that exhibit a slower growth rate; therefore, our study revealed that filial cannibalism by the female parent plays a role in eliminating later-arriving, slower-growing, and hence less-adaptive offspring, although its primary role is thought to be to regulate the number of offspring to the amount of carcass provided as food (Bartlett 1987).

In this study, clutch size did not have a significant effect on offspring survival, hatching spread and hatching skew. Moreover, there was no significant correlation between hatching spread and hatching skew. These findings suggest that hatching spread and hatching skew are independent variables. Thus, future studies to investigate the adaptive consequence of asynchronous hatching need to consider not only hatching spread but also hatching skew. Similar results was reported from Smiseth et al. (2008). They investigated the effect of clutch size on hatching spread and hatching skew and the correlation between hatching spread and hatching skew on five different carcass sizes in *N. vespilloides*. They found that there was no correlation between hatching spread and hatching skew, but these two indices were significantly influenced by clutch size. In contrast to Smiseth et al. (2008), clutch size did not have significant effect on hatching spread and hatching skew in this study. This difference may be due to a difference in methodology. Smiseth et al. (2008) have tested the effect on five different carcass sizes (5 – 25 g), while our study tested the effect of clutch size on hatching spread and hatching skew only on 15 g carcass. Therefore, the smaller variations in clutch size may reduce statistical power in our study. Further studies are needed to investigate the potential linkage between hatching spread and hatching skew.

Our study demonstrated that the female parent rears larvae of different ages. Smiseth et al. (2008) and Smiseth and Morgan (2009) established three types of broods with different hatching pattern, synchronous, moderately asynchronous and highly asynchronous, with a hatching span of 0, 24 and 48 h. Smiseth and Morgan (2009) found that offspring survival is lower in highly asynchronous broods than in synchronous or aynchronous broods and Smiseth et al. (2008) found that offspring survival is higher in moderately asynchronous broods than in either synchronous or highly asynchronous broods. These findings suggest that there is an optimal length of hatching spread. In this study, the hatching spread across larvae that survived was lower than the hatching spread across the entire brood. Furthermore, larvae hatching at latter intervals had lower survival. Our results suggest that the female parent decreases hatching spread by filial cannibalism. These findings imply that the optimal length of hatching span in *N. quadripunctatus* is lower than the observed length of hatching span and female parent regulate the length of hatching span to optimal length by filial cannibalism. To understand the adaptive significance of asynchronous hatching in *Nicrophorus*, further studies are needed to investigate the effect of hatching pattern on offspring survival and growth. Additionally, in this study, we focused on the effect of filial cannibalism by the female parent on age structure of offspring, and male was removed from experimental system. Although male care has no effect on larval growth or survival under laboratory conditions (Takata, unpublished study), the presence of male may affect pattern of filial cannibalism by female parent. Further studies are needed to investigate the potential effects of male presence on the pattern of filial cannibalism by female.

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Ethics declarations

Ethics approval

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Figures

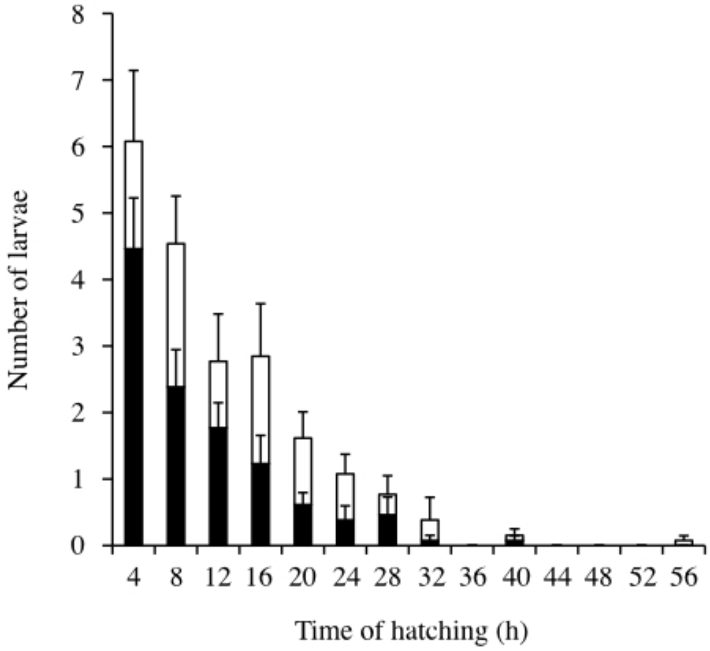

Figure 1 Number of hatching larvae and larvae that survived during 4-h intervals. The black bars denote the mean number of larvae that survived; the white bars denote the mean number of dead larvae. Data are presented as mean + SE.

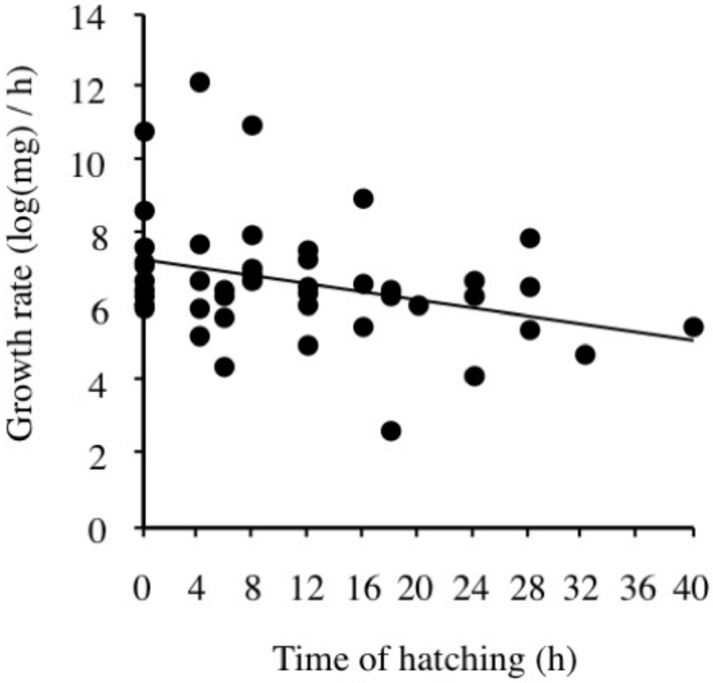

Figure 2 The effect of the point in time at which each group of offspring hatched on offspring growth rate. Each plot denotes the mean growth rate of larvae in each brood at 4-h intervals. The growth rate was calculated as the slope of the regression line of log (body mass) on time for each group of offspring.