Trophic eggs compensate for poor offspring feeding capacity in a subsocial burrower bug

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Various animals produce inviable eggs or egg-like structures called trophic eggs, which are presumed to be an extended maternal investment for the offspring. However, there is little knowledge about the ecological or physiological constraints associated with their evolutionary origin. Trophic eggs of the seminivorous subsocial burrower bug (*Canthophorus niveimarginatus*) have some unique characteristics. Trophic eggs are obligate for nymphal survival, and first-instar nymphs die without them. To identify the cause of nymphal death, we hypothesized that first-instar nymphs starve to death because they cannot feed on anything but trophic eggs. Although first-instar nymphs fed on artificially exposed endosperm did survive, nymphs that were provided with intact seed were not able to penetrate the seed vessel and starved to death. Another hypothesis that trophic eggs play a role in transferring the midgut symbiont, essential for survival in heteropteran bugs, from mother to offspring was rejected because almost all nymphs had retained the symbiont without feeding on trophic eggs. These results suggest that poor feeding capacity of the offspring is the cause of nymphal death, and the important constraint that promotes the evolution of the curious trophic egg system in *C. niveimarginatus*.

Keywords: maternal investment; constraints; starvation; *Canthophorus niveimarginatus*

1. INTRODUCTION

Trophic eggs are inviable eggs or egg-like structures that usually function as a food supply for hatched siblings [1]. They are reported in various animals including fishes, amphibians, arthropods and gastropods, and can be regarded as an extended maternal investment [2,3]. Although these species vary immensely in life history, they share an extraordinary maternal tactic: reassigning the ovarian machinery to producing food for the offspring, while occasionally risking the loss of viable offspring [3]. The experiments

with animals whose offspring's survival and growth strongly depend on trophic eggs may allow us to investigate why such a phenotype evolved.

In the present study, we focus on a subsocial burrower bug, *Canthophorus niveimarginatus* (Heteroptera: Cydnidae), which has a unique characteristic; nymphs prevented from feeding on trophic eggs during the first-instar stage die, unlike related species [4]. This insect is about 7 mm in length, and inhabits highland grasslands where its sole host plant, *Thesium chinense* (Santalaceae), also resides. A female protects an egg mass and provisions nymphs with trophic eggs and host seeds [4]. Although trophic eggs have been reported in related bugs (e.g. *Adomerus triguttulus* [5] and *Parastrachia japonensis* [6]), *C. niveimarginatus* has two types of trophic eggs. One is the pre-hatch trophic egg (pre-TE), placed on the egg mass in a low proportion, and the other is the post-hatch trophic egg (post-TE), continuously produced for about four days during the first-instar stage, up to a total number of around 30 [4]. The function of pre-TEs as a nutritional resource is yet unclear because nymphs can survive uneventfully without pre-TEs but not without post-TEs. First-instar nymphs would be left without food unless post-TEs were given because the females start transporting host seeds from outside to feed the nymphs after moulting to second instars [4]. Such obligate dependency on trophic eggs is extremely rare in insects. Thus, we focus on the feature of post-TEs, which is specific to *C. niveimarginatus*, and tested the following hypothesis: why trophic eggs have evolved in certain species, and to develop evolutionary ideas on the adaptive value of trophic eggs.

Filippi *et al.* [4] suggested three possibilities for nymphal death in *C. niveimarginatus*: the starvation hypothesis is that first-instar nymphs die of starvation because they cannot feed on host seeds as they have tough seed vessels; the symbiont transmission hypothesis is that trophic eggs are the vectors that vertically transmit midgut symbionts, like the symbiont capsules of plataspid stinkbugs [7]; and the nutritional essentials hypothesis is that some nutrients that are found in trophic eggs, but not in regular foods, directly affect nymphal survival. We tested the three hypotheses with laboratory experiments and demonstrated which ecological and physiological constraints negatively affect nymphal survival and how trophic eggs compensate for this.

2. MATERIAL AND METHODS

Canthophorus niveimarginatus were collected in Ohnohara grassland (Nagasaki, Japan) and reared in plastic cases (35 × 40 × 12 cm) with the host plant *T. chinense* collected in Hisayama (Fukuoka, Japan). Gravid females with swollen abdomens were transferred individually to plastic cups (10 cm diameter × 6 cm in height) with moderately moist filter paper and sufficient host seeds. The cups were kept at 22°C with a 16 L : 8 D (light : dark regime).

First, we examined the starvation hypothesis by observing three groups of clutches maintained with different food conditions. Thirty-nine females and their offspring were divided into three groups of 13 offspring, each of which contained clutches of 10 nymphs. Group A was the control group (regular maternal care with post-TEs during the first-instar stage). Five intact seeds were placed in each cup and were replaced with new seeds daily. In group B, mothers were removed just before hatching (10 days after oviposition), and the nymphs were hand-reared with regular intact seeds but no post-TEs were given. Mothers of group C were also removed just before hatching, and the nymphs were not allowed to

Figure 1. (a) Nymphal survival rates after hatching in relation to food availability in groups A (open circles) (under regular maternal care with post-TEs, $n = 13$), B (filled triangles) (hand-reared with regular intact seeds but without post-TEs, $n = 13$) and C (filled circles) (without post-TEs but with processed seeds, $n = 13$). (b) A first-instar nymph directly feeding on the endosperm of a processed host seed split in half. Bugs usually feed on seeds covered with tough seed vessels as shown in the inset.

Table 1. Results of PCR analysis for detecting the midgut symbiont in egg masses and nymphs with and without trophic eggs.

	egg masses		nymphs	
	just after oviposition	just before hatching	regular maternal care	without trophic eggs
sample size	three egg masses	three egg masses	66 individuals	101 individuals
symbiont retention rate (%)	100	100	100	98

feed on post-TEs. Instead, five host seeds that had been split in half to expose the endosperm (processed seeds) were provided, because we predicted that the seed vessels of the host seeds were too tough for the first-instar nymphs to feed on them. Five intact seeds were placed in each cup after all nymphs in a cup had moulted to the second-instar stage. Seeds were replaced with new ones daily. In all groups, pre-TEs were removed from the egg masses before hatching. We compared the nymphal daily survival for 15 days after hatching and the proportion of offspring developing to adulthood. Survival analyses using log-rank tests with the proportional hazards model were performed with JMP 7.0 (SAS Institute).

Secondly, we examined the symbiont transmission hypothesis. We preliminarily confirmed that *C. niveimarginatus* harboured a single γ -proteobacterial symbiont in the midgut, using the methods that have been applied to other stink bugs [7]. The symbiont 16S rRNA gene sequence was deposited in the DNA Data Bank of Japan database (<http://www.ddbj.nig.ac.jp>) with accession number AB478043. We examined whether the symbionts were present in: egg masses within 24 h after oviposition ($n = 3$), egg masses 1 or 2 days before hatching ($n = 3$), first-instar nymphs that had fed on post-TEs ($n = 66$ from five families) and those that had been prevented from feeding on post-TEs ($n = 101$ from 13 families). DNA was extracted from each egg mass or nymph with the NucleoSpin Tissue Kit (MACHERY-NAGEL) and subjected to diagnostic PCR with primers CnSymF (5'-CGATGTGGTTAATAACCGAGT-3') and 16SB1 (5'-TACGGYTACCTTGTTACGACTT-3') under a temperature profile of 95°C for 10 min, followed by 35 cycles of 94°C for 30 s, 55°C for 1 min and 72°C for 1 min, which specifically amplified the symbiont 16S rRNA gene. If post-TEs mediated symbiont transmission, PCR amplification would only be found in the nymphs that had fed on post-TEs.

3. RESULTS

Figure 1a shows the comparison of nymphal survival among the three groups maintained with different food conditions. The nymphs of group A, which

received regular maternal care, showed the highest survival during the 15 experimental days, and 88.0 per cent of them normally developed to adulthood. In the absence of mothers (post-TEs not available) but with sufficient intact seeds, 97.2 per cent of group B died before moulting to second instars ($z = 3.12$, $p < 0.0001$) and none became adults. By contrast, 77.6 per cent of nymphs of group C, which had been allowed to feed on processed seeds, became adults. The percentages of survival were not significantly different between groups A and C ($z = 2.98$, $p = 0.07$). We could not observe first-instar nymphs sucking intact seeds in group B, but observed first instars actively sucking endosperm of processed seeds in group C (figure 1b). Nymphs after the second-instar stage in groups A and C fed actively on intact seeds and developed normally.

The midgut symbionts were detected in egg masses both just after oviposition and before hatching. All nymphs with regular maternal care retained sufficient symbionts. Moreover, 98.2 per cent of nymphs without a mother (post-TEs not available) also retained sufficient symbionts (table 1).

4. DISCUSSION

We first demonstrated that the majority of *C. niveimarginatus* nymphs cannot survive when post-TEs are not available. We then examined the ability of the starvation, symbiont transmission and nutritional essentials hypotheses to explain the contribution of

post-TEs to nymphal survival. We showed that nymphs that had no access to post-TEs could survive well if they were allowed to feed on processed seeds. This fact supports the starvation hypothesis and reveals the indispensability of feeding during the first-instar stage and the incapability of first-instar nymphs to feed on regular host seeds. The nutritional essentials hypothesis, which assumes that some nutrients in trophic eggs are essential for nymphal growth and survival, was not strongly supported in *C. niveimarginatus* because the nymphs did survive on alternative nutrition. The small difference in survival rate between groups A and C may indicate that trophic eggs have a possible small benefit over the availability of processed seeds. The symbiont transmission hypothesis assumes that the midgut symbionts involved in metabolism or growth of the offspring are vertically transmitted via trophic eggs. However, the majority of nymphs retained sufficient symbionts, even if post-TEs had been unavailable. Therefore, nymphal death is not caused by the failure of symbiont transmission.

The major remaining issues in understanding why trophic eggs have evolved in various ovipara are to identify the specific ecological, physiological and life-history backgrounds in which trophic egg production is advantageous [8,9]. The icebox hypothesis assumes that trophic eggs may evolve in species that experience abundant food during reproduction but limited food during parental provisioning or offspring foraging [2,10]. Food limitation is likely to occur when food availability largely depends on the seasons or weather conditions or when nymphal foraging ability is limited [1,3]. Several authors indicate that trophic eggs are observed in animals whose offspring seem to have limited mobility for acquiring foods (e.g. tree frogs [9], prosobranchia [11], coccinellids [12]) or limited feeding ability (e.g. polychaetes [13], burrower bugs [4–6], spiders [14]). Thus, trophic eggs may evolve in species that suffer ecological or physiological constraints associated with food during the immature stage. However, no study has demonstrated such constraints in the foraging ability of immatures. Besides, it is unknown which type of feeding ability (sucking, chewing, swallowing, digesting and/or metabolism) is affected by the constraints.

First instars of *C. niveimarginatus* were capable of sucking and digesting food if tough seed vessels were removed. Their foraging ability does not appear to be restricted because the nymphs show high mobility (N. Baba 2007, personal observation). Consequently, nymphal survival is suppressed by their poor feeding capacity: nymphs cannot suck host seeds during the first-instar stage, which is compensated for by post-TEs. Through an experiment with an artificial alternative food source, we are the first to demonstrate a constraint that may represent one of the evolutionary origins of trophic eggs. Comparative data collected from host plants which support species both with and without trophic eggs may reveal that trophic egg production is a co-evolutionary strategy for responding to the harder seeds in some host plants.

Canthophorus niveimarginatus has three types of maternal provisioning resources, i.e. pre-TE, post-TE

and host seeds. In subsocial or eusocial species, it is not uncommon that care givers have diversified options for provisioning the offspring with foods depending on the offspring status [2]. Both trophic eggs of *C. niveimarginatus* are likely to be morphologically or nutritionally specialized for offspring ease of consumption (N. Baba, 2007, 2008, unpublished data). Such diversification and specialization of parental investment forms may be caused by the life-history constraints of care givers and/or the offspring.

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