

Variation in the geometry of foreleg claws in sympatric giant water bug species: an adaptive trait for catching prey?

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Abstract

When giant water bugs (Heteroptera: Belostomatidae) encounter prey animals that are larger than they are themselves, they first hook the claw of their raptorial legs onto the animal, and then use all their legs to pin it. The claws of the raptorial legs in giant water bugs play an important role in catching larger prey, but the relationship between the claws, body lengths of predators, and prey size has not been fully investigated. To elucidate the functioning of claws in catching prey, we investigated prey body size relative to predator size in nymphs of two sympatric belostomatid giant water bug species, the vertebrate eater *Kirkaldyia* (= *Lethocerus*) *deyrolli* Vuillefroy and the invertebrate eater *Appasus japonicus* Vuillefroy, captured in rice fields. The younger nymphs of *K. deyrolli* caught preys that were larger than themselves, whereas those of *A. japonicus* caught preys that were smaller. Younger nymphs of *K. deyrolli* had claws that were curved more sharply than those of *A. japonicus*. The more curved claws of younger nymphs of *K. deyrolli* probably hook more easily onto larger vertebrates and thus this shape represents an adaptation for acquiring such prey.

Introduction

Differences in size and shape of the feeding apparatus among sympatric species are considered to have evolved under the context of prey selection (Houston & Shine, 1993). Competition for food resources induced by niche overlap may promote divergence in function of traits that are used for gathering food, eventually leading to ecological character displacement (McPhail, 1992; Schluter, 1994). For instance, in the larvae of water-scavenger beetles, the mandibles of insectivorous species are symmetric, but those of snail-eating species are asymmetric (Inoda et al., 2003). Thus, knowledge of the feeding apparatus aids in

understanding how these body parts evolve under resource competition.

Here, we focus on the relationship between the shape of the feeding apparatus and feeding behaviour in two sympatric Belostomatidae (Heteroptera) species, *Kirkaldyia* (= *Lethocerus*) *deyrolli* Vuillefroy (Lethocerinae) and *Appasus japonicus* Vuillefroy (Belostomatinae). The diets of these two species differ in Japanese rice fields (Ohba & Nakasuji, 2006). Nymphs of *A. japonicus* eat invertebrates such as snails and aquatic insects, whereas nymphs of *K. deyrolli* mainly feed on vertebrates such as tadpoles and fish (Ohba & Nakasuji, 2006; Ohba et al., 2008). Unlike *K. deyrolli*, *A. japonicus* does not digest vertebrates well, because, in general, the saliva of Belostomatinae does not have enzymes capable of digesting protein-rich meals (Swart et al., 2006).

Belostomatid bugs use all their legs to catch animals larger than they are, possibly to ensure success (Smith, 1997; Ohba et al., 2006, Figure 1B). When bugs encounter a prey that is larger than they are, they first hook the claws of their raptorial legs onto the animal, and then use all their legs to pin it (Figure 1B). Consequently, species that feed on prey larger than

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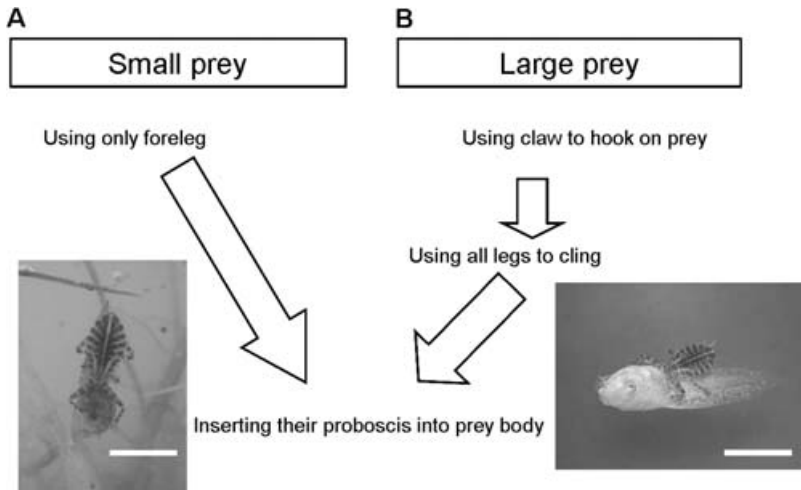


Figure 1 Scheme of predatory behaviour in Belostomatidae. Photos indicate the first instar nymph of *Kirkaldyia deyrolli* capturing a tadpole that is (A) smaller or (B) larger than itself. Bar = 1.0 mm.

themselves may need more sharply curved claws that hook easily onto the prey. Thus, it is expected that the geometry of claws will differ by food habits in the two sympatric species.

The primary goal of the present study was to reveal whether the shape of curved claws on raptorial legs was associated with prey body size relative to predator size in the two belostomatid species. For that purpose, we coupled field observation with a laboratory experiment. We then discuss interspecific differences in the functioning of the feeding apparatus in the context of prey size.

Materials and methods

Study sites and field observations

To investigate prey body size relative to predator size, we conducted a field census at 1- to 3-days intervals in rice fields at Uekidani (35°1'N, 134°19'E) in Hyogo from June to mid-August in 2004 and 2005, and at Chuoh (34°58'N, 133°58'E) in Okayama, Honshu, Japan, from June to mid-August in 2004 and 2005. Census plots were set up in ditches along the ridge of the rice fields. The plot areas were 118 m² in Uekidani and 100 m² in Chuoh.

We searched the ridges around the rice fields for belostomatids, using a flashlight every 2 h between 20:00 and 01:00 hours. Belostomatids are primarily nocturnal, and they ambush prey at the water surface; flashlights do not interfere with their foraging behaviour (S Ohba, unpubl.). We identified dietary items to be those captured individuals into which the bug inserted its proboscis. After recording the species of predator and prey, we captured them. We measured the length from top (head or proboscis) to tip of abdomen as total body length. The total body lengths of both predator (*K. deyrolli* or *A. japonicus*) and prey, or shell lengths in the case of snails, were measured as indices of body size with calipers. Wilcoxon's signed-rank test was

used to compare body lengths of predators and their prey animals at each nymphal stage.

Younger nymphs (first to third instar) of *K. deyrolli* caught preys, mainly tadpoles, that were larger than themselves (see Results). To clarify the approximate size of captured prey by *K. deyrolli* compared to the size of available (i.e., captured and non-captured) prey individuals, we measured available and captured tadpole size in each instar nymph. A D-frame dipnet (28 cm in width, 3 mm mesh) was used for sweeping. The dipnet was slowly pulled along the paddy bottom so as to collect tadpoles. Tadpole total length was measured and classified into six categories: <5, 5–10, 11–15, 16–20, 21–25, and >25 mm. After measurement, the tadpole was released into the paddy. This work was done weekly during the survey periods. The median of tadpole total length in present and captured nymphs in each instar was compared using a Mann–Whitney U-test.

Rearing experiments

To compare the growth patterns of curvature of the claw on the forelegs between the two species, rearing experiments were conducted. Two male–female pairs collected from rice fields in May 2004 were released in an aquarium (60 × 45 cm for *K. deyrolli* and 30 × 20 cm for *A. japonicus*, both 10 cm deep) and females were allowed to lay eggs. Because hatched nymphs of *K. deyrolli* are often cannibalistic, individual hatchlings were isolated in a cage (10 × 10 cm, 20 cm high, 3 mm mesh size) and placed in an aquarium (10 cm water). Nymphs were reared at 25.0 °C with a photoperiod of L16:D8 until moulting to adults (n = 43). The nymphs were provided with various animals including tadpoles [*Hyla japonica* Günther; Gosner stage 35–42 (Gosner, 1960)] and Odonata nymphs (Platycnemididae: *Copera annulata* Selys; Lestidae: *Lestes* spp., 10–20 mm in total length; Libellulidae: *Orthetrum*

albistylum speciosum Uhler, *Sympetrum frequens* Selys, and *Sympetrum infuscatum* Selys; and Aeshnidae: *Anax parthenope julius* Brauer, *Anax nigrofasciatus nigrofasciatus* Oguma, and *Gynacantha japonica* Barteneff, 20–40 mm). Prey density was kept constant in each cage (six individuals per container for the first–second instars and 10 individuals per container for the third–fifth instars). Because claw developmental patterns were almost identical irrespective of food items (Ohba et al., 2006), the data from three prey treatments (tadpoles, Odonata nymphs, and tadpole–Odonata nymph mixture) were pooled. The dead bodies of prey were removed daily and replaced with live prey.

Hatched nymphs of *A. japonicus* were reared individually in Petri dishes (9 cm diameter, 1.5 cm high) at 25.0 °C with a photoperiod of L16:D8; a piece of floating net cloth was used as a perching site (n = 47). The nymphs were provided with various prey, namely, aquatic isopods (*Asellus* spec., 2–10 mm in total length) and aquatic snails (Lymnaeidae and Physidae, 2–10 mm in shell length). The prey density in each cage was kept constant [five aquatic isopods (*Asellus* spec.) and five aquatic snails per container for the first–second instars, along with 10 aquatic isopods and 10 aquatic snails per container for the third–fifth instars]. The dead bodies of prey were removed daily and replaced with live prey.

Measurements of morphological characteristics

Exuviae of each instar of *K. deyrolli* and *A. japonicus* were collected from the rearing cage or Petri dish and preserved in 70% ethanol. Moulting and the preservation process do not change the claw's shape (S Ohba, unpubl.). The curvature of the claw on the right foreleg from the dorsal side of the exuviae was measured as follows. First, a line was drawn from the claw tip (A) to the base of the claw (B) to give the claw-arc (Type 1 landmark, see Bookstein, 1991; Figure 2). Second, the farthest part from claw-arc on the inner claw was pointed as C (Type 2 landmark, Bookstein, 1991). Two lines were drawn from C to both A and B, and the angle of ACB was measured as the curvature of the claw. Claws of the same individual were measured from the first to the fifth instar.

To evaluate differences in angles of the curvature of claws, we performed repeated-measures one-way analysis of variance (ANOVA), with species as the between-subject factor and nymphal stages (first–fifth instar) as the within-subject factor. Mauchly's test for sphericity indicated a significant violation of the assumption in all characteristics ($P < 0.05$). For this reason, significance levels for within-subject effects of the claw angle of curvature were determined using the Greenhouse–Geisser correction (G-G ϵ). Scheffé's tests were carried out to assess differences among nymphal stages when significant effects were detected.

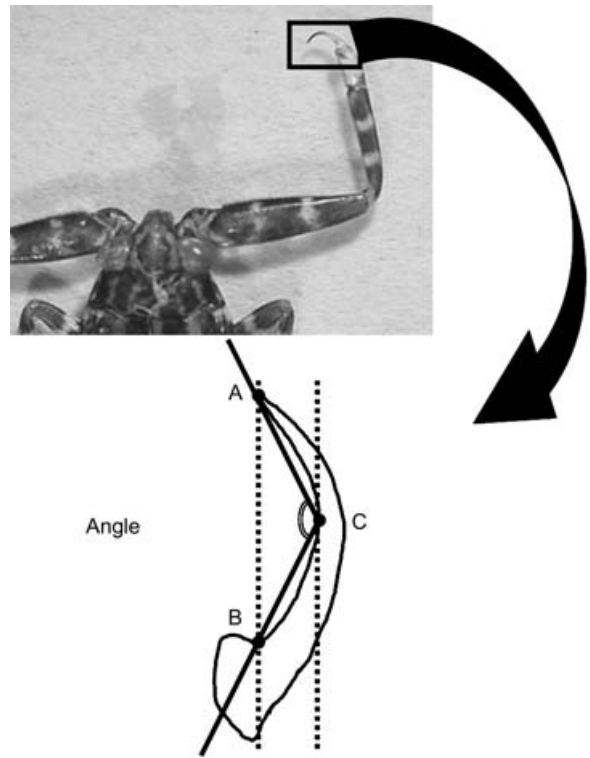


Figure 2 Measurement of the claw angle of a *Kirkaldyia deyrolli* nymph. A, claw tip; B, base of the claw; C, the furthest part from the claw-arc, AB, on the inner claw.

All statistical tests were performed using SPSS for Windows version 11.0 (SPSS, 2001).

Results

Comparison of predator and prey size

The bodies of *A. japonicus* were significantly larger than those of their prey, except when the predator was the first instar (Figure 3). In contrast, the body lengths of the first, second, and third instars of *K. deyrolli* were significantly smaller than those of the prey. Fourth instars of *K. deyrolli* were the same size as their prey and fifth instars were larger than the prey (Figure 3).

Frequency distribution of medians of each body size range of available and captured tadpoles is shown in Figure 4. Mean total body length of tadpoles captured by *K. deyrolli* was significantly larger than that of tadpole present in all nymphal stages (Mann–Whitney U-test: $P < 0.001$; Figure 4).

Variation in claw angle

The repeated-measures one-way ANOVA revealed that effects for species, nymphal stage, and species*nymphal

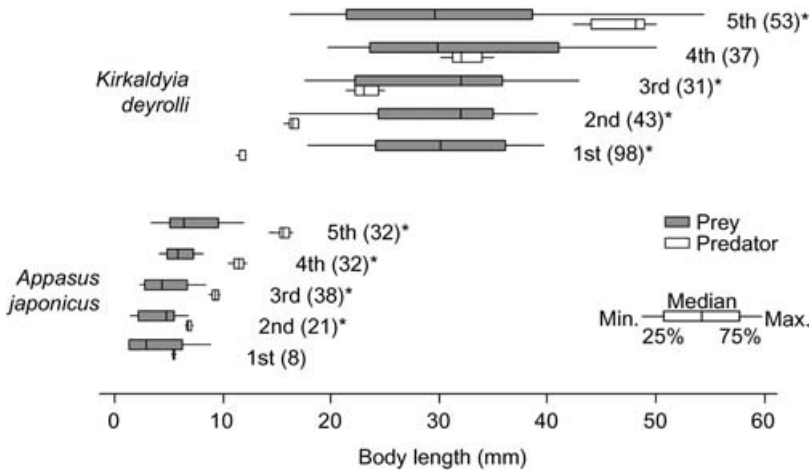


Figure 3 Body lengths of *Kirkaldyia deyrolli* and *Appasus japonicus* predators (first–fifth instar) and their captured prey in a rice paddy field. Box plots are based on medians and quartiles. Numbers in parentheses show sample size. Asterisks denote significantly different sizes of the predator and its prey (Wilcoxon’s signed-rank test: $P < 0.05$).

stage interaction were all significant, indicating different developmental patterns for the angles of claws [species: $F_{1,8} = 787.7$, $P < 0.001$; nymphal stage: $F_{3,5,305,3} = 104.7$, $P < 0.001$ (after Greenhouse–Geisser correction); species*nymphal stage: $F_{3,5,305,3} = 203.2$, $P < 0.001$ (after Greenhouse–Geisser correction), G–G $\epsilon = 0.867$ for log-transformed data]. One-way ANOVA for each belostomatid species revealed that the claw angles differed significantly among nymphal stages (*K. deyrolli*: $F_{4,210} = 286.8$, $P < 0.001$; *A. japonicus*: $F_{4,230} = 6.1$, $P < 0.001$). In *K. deyrolli*, claws became less curved as nymphs grew and nymphs tended to catch smaller prey than their body size. What is more, the claw angle of the first-instar nymph was considerably smaller than that of other instars (Figure 5). In contrast, the claw angle of the first instar of *A. japonicus*

was significantly larger than that of the fifth instar, with those of the second, third, and fourth instars being intermediate ($P < 0.05$).

Discussion

It is frequently seen that predators catch preys that are smaller than they are themselves (Pearson & Mury, 1979; Brodie & Formanowicz, 1983; Ganeshiah & Belavadi, 1986; Nentwig & Wissel, 1986; Warren & Lawton, 1987). Prey size increases as predator nymphs grow (Cloarec, 1992; Perez-Goodwyn, 2001). The present study revealed that these general tendencies applied to the case of the nymphs of the invertebrate eater *A. japonicus*. Nymphs of the vertebrate eater *K. deyrolli*, however, did not conform to this pattern (Figure 3). In particular, observations in the field demonstrated that the first, second, and third instars of *K. deyrolli* caught preys, mainly tadpoles, that were larger than they were themselves.

The eggs of anurans, such as *H. japonica*, *Rhacophorus schlegelii* (Günther), and *Rana nigromaculata* (Hallowell), species that are principal dietary items for *K. deyrolli* hatch in rice fields in mid-May. Consequently, tadpoles grow large enough when the hatchlings of *K. deyrolli* occur from June to July (Figure 4). Under these feeding circumstances, curvier claws of *K. deyrolli* might be expedient to hook larger prey. Younger nymphs of *K. deyrolli* possessing sharply curved claws hook more easily onto tadpoles and other preys that are larger than the predator itself and cling to prey with all legs (Figure 1B; Smith, 1997, Ohba et al., 2006). The same feeding behaviour was observed in another experiment in the laboratory (S Ohba et al., unpubl.). On the other hand, the invertebrate eater *A. japonica* mainly use the forelegs to capture prey smaller than their own body size (Figures 1A and 3) and they hardly employ the behaviour of claw hooking and clinging using all legs as

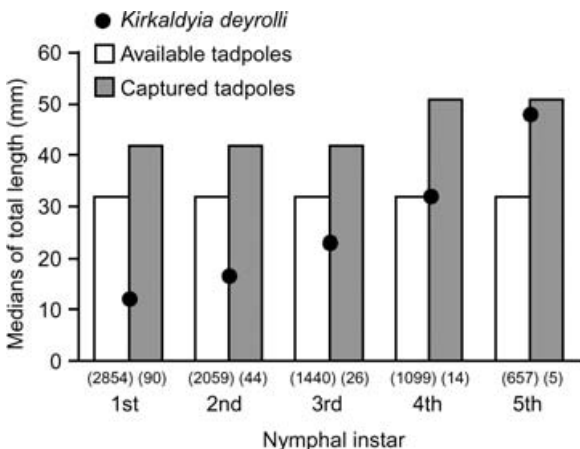
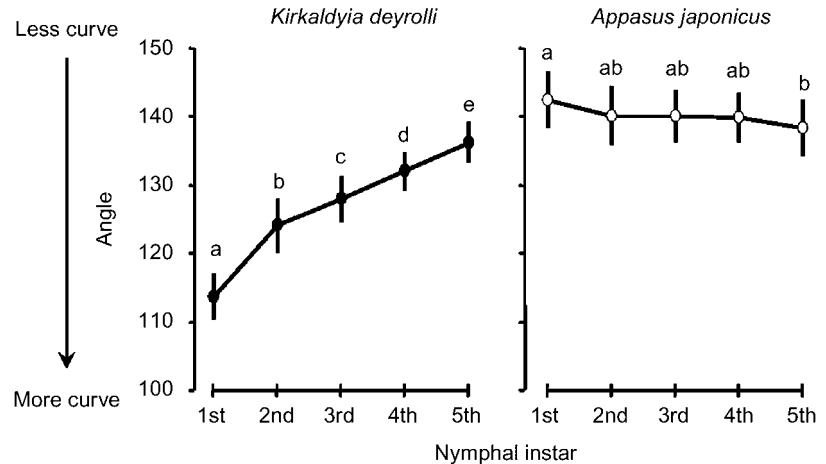


Figure 4 Comparison of medians of total size of available and captured tadpoles in each of five instars of *Kirkaldyia deyrolli*. Numbers in parentheses show sample size. The difference between available and captured tadpoles was significant in all instars (Mann–Whitney U-test: $P < 0.001$).

Figure 5 Changes in the claw angle in *Kirkaldyia deyrolli* and *Appasus japonicus* in five nymphal instars. Data indicate means \pm SD. Different letters indicate significant differences between nymphal stages in a species (Scheffé's test: $P < 0.05$).



shown in Figure 1B. Thus, geometric differences in foreleg claws between the two species may be relevant to the manner of catching prey.

Conspicuous differences in the geometry of curved claws between the two species provide circumstantial evidence that the diets of these two species differ in Japanese rice fields. According to Smith (1997), members of the genus *Lethocerus*, including *Kirkaldyia*, are vertebrate specialists. He argued that selective regimes to facilitate the handling of progressively larger aquatic vertebrate prey tend to expedite the evolution of size and shape of the claws (Smith, 1997). The present study strongly suggests that *K. deyrolli* nymphs preferred larger tadpoles among available ones (Figure 4). Our findings of catching larger prey in the nymphs of *K. deyrolli* are in line with Smith's (1997) evolutionary scenario. To reveal the evolutionary process in Belostomatidae, phylogenetic information as well as further comparative data of feeding behaviour are necessary.

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