Full title

Age-dependent walking and feeding of the assassin bug *Amphibolus venator*

Short title

Age-dependent walking and feeding

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Abstract

Animal behaviours often dependent on age. In many insect species, walking shows an age-dependent decline, and food intake may also be dependent on age. However, few studies have investigated the relationship between age and walking or food intake. In the present study, we compared walking traits and food intake among individuals of different ages in the assassin bug *Amphibolus venator* (Hemiptera, Reduviidae). The present results showed an age-dependent decline in walking, similar to findings in many animal species. On the other hand, food intake showed a positive correlation with age. Therefore, the decline in walking did not lead to a decline in feeding. The positive relationship between food intake and age may be related to the type of predation, sit-and-wait, used by *A. venator* via alterations in investment in reproductive traits with age.

Keywords

Age-dependent, walking, food intake, sit-and-wait predator, *Amphibolus venator*

1. Introduction

Senescence often depends on the age at which deterioration of physiological function occurs (Rose, 1991; Arking, 1998; López-Otín et al., 2013). Many previous studies have reported that behaviours of vertebrate and invertebrate animals often show an age-dependent decline (Leffelaar & Grigliatti, 1983; Le Bourg & Minois, 1999; Grotewiel et al., 2005; Murakami & Murakami, 2005; Gargano et al., 2005; Ridgel & Ritzmann, 2005; Martines et al., 2007). Among invertebrates, the fruit fly *Drosophila melanogaster* has been used extensively to study age-related behavioural changes (Grotewiel et al., 2005). Previous studies have focused on the decline in behavioural traits with age in flies, including duration of flight (Leffelaar & Grigliatti, 1983) and locomotor activity (Gargano et al., 2005; Martinez et al., 2007) in insect species. In insects, negative correlations between behaviour and age are considered to occur due to damage to an appendage, including the legs and the cuticula that make up their structure (Ridgel & Ritzmann, 2005).

In mammals, food consumption may be negatively correlated with age (McCue, 1995; Blanton et al., 1998). A loss of mobility could affect an animal's ability to acquire resources. Increased age has also been shown to be associated with a decrease in foraging efficiency in some invertebrate species (Tofilsky, 2000; Moya-Larano, 2002; Grotewiel et al., 2005). Moreover, the foraging style of a predator may also affect to the food intake. The foraging efficiency of an actively hunting predator may decrease with age due to age-dependent decline in moving, whereas a sit-and-wait predator that does not require moving to forage is expected to be unaffected by an age-dependent decline of mobility foraging efficiency. However, few studies have examined the effects of an age-dependent decline in mobility on the foraging efficiency and food intake in animals (but see Anotaux et al., 2014).

In the present study, we tested whether the walking behaviours and food intake are correlated with age in the assassin bug *Amphibolus venator* (Klug) [Hemiptera, Reduviidae]. *A. venator* often eats stored-grain insects including the red flour beetle *Tribolium castaneum* (Nishi et al., 2004). We hypothesized that if behavioural performance of *A. venator* declined with age, then walking traits would be negatively correlated with age. Furthermore, food intake may also (positively or negatively) correlate with age. To test this hypothesis, we investigated walking traits and food intake using adults of *A. venator* of various ages. To measure walking traits, we used a treadmill system. We also measured food intake of *A. venator* in small and large containers because of a possible difference in density of beetles in food. We also examined the effects of sex on aging.

2. Materials and Methods

2.1. Insect and culture

The population of *A. venator* used for the present study was collected from a grain store in Urasoe City, Okinawa, Japan, in 2015 by T. Miyatake, and this population has been maintained in the laboratory of Okayama University (see Matsumura et al., 2019). Each bug was reared in an incubator maintained at 29°C and 16L:8D (light on at 7:00, light off at 23:00) light. We fed *T. castaneum* to each bug ad libitum. In this study, we defined the age of this insect as the number of days from the date of moulting of the last instar larva, and we used *A. venator* individuals with a large variation of ages (about 1 to 300 days old). A previous study revealed that the median longevity of *A. venator* was 297 days old (n = 246) under a laboratory conditions (Matsumura et al., 2019). Therefore, the bugs used in this study were relatively young. However, a previous study showed that the longevity of *A. venator* is shortened at high temperatures (Youssef and Abd-Elgayed, 2015). Because the insects used in this study were reared at 29°C, whereas in the previous study they were reared at 25°C, the median of longevity may be shorter than 297 days old.

2.2. Locomotor activity

To measure the walking traits of *A. venator*, we used a treadmill system, ANTAM (Nagaya et al., 2017). The ANTAM was developed from an omnidirectional treadmill mechanism system in which animal movements can be continuously recorded and compensated for in such a way that the animal is always located on the top of the sphere and experiences a virtual unbounded two-dimensional field (Nagaya et al., 2017). Therefore, this system is able to measure the free walking trajectories of small animals such as insects (Shoji et al., 2019). The walking speed of *A. venator* is 37.25 ± 12.55 mm / sec (mean ± *s.d.*, n = 133; unpublished), which is within the allowable range of the movement speed of the system used (for example, ANTAM can measure the walking speed of 55.2 ± 34.3 mm/sec (mean ± *s.d.*)

of the pill bug, *Armadillidium vulgare* (Nagaya et al., 2017)).

Virgin males (n = 59) and females (n = 74) were randomly collected from stock cultures, and each bug was placed on the ANTAM system. When a bug was moving, we recorded walking traits for 10 min. Measurements were conducted between 10:00 and 18:00.

2.3. Predation

We measured the food intake of *A. venator* in small and large scale containers over 10 days. In the small container experiment, virgin males (n = 19) and females (n = 37) were randomly collected from the stock culture, and each bug was placed in a cylindrical container (35 mm in diameter, 10 mm in height). All bugs were starved for 7 days before the experiment (Matsumura & Miyatake 2015). Five *T. castaneum* adults were randomly collected from the stock culture and put into a Petri dish along with an *A. venator* adult, and we counted the number of beetles in each Petri dish that were eaten by the predatory every two days. When the beetles were gone, we replaced them with live beetles.

In the large container experiment, virgin males (n = 17) and females (n

= 24) were randomly collected from the stock culture, and each bug was placed in a cylindrical container (149 mm in diameter, 65 mm in height). Bugs were not provided with food for seven days before the experiment. Five *T. castaneum* adults were randomly collected from the stock culture and put into each Petri-dish along with an *A. venator* adult, and we counted the number of beetles in each Petri-dish that were eaten by the predatory bug every two days. All predation experiments were conducted in the incubator described above.

2.4. Statistical analysis

In the analysis for walking traits, we separated data from the ANTAM system: (a) total distance walked, (b) total displacement (i.e., direct distance from the start point), (c) average speed (i.e., total distance/duration of walking), (d) average velocity (i.e., total displacement/total distance), and (e) walking rate (i.e., distance walked/600 sec). To analyse these data, we used a generalized linear model (GLM) of gamma distribution with a log link function, and age, sex, and the interaction between age and sex as explanatory variables.

For the analysis of food intake, we used a GLM with a Poisson distribution with a log link function. In this analysis, age, sex, and the interaction between age and sex were used as explanatory variables. Analysis of food intake was conducted separately for small- and large-scale experiments. Because a significant effect of interaction between age and sex was shown in food intake in the small container (Table 2), the male and female were analysed separately.

All analyses were conducted using R version 3.4.3 (R Core Team 2017). We used *lme4* package (Bates et al., 2015) for conducting the GLM and *car* package (Fox & Weisberg, 2011) for additional tests.

3. Results

Figure 1 shows the results of walking traits. GLMs showed that age had a significant negative effect on total distance, total displacement, average speed, and walking rate (Fig. 1, Table 1). There were no significant effects of sex or interaction on any trait. There were no significant effects for all factors in the average velocity result (Table 1).

Figure 2 shows the results of predation experiments on the small and large scales. In the small container, females showed significantly higher food intake than males (Fig. 2, Table 2). Age had a significant effect on food intake, and an interaction between age and sex was also shown in the small-scale experiment (Table 2). Therefore, we also analysed the data for sex separately, and a positive significant correlation between age and food intake was found in females ($\chi^2_{1,35} = 9.76$, $p = 0.0018$), but not in males ($\chi^2_{1,17} = 0.71$, $p = 0.3994$). In the large-scale experiment, there was a positive correlation between predation and age (Fig. 2b, Table 2), but no significant effect of sex and interaction was found (Fig. 2b, Table 2).

4. Discussion

An age-dependent decline in walking is found in many animal species (McCue, 1995; Blanton et al., 1998; Tofilsky, 2000; Moya-Larano, 2002; Grotewiel et al., 2005). In the present study, walking traits showed an age-dependent decline in *A. venator*. Meanwhile, food intake showed an age-dependent increase. In the small-scale experiment, a positive correlation between food intake and age was found only in females. However, in the large-scale experiment, insects of both sexes showed positive correlations between food intake and age.

Although the present results suggested that walking and feeding by *A. venator* were negatively correlated with age, we did not investigate intra-individual changes in behavioural traits with age. Therefore, the present results are difficult to associates with the effects of aging on the behaviour. We need additional studies that investigate the effects of aging on behavioural traits on an intra-individual level in the future.

In the present study, a negative correlation between walking traits and age was found in *A. venator*. Therefore, the present results for *A. venator* are in accordance with those of previous studies (Rose, 1991; Larsson et al., 1997; Grotewiel et al., 2005; Ridgel & Ritzmann, 2005; Gargano et al., 2005; Martinez et al., 2007; Miller et al., 2008). The average speed was negatively correlated with age (Fig. 1c), whereas the average velocity was not (Fig. 1d). This suggests that an age-dependent decline in walking might not have caused of alterations of the walking pattern with age. This is consistent with an age-related decline in movement, which in some insects has been mechanistically attributed to damage to walking appendages/legs (Ridgel & Ritzmann, 2005). That is, the present results suggest that older aged adults of *A. venator* decrease their locomotor activity due to leg damage with aging. Moreover, because the moving rate also showed an age-dependent decline, the decrease in walking performance might have been affected by physiological factors in addition to mechanical damage.

Although some previous studies reported that food intake was negatively correlated with age (McCue, 1995; Blanton et al., 1998; Tofilsky, 2000; Moya-Larano, 2002; Grotewiel et al., 2005), the present predation experiment showed that the food intake of *A. venator* was not. Therefore, the present result does not agree with those of previous studies. This suggests an age-dependent decline in food intake by *A. venator*. These differences in results may be caused by the foraging type predator used in the present study: *A. venator* is a sit-and-wait predator, which is a strategy of waiting until prey approach the predator, and it does not actively search for prey (Huey & Pianka, 1981). A decline in moving by an actively searching predator is expected to have negative effects on foraging success. On the other hand, the food intake of a sit-and-wait predator may not depend on age, even when walking traits showed a negative correlation with age. A previous study that used the orb-web spider *Zygiella x-notata*, which is a sit-and-wait predator, showed that the foraging rate did not decrease with age, although the foraging speed did decrease with age (Anotaux et al., 2014). A possible explanation for the positive correlation between the amount of predation and age is that the *A. venator* may have experienced a long period of predation on *T. castaneum* in the laboratory, which may have increased their predatory abilities over time. For example, because foraging efficiency may increase with age by experience and learning, bugd that lived longer walked more slowly, but took more direct routes, and these bugs might have succeeded more often in foraging. An age-dependent decline in appetite in *A. venator* is also possible. We need additional studies investigating the relationship between appetite and age in *A. venator*.

There were no sex differences in walking-related traits including distance and linearity, and no sex differences of the age-dependent increase in walking related traits. On the other hand, in the small-scale experiment, females showed significantly higher food intake than males, and positive correlation between age and food intake was found in females but not males. It is suggested that because females may need more resources for reproduction than males, they showed clear responses even in the small-scale experiment.

Our results suggested that walking traits and food intake are dependent on age in *A. venator*. Moreover, these results suggest that feeding did not depend on moving, and it may be affected by the foraging style of the predator. The present study suggested that differences in performance among individuals at various ages may depend on differences in behaviour types. We need additional studies that investigate factors concerning the present results in near the future.

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Tables and Figures

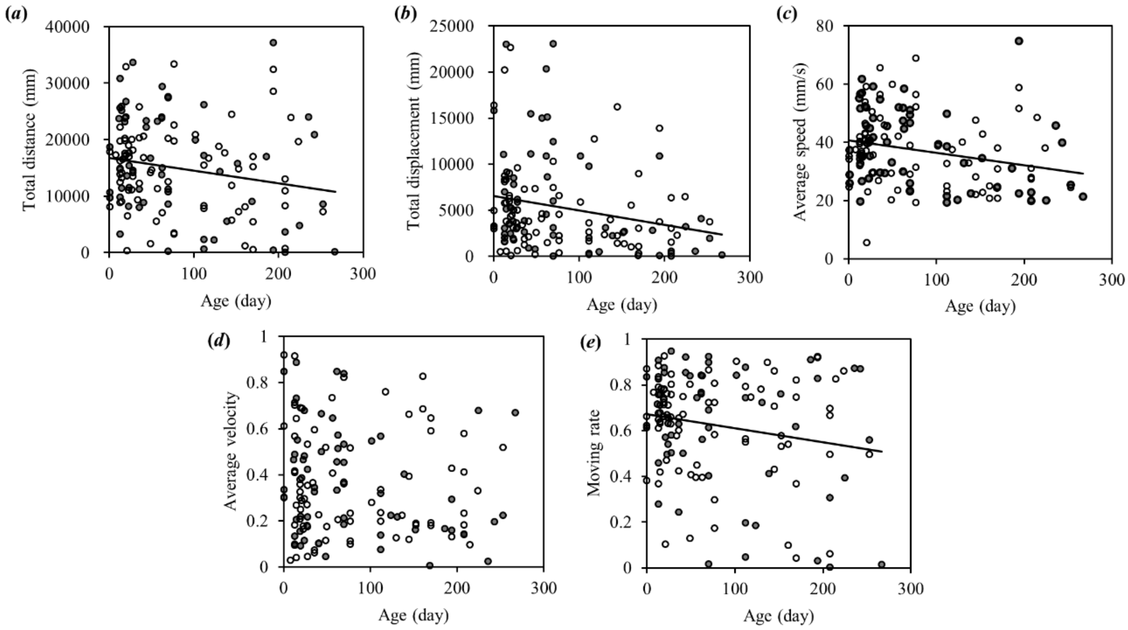

Figure 1. Relationships between walking traits (*a*: total distance, *b*: total displacement, *c*: average speed, *d*: average velocity, and *e*: moving rate) and age of *A. venator*. Filled and open circles show male and female, respectively. Regression lines in the figures (*a*, *b*, *c*, and *d*) showed when a relationship is significant (see Table 1).

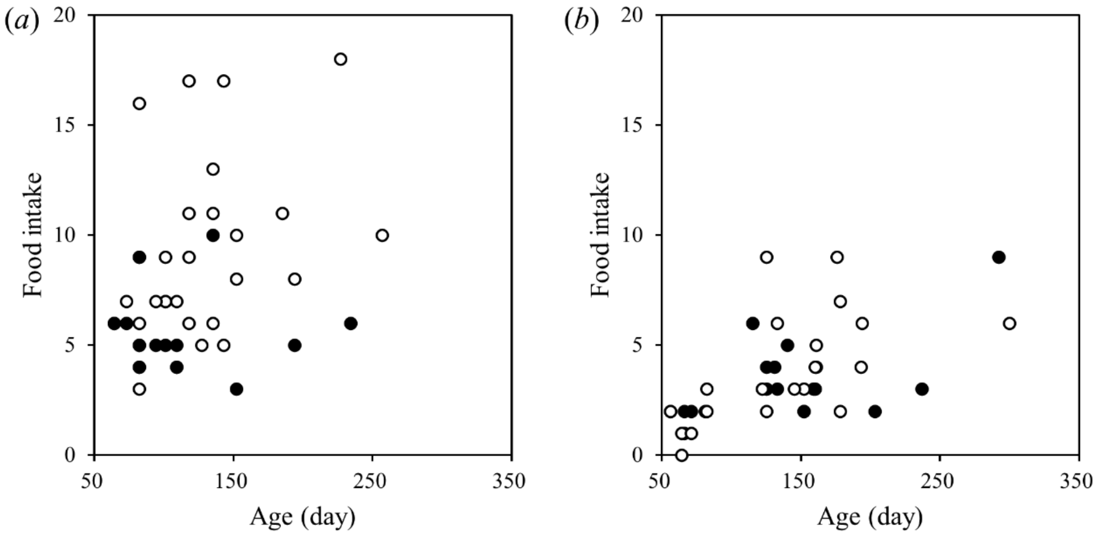

Figure 2. Relationship between age and feeding in (*a*) small- and (*b*) large-scale experiments with *A. venator*. Filled and open circles show males and females, respectively.

Table 1. Results of GLM for walking traits of *A. venator*.

Trait	Factor	*d.f.*	χ^2	*p*
Total distance	Sex	1	0.53	0.4671
	Age	1	5.06	**0.0245**
	Sex*Age	1	1.44	0.2296
	Error	135		
Total displacement	Sex	1	1.11	0.292
	Age	1	8.01	**0.0046**
	Sex*Age	1	1.53	0.2161
	Error	135		
Average speed	Sex	1	0.81	0.3676
	Age	1	8.93	**0.0028**
	Sex*Age	1	0.90	0.3436
	Error	135		
Average velocity	Sex	1	0.05	0.8155
	Age	1	1.62	0.2025
	Sex*Age	1	1.69	0.1935
	Error	135		
Moving rate	Sex	1	0.01	0.9176
	Age	1	4.63	**0.0314**
	Sex*Age	1	2.20	0.1378
	Error	135		

Table 2. Results of GLM for predation in small- and large-scale experiments.

Scale	Factor	*d.f.*	χ^2	*p*
Small	Sex	1	10.33	**0.0013**
	Age	1	5.09	**0.0240**
	Sex*Age	1	5.37	**0.0204**
	Error	52		
Large	Sex	1	1.24	0.2655
	Age	1	15.25	< **0.0001**
	Sex*Age	1	0.17	0.6822
	Error	37		