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Full title

Age-dependent walking and feeding of the assassin bug

Amphibolus venator

Short title

Age-dependent walking and feeding

Author

Kentarou Matsumura ^a, Mana Iwaya ^b, Naohisa Nagaya ^c, Ryusuke

Fujisawa ^d, Takahisa Miyatake ^{a*}

a. Graduate School of Environmental and Life Science, Okayama

University

b. Faculty of Agriculture, Okayama University

c. Kyoto Sangyo University

d. Kyushu Institute of Technology

*corresponding author

E-mail: miyatake@okayama-u.ac.jp

19

20 **Abstract**

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

33

34 **Keywords**

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

37

38 **1. Introduction**

39 Senescence often depends on the age at which deterioration of physiological
40 function occurs (Rose, 1991; Arking, 1998; López-Otín et al., 2013). Many
41 previous studies have reported that behaviours of vertebrate and invertebrate
42 animals often show an age-dependent decline (Leffelaar & Grigliatti, 1983;
43 Le Bourg & Minois, 1999; Grotewiel et al., 2005; Murakami & Murakami,
44 2005; Gargano et al., 2005; Ridgel & Ritzmann, 2005; Martines et al., 2007).
45 Among invertebrates, the fruit fly *Drosophila melanogaster* has been used
46 extensively to study age-related behavioural changes (Grotewiel et al., 2005).
47 Previous studies have focused on the decline in behavioural traits with age
48 in flies, including duration of flight (Leffelaar & Grigliatti, 1983) and
49 locomotor activity (Gargano et al., 2005; Martinez et al., 2007) in insect
50 species. In insects, negative correlations between behaviour and age are
51 considered to occur due to damage to an appendage, including the legs and
52 the cuticula that make up their structure (Ridgel & Ritzmann, 2005).

53 In mammals, food consumption may be negatively correlated with age
54 (McCue, 1995; Blanton et al., 1998). A loss of mobility could affect an

55 animal's ability to acquire resources. Increased age has also been shown to
56 be associated with a decrease in foraging efficiency in some invertebrate
57 species (Tofilsky, 2000; Moya-Larano, 2002; Grotewiel et al., 2005).
58 Moreover, the foraging style of a predator may also affect to the food intake.
59 The foraging efficiency of an actively hunting predator may decrease with
60 age due to age-dependent decline in moving, whereas a sit-and-wait predator
61 that does not require moving to forage is expected to be unaffected by an
62 age-dependent decline of mobility foraging efficiency. However, few studies
63 have examined the effects of an age-dependent decline in mobility on the
64 foraging efficiency and food intake in animals (but see Anotaux et al., 2014).

65 In the present study, we tested whether the walking behaviours and food
66 intake are correlated with age in the assassin bug *Amphibolus venator* (Klug)
67 [Hemiptera, Reduviidae]. *A. venator* often eats stored-grain insects including
68 the red flour beetle *Tribolium castaneum* (Nishi et al., 2004). We
69 hypothesized that if behavioural performance of *A. venator* declined with age,
70 then walking traits would be negatively correlated with age. Furthermore,
71 food intake may also (positively or negatively) correlate with age. To test
72 this hypothesis, we investigated walking traits and food intake using adults

73 of *A. venator* of various ages. To measure walking traits, we used a treadmill
74 system. We also measured food intake of *A. venator* in small and large
75 containers because of a possible difference in density of beetles in food. We
76 also examined the effects of sex on aging.

77

78 **2. Materials and Methods**

79 *2.1. Insect and culture*

80 The population of *A. venator* used for the present study was collected
81 from a grain store in Urasoe City, Okinawa, Japan, in 2015 by T. Miyatake,
82 and this population has been maintained in the laboratory of Okayama
83 University (see Matsumura et al., 2019). Each bug was reared in an incubator
84 maintained at 29°C and 16L:8D (light on at 7:00, light off at 23:00) light. We
85 fed *T. castaneum* to each bug ad libitum. In this study, we defined the age of
86 this insect as the number of days from the date of moulting of the last instar
87 larva, and we used *A. venator* individuals with a large variation of ages
88 (about 1 to 300 days old). A previous study revealed that the median
89 longevity of *A. venator* was 297 days old ($n = 246$) under a laboratory
90 conditions (Matsumura et al., 2019). Therefore, the bugs used in this study

91 were relatively young. However, a previous study showed that the longevity
92 of *A. venator* is shortened at high temperatures (Youssef and Abd-Elgayed,
93 2015). Because the insects used in this study were reared at 29°C, whereas
94 in the previous study they were reared at 25°C, the median of longevity may
95 be shorter than 297 days old.

96

97 *2.2. Locomotor activity*

98 To measure the walking traits of *A. venator*, we used a treadmill system,
99 ANTAM (Nagaya et al., 2017). The ANTAM was developed from an
100 omnidirectional treadmill mechanism system in which animal movements
101 can be continuously recorded and compensated for in such a way that the
102 animal is always located on the top of the sphere and experiences a virtual
103 unbounded two-dimensional field (Nagaya et al., 2017). Therefore, this
104 system is able to measure the free walking trajectories of small animals such
105 as insects (Shoji et al., 2019). The walking speed of *A. venator* is $37.25 \pm$
106 12.55 mm / sec (mean \pm *s.d.*, $n = 133$; unpublished), which is within the
107 allowable range of the movement speed of the system used (for example,
108 ANTAM can measure the walking speed of 55.2 ± 34.3 mm/sec (mean \pm *s.d.*)

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

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

114

115 2.3. Predation

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

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

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

135

136 *2.4. Statistical analysis*

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

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

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

155

156 **3. Results**

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

162 Figure 2 shows the results of predation experiments on the small and

163 large scales. In the small container, females showed significantly higher food
164 intake than males (Fig. 2, Table 2). Age had a significant effect on food intake,
165 and an interaction between age and sex was also shown in the small-scale
166 experiment (Table 2). Therefore, we also analysed the data for sex separately,
167 and a positive significant correlation between age and food intake was found
168 in females ($\chi^2_{1,35} = 9.76, p = 0.0018$), but not in males ($\chi^2_{1,17} = 0.71, p =$
169 0.3994). In the large-scale experiment, there was a positive correlation
170 between predation and age (Fig. 2b, Table 2), but no significant effect of sex
171 and interaction was found (Fig. 2b, Table 2).

172

173 **4. Discussion**

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

181 between food intake and age.

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

188 In the present study, a negative correlation between walking traits and
189 age was found in *A. venator*. Therefore, the present results for *A. venator* are
190 in accordance with those of previous studies (Rose, 1991; Larsson et al.,
191 1997; Grotewiel et al., 2005; Ridgel & Ritzmann, 2005; Gargano et al., 2005;
192 Martinez et al., 2007; Miller et al., 2008). **The average** speed was negatively
193 correlated with age (**Fig. 1c**), whereas the average velocity was not (**Fig. 1d**).
194 This suggests that an age-dependent decline in walking might not have
195 caused alterations of the walking pattern with age. This is consistent with
196 an age-related decline in movement, which in some insects has been
197 mechanistically attributed to damage to walking appendages/legs (Ridgel &
198 Ritzmann, 2005). That is, the present results suggest that older aged adults

199 of *A. venator* decrease their locomotor activity due to leg damage with aging.
200 Moreover, because the moving rate also showed an age-dependent decline,
201 the decrease in walking performance might have been affected by
202 physiological factors in addition to mechanical damage.

203 Although some previous studies reported that food intake was
204 negatively correlated with age (McCue, 1995; Blanton et al., 1998; Tofilsky,
205 2000; Moya-Larano, 2002; Grotewiel et al., 2005), the present predation
206 experiment showed that the food intake of *A. venator* was not. Therefore, the
207 present result does not agree with those of previous studies. This suggests an
208 age-dependent decline in food intake by *A. venator*. These differences in
209 results may be caused by the foraging type predator used in the present study:
210 *A. venator* is a sit-and-wait predator, which is a strategy of waiting until prey
211 approach the predator, and it does not actively search for prey (Huey &
212 Pianka, 1981). A decline in moving by an actively searching predator is
213 expected to have negative effects on foraging success. On the other hand, the
214 food intake of a sit-and-wait predator may not depend on age, even when
215 walking traits showed a negative correlation with age. A previous study that
216 used the orb-web spider *Zygiella x-notata*, which is a sit-and-wait predator,

217 showed that the foraging rate did not decrease with age, although the
218 foraging speed did decrease with age (Anotaux et al., 2014). A possible
219 explanation for the positive correlation between the amount of predation and
220 age is that the *A. venator* may have experienced a long period of predation
221 on *T. castaneum* in the laboratory, which may have increased their predatory
222 abilities over time. For example, because foraging efficiency may increase
223 with age by experience and learning, bugs that lived longer walked more
224 slowly, but took more direct routes, and these bugs might have succeeded
225 more often in foraging. An age-dependent decline in appetite in *A. venator*
226 is also possible. We need additional studies investigating the relationship
227 between appetite and age in *A. venator*.

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

235 experiment.

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

243

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250 to TM.

251

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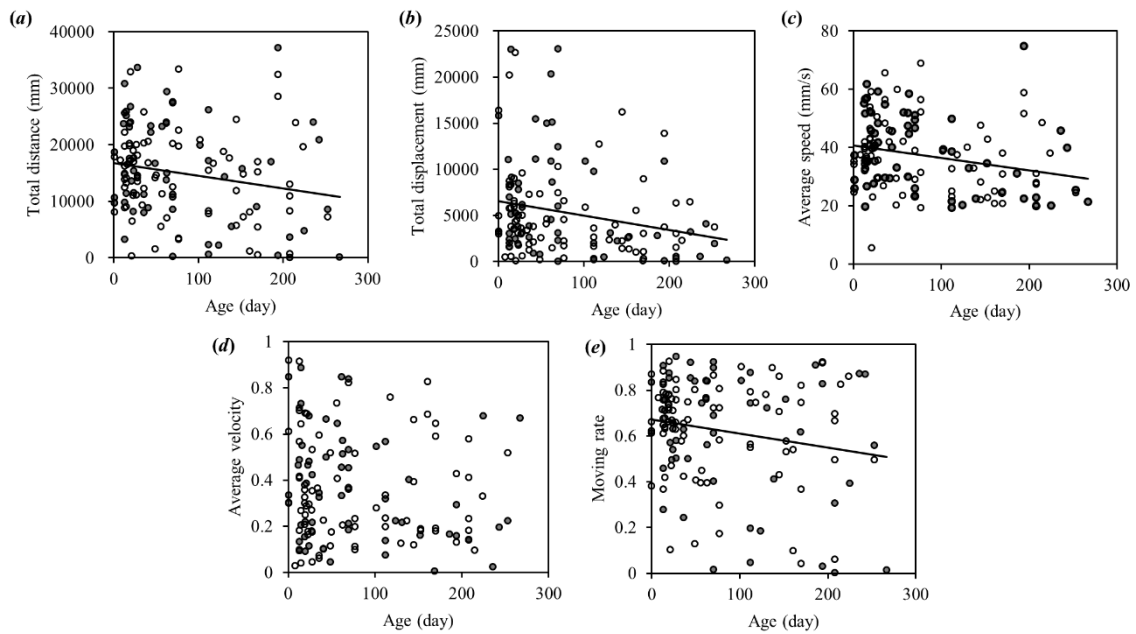
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333

334 **Tables and Figures**

335



336

337

338 **Figure 1.** Relationships between walking traits (*a*: total distance, *b*: total

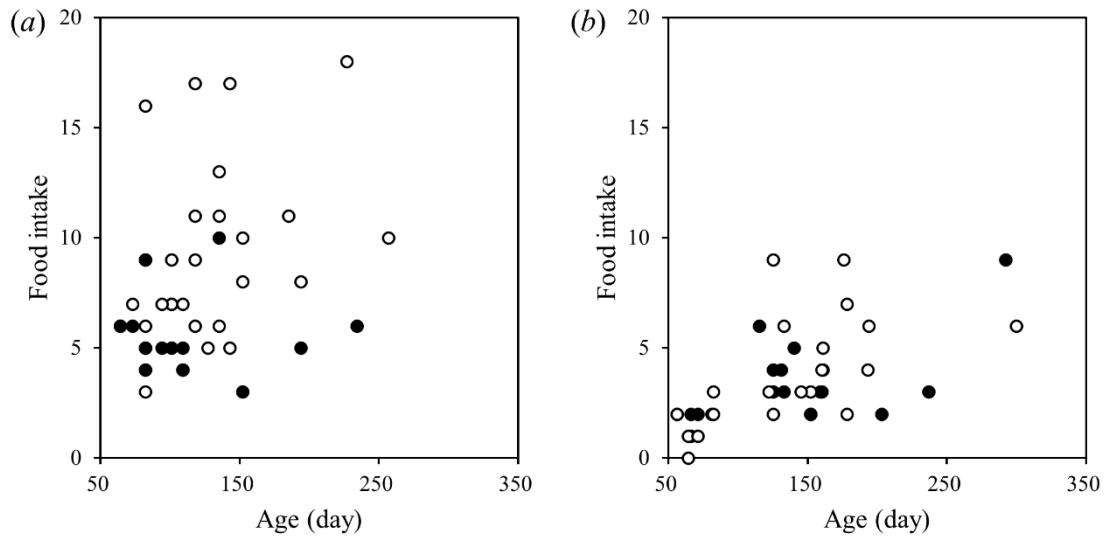
339 displacement, *c*: average speed, *d*: average velocity, and *e*: moving rate) and

340 age of *A. venator*. Filled and open circles show male and female, respectively.

341 Regression lines in the figures (*a*, *b*, *c*, and *e*) showed when a relationship is

342 significant (see Table 1).

343



344

345

346 **Figure 2.** Relationship between age and feeding in (a) small- and (b) large-

347 scale experiments with *A. venator*. Filled and open circles show males and

348 females, respectively.

349

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

Trait	Factor	<i>d.f.</i>	χ^2	<i>p</i>
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		

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

Scale	Factor	<i>d.f.</i>	χ^2	<i>p</i>
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		