

1

Title

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Is there a genetic correlation between movement and

3

immobility in field populations of a beetle?

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17

Abstract

18 Genetic correlations among behavioural traits are often controlled by pleiotropic genes.
19 Many studies suggest the existence of genetic correlations among behavioural traits
20 based on artificial selection experiments in the laboratory. However, few studies have
21 examined whether behavioural correlations in the laboratory are maintained in the field,
22 where natural selection works. Artificial selection experiments showed a behavioural
23 correlation among death feigning, walking movement, and locomotor activity in the red
24 flour beetle (*Tribolium castaneum*). This study investigated whether this behavioural
25 correlation is observed in wild *T. castaneum* populations. We also collected beetles from
26 various regions in Japan and investigated the geographic variation in these traits. There
27 was geographic variation in the three behavioural traits. However, these behavioural
28 traits were not correlated. The results suggest that the genetic correlations among
29 behavioural traits are not maintained in the field. Therefore, the results derived from
30 laboratory experiments may be overestimated. The same correlation between traits was
31 not believed to arise in the field, as the indoor results may have been caused by
32 unrealistic selection pressures. Further laboratory and field investigations are both
33 needed.

34

35 **Keyword:** behavioural correlation, genetic correlation, death feigning, moving,

36 locomotor activity, geographic variation

37

38 **Introduction**

39 Animal behaviours are often correlated with other behavioural traits due to
40 environmental and genetic factors (Lande, 1979; Lande & Arnold, 1983; Bell, 2005).

41 Correlations among behavioural traits by genetic factors (*i.e.*, genetic correlation) are
42 often controlled by pleiotropic genes (Lande, 1979; Lande & Arnold, 1983; Bell, 2005).

43 If a correlation among behavioural traits has a genetic basis when natural selection
44 favors a behaviour, other behavioural traits genetically correlated with the behavioural
45 trait may also evolve, even if the correlated behaviours decrease fitness. This may
46 decrease the variation in behavioural traits. That is, a genetic correlation among
47 behavioural traits may maintain the variation in behavioural traits within a population.

48 Therefore, studies of genetic correlations among behavioural traits are important in
49 behavioural ecology.

50 Artificial selection is an experimental method that examines genetic correlations
51 among behavioural traits (Hill & Caballero, 1992; Garland Jr & Carter, 1994). When
52 behavioural traits respond to artificial selection for another behavioural trait, the
53 relationship among these behavioural traits may be genetic (Falconer & Mackay, 1996).

54 Many artificial selection experiments have examined animal behaviours. For example,
55 risk-taking behaviour in the great tit, *Parus major* was correlated with artificial
56 selection for exploration behaviour (van Oers, Drent, de Goede, & van Noordwijk,
57 2004). In the adzuki bean beetle, *Callosobruchus chinensis*, when the duration of
58 death-feigning behaviour, which may be adaptive anti-predator behaviour, is selected
59 artificially, flight activity responds negatively as a correlated trait (Ohno & Miyatake,
60 2007). However, because the pressure due to artificial selection may not occur in the
61 field, these studies may have overestimated the correlations among behavioural traits.
62 Therefore, comparative investigations using wild populations, not only artificial
63 selection, are important to explain the evolution of correlations among behavioural
64 traits.

65 Wild populations may be under many selection pressures compared with
66 populations selected artificially (Mousseau, Sinervo, & Endler, 2000). If the genetic
67 correlation among behavioural traits is controlled by pleiotropic genes, this behavioural
68 correlation may be observed in wild populations. However, few studies have
69 investigated behavioural correlations using both artificial selection and wild populations
70 (but see Ohno & Miyatake, 2007).

71 The intensity and direction of selection pressure may differ among
72 geographically different populations. Geographic variation in morphological and
73 life-history traits has been observed in many species (*e.g.*, Bergmann, 1848;
74 Blanckenhorn & Demont, 2004; Blanckenhorn, Stillwell, Young, Fox, & Ashton, 2006).
75 Furthermore, many studies have reported geographic variation in behavioural traits
76 (*e.g.*, Lankinen, 1986; Foster & Endler, 1999; Mathias, Jacky, Bradshaw, & Holzapfel,
77 2005; Lankinen & Forsman, 2006). Therefore, if the relationship among behavioural
78 traits is genetic, geographic variation may also be observed in the behavioural
79 correlation. However, few studies have investigated behavioural correlations in various
80 wild populations.

81 In this study, we examined the red flour beetle *Tribolium castaneum*, a common
82 cereal storage pest worldwide (Sokoloff, 1977). Previous studies induced artificial
83 selection on two behavioural traits in *T. castaneum*: death-feigning behaviour and
84 moving ability (Miyatake, Katayama, Takeda, Nakashima, Sugita, & Mizumoto, 2004;
85 Matsumura & Miyatake, 2015). Miyatake, Katayama, Takeda, Nakashima, Sugita, &
86 Mizumoto (2004) artificially selected death-feigning duration and established genetic
87 strains with longer (LD) and shorter (SD) durations of death-feigning; in a predation
88 experiment, LD individuals had a higher survival rate than the SD strain in encounters

89 with the jumping spider, *Hasarius adansoni*. Moreover, individuals from the LD strain
90 were significantly less mobile than the SD strain (Miyatake, Tabuchi, Sasaki, Okada,
91 Katayama, & Moriya, 2008b). Matsumura and Miyatake (2015) artificially selected
92 moving ability and established higher (HM) and lower (LM) mobility strains.
93 Individuals from the HM strain had significantly shorter death feigning and greater
94 locomotor activity than the LM strain (Matsumura, Sasaki, & Miyatake, 2016). These
95 studies suggested that the relationship among death feigning, moving ability, and
96 locomotor activity is genetic in *T. castaneum*. The relationship among these behavioural
97 traits was also demonstrated in *Tribolium confusum* (Nakayama, Nishi, & Miyatake,
98 2010; Nakayama, Sasaki, Matsumura, Lewis, & Miyatake, 2012) and *C. chinensis*
99 (Ohno & Miyatake, 2007; Nakayama & Miyatake, 2010) in artificial selection
100 experiments. If these three behavioural traits are correlated genetically, such
101 correlations may be observed in wild insect populations and geographic variation might
102 be found in nature. Therefore, in this study, we measured the death feigning, moving
103 ability, and locomotor activity of *T. castaneum* in wild populations at 36 locations in
104 Japan. To investigate the role of genetic factors in the behavioural correlations, we used
105 wild populations maintained for at least two generations after collection from the field.
106 We investigated whether the behavioural correlation was observed in wild populations.

107

108 **Materials & Methods**

109 *Insect*

110 *Tribolium castaneum* was collected at 36 locations in Japan (Fig. 1). Table S1 shows the
111 latitude and longitude of each. The northernmost is Aomori (40°89'N, 140°46'E) and
112 the southernmost is Okinawa (26°25'N, 127°69'E) (Appendix Table A1). Collection
113 was done in 2016 and 2017. Each beetle was reared in an incubator (Sanyo, Tokyo,
114 Japan) maintained 25°C and 16L:8D light cycle (light on at 07:00, off at 23:00). Food is
115 a mixture of whole meal (Nisshin Seifun, Tokyo, Japan) with brewer's yeast (Asahi
116 Beer, Tokyo).

117

118 *Measurements of each behavioural trait*

119 We measured frequency and duration of death feigning of *T. castaneum*, in
120 accordance with Miyatake, Katayama, Takeda, Nakashima, Sugita, & Mizumoto (2004).
121 Virgin males and females (21–28 days old) were randomly collected from each wild
122 population. When the beetle shows death-feigning behaviour by touching the beetle's
123 abdomen with a stick, we measured the duration with a stopwatch (the duration was
124 defined as the time until detecting its first visible movement). If the beetle did not show

125 death feigning, the stimuli was repeated up to three times. Details of the methods for
126 observation of death feigning are described in Miyatake, Katayama, Takeda,
127 Nakashima, Sugita, & Mizumoto (2004).

128 Walking distance as the moving ability of *T. castaneum* was measured using an image
129 tracker system (Digimo, Osaka, Japan), in accordance with Matsumura and Miyatake
130 (2015). Virgin beetles (21–28 days old) were collected from each population and
131 measured walking distance. The moving behaviour were recorded for 30 min, and
132 measure the walking distance of each beetle on the recorded image, we used analysis
133 software (2D-PTV Ver. 9.0, Digimo, Osaka, Japan). Details of the methods for
134 measurement of moving ability are described in Matsumura and Miyatake (2015).

135 To measurement of locomotor activity, virgin males and females (21–28 days old)
136 were randomly collected from each wild population, and measured locomotor activity
137 used by an infrared actograph system. When the beetle passed the midpoint of the dish,
138 the infrared light between emitter and detector device (E3R-5E4/R2E4DS30E4; Omron,
139 Kyoto, Japan) was interrupted. We measured the number of interruptions of the infrared
140 light for 24 h as locomotor activity of the beetle. To remove artificial effect on behavior,
141 we removed first 2 h data (i.e., we used 22 h data for statistical analysis). Details of the
142 methods for measurement of locomotor activity are described in Matsumura, Sasaki, &

143 Miyatake (2016).

144

145 *Statistical analysis*

146 Death-feigning duration (+ 1 s) and moving ability (+ 1 mm) were analyzed using a
147 generalized linear model (GLM) with gamma distribution, and population, sex, and the
148 interaction between population and sex as experimental variables. The frequencies of
149 death feigning and locomotor activity were analyzed by GLMs with binomial and
150 Poisson distributions, respectively. To analyze the relationship between duration of
151 death feigning and moving ability within individual level, we used GLM with gamma
152 distribution, and duration of death feigning as a dependent variable, and moving ability
153 and population as experimental variables. Because individuals used measurement of
154 locomotor activity are differed with individuals used measurement of death feigning and
155 moving ability, we did not analysis of relationship between death feigning and
156 locomotor activity, and moving and locomotor activity within individual. To analyze the
157 relationship between death feigning, moving ability, and locomotor activity inter
158 populations, we used Spearman's rank correlation coefficient for mean values of three
159 behavioural traits in each wild population. To analyze the effects of latitude and
160 longitude on mean values of each behavioural trait, we used analysis of covariance

161 (ANCOVA) with population, sex, and the interaction between the two as covariates. All
162 analyses were done using R ver. 3.4.3 (R Core Team, 2017).

163

164 *Ethical Note*

165 The laboratory population of *T. castaneum* used in this study have maintained at
166 Okayama University for over 15 years. This population has been maintained on whole
167 meal flour with yeast (see Miyatake et al. 2004). We reared this population at 25 °C,
168 which resemble natural conditions for this insect. All animals in the study were handled
169 more carefully. The use of these animals conforms to the Animal Ethics Policy of
170 Okayama University.

171

172 **Results**

173 Figure 2 shows the duration and frequency of death-feigning behaviour. Figure 3 shows
174 the moving ability and locomotor activity. Table 1 shows the mean values of each
175 behaviour. Death-feigning duration differed significantly among wild populations, but
176 not the sexes (Fig. 2a, Table 2). The frequency of death feigning also differed
177 significantly among wild populations, and was significantly more frequent in males
178 (Fig. 2b, Table 2). Moving ability differed significantly among wild populations, but not

179 the sexes (Fig. 3a, Table 2). Locomotor activity differed significantly among wild
180 populations, and females were significantly more active than males (Fig. 3b, Table 2).
181 The interaction between wild population and sex was not significant in any behavioural
182 trait (Table 2). Appendix Figure A1 shows the relationship between latitude and each
183 behavioural trait. There were no significant associations between latitude and each
184 behavioural trait (Table 3). Appendix Figure A2 shows the relationship between
185 longitude and each behavioural trait. There were no significant associations between
186 longitude and each behavioural trait (Table 3). Duration of death feigning did not show
187 significantly correlated with moving ability within individual level ($\chi^2_{1,1044} = 3.80, p =$
188 0.05112). In inter population level, no behaviour was significantly correlated with
189 another (Table 4).

190

191 **Discussion**

192 This study found geographic variation in death-feigning, moving ability, and locomotor
193 activity among 36 wild populations collected in Japan (Figs. 2, 3). Because we used
194 each population maintained at the laboratory for at least two generations, these
195 behavioural differences among populations may be caused by genetic factors, rather
196 than environmental or maternal factors. Moreover, the geographic variation suggests

197 that the selection pressures on the three behaviours differ among wild populations.
198 However, although previous studies suggested genetic correlations among the three
199 behaviours in artificial selection experiments, we did not find any correlations among
200 the three behaviours in the wild populations (Fig. 4). This suggests that the intensity of
201 the relationships among these behavioural traits is lower in the field, even if the genetic
202 correlations were shown by artificial selection.

203 First, we discuss the results for the death-feigning behaviour. Prohammer and
204 Wade (1981) reported geographic variation in death-feigning behaviour among
205 populations from Spain, Japan, and USA. Our results are in accord regarding
206 geographic variation in the duration of death feigning (Prohammer & Wade, 1981). We
207 also found that geographic variation in death feigning was seen over a relatively narrow
208 range compared with Prohammer & Wade (1981).

209 We considered three hypotheses to explain the death-feigning behaviour results.
210 The first is the difference in predation pressure on each wild population. Death feigning
211 is an adaptive anti-predator behaviour (Humphreys & Ruxton, 2018). However, the
212 optimum death-feigning duration may be affected by predator type or density. In the
213 great tit *Parus major*, the egg ejection rate showed geographic variation, and this pattern
214 matched the geographic pattern of parasitism risk (Liang et al., 2016). In *T. castaneum*,

215 individuals with longer death-feigning duration increase their survival rate in encounters
216 with predators (Miyatake, Katayama, Takeda, Nakashima, Sugita, & Mizumoto, 2004).
217 Similarly, the optimum death-feigning duration may be altered by the density of
218 predators. For example, individuals with longer death-feigning duration may increase
219 the survival rate in places under higher predation pressure, whereas individuals with a
220 shorter duration of this behaviour may increase foraging or reproductive success at
221 places with lower predation pressure (Nakayama & Miyatake, 2010a, b). Moreover,
222 differences in the type of predation may affect the evolution of this behaviour (Honma,
223 Oku, & Nishida, 2006). For example, individuals with longer death-feigning duration
224 may increase their survival rate when encountering active-hunting predators, whereas
225 they may decrease the survival rate when encountering sit-and-wait predators (Honma,
226 Oku, & Nishida, 2006). Therefore, in locations with sit-and-wait predators, shorter
227 death-feigning duration may evolve. Populations with longer death-feigning duration
228 might suffer predation pressure by active hunting predators, whereas populations with
229 shorter death feigning might suffer predation pressure by sit-and-wait predators. Ohno
230 & Miyatake (2007) also reported geographic variation in the duration of death feigning
231 in *C. chinensis*, and suggested that this geographic variation was a result of differences

232 in predation pressure among these wild populations. Future studies should investigate
233 the density and type of predators.

234 The second hypothesis is the effects of prey density. In *T. castaneum*, the
235 optimal death-feigning duration may depend on the conspecific density. Miyatake,
236 Nakayama, Nishi, & Nakajima (2009) reported that beetles with longer death-feigning
237 duration had a higher survival rate in the presence of non-feigners or prey of a different
238 species, compared to when alone, confirming the selfish-prey hypothesis. The prey
239 density might have important effects on death-feigning behaviour. Conspecific or
240 heterospecific density in populations with longer death-feigning durations may be
241 higher, whereas it may be lower in populations with shorter death-feigning durations.
242 Studies need to investigate the effects of population density.

243 The third hypothesis is founder effects on the behaviour. The founder effect, *i.e.*,
244 the loss of genetic variation when a new population is established by a small number of
245 individuals (Templeton, 1980), is found in animal behaviour (Suarez, Tsutui, Holway,
246 & Case, 1999). In populations with longer death-feigning duration, populations may
247 have been established by individuals with longer death-feigning durations. This
248 hypothesis is based on the supposition that gene flow is infrequent among *T. castaneum*
249 populations. Although a study reported a bottleneck in a wild population (Semeao,

250 Campbell, Beeman, Lorenzen, Whitworth, & Sloderbeck, 2012), another suggested that
251 gene flow often occurs over a wide range in *T. castaneum* (Ridley, Hereward, Daglish,
252 Raghu, Collins, & Walter, 2011). Additional studies should investigate genetic
253 differences among wild populations.

254 Moving ability and locomotor activity also showed geographic variation among
255 wild populations in *T. castaneum*. A previous study revealed that *T. castaneum* with
256 genetically higher moving ability had a lower survival rate when predators were present
257 (Matsumura & Miyatake, 2015). Similarly, individuals with higher locomotor activity
258 are considered at increased risk of predation in many animals (Sih, Bell, & Johnson,
259 2004; Réale, Reader, Sol, McDougall, & Dingemanse, 2007). Therefore, predation
260 pressure on moving ability and locomotor activity may also differ among wild
261 populations, as with death-feigning behaviour. Moreover, moving ability affects
262 reproductive success, and there was a trade-off between survival rate and reproductive
263 success between strains selected for higher and lower moving ability in *T. castaneum*
264 (Matsumura & Miyatake, 2015, 2018a; Matsumura, Archer, Hosken, & Miyatake,
265 2019). Therefore, these behavioural traits may be affected by balancing selection
266 between predation avoidance and reproduction among wild populations. Moving ability
267 seems to be similar to locomotor activity and vice versa. Nevertheless, these behaviours

268 were not significantly correlated. These results suggest that these behaviours evolved
269 independently in the field. In the parasitoid wasp *Leptopilina heterotoma*, geographic
270 variation was found in locomotor activity (Fleury, Allemand, Fouillet, & Boulétreau,
271 1995). Furthermore, some insect studies reported that the circadian rhythm of locomotor
272 activity showed geographic variation, with a clear rhythm at lower latitudes and no
273 rhythmic activity at higher latitudes (*e.g.*, Fleury, Allemand, Fouillet, & Boulétreau,
274 1995; Joshi, 1999). Additional studies should investigate the circadian rhythm of *T.*
275 *castaneum* in each wild population.

276 Although this study revealed geographic variation in three behavioural traits,
277 these behavioural traits did not show latitudinal or longitudinal clines (Appendix Figs.
278 A1, A2). In the medaka *Oryzias latipes* complex, courtship behaviour by males and
279 female preference for males showed latitudinal variation such that populations from
280 lower latitudes showed greater intensity of these behaviours (Fujimoto, Miyake, &
281 Yamahira, 2015). These results suggest that because the reproductive season is
282 relatively shorter at lower latitudes, a latitudinal cline develops in the operational sex
283 ratio among wild populations, which ultimately results in a latitudinal cline in sexual
284 selection pressures (Fujimoto, Miyake, & Yamahira, 2015). Therefore, the intensity or
285 direction of selection pressure on the behavioural traits may differ among wild

286 populations from various latitudes. Moreover, some studies reported that individuals
287 had a significantly longer death-feigning duration under lower temperatures than under
288 higher temperatures in other insect species (Holmes, 1906; Miyatake, Okada, & Harano,
289 2008a). However, we did not find a latitudinal or longitudinal cline in death-feigning
290 behaviour. That is, the effects of temperature on death feigning may be relatively
291 smaller in *T. castaneum*, at least, within the temperature range examined in this study.
292 Furthermore, moving ability and locomotor activity did not show latitudinal or
293 longitudinal clines, which also suggested that environmental factors, such as
294 temperature, do not affect these behavioural traits in this beetle.

295 Although the three behavioural traits showed geographic variation among the
296 wild populations, we did not find a significant correlation among these behaviours (Fig.
297 4). Some artificial selection studies in laboratories suggested a genetic correlation
298 among death-feigning, moving ability, and locomotor activity in some insects (Ohno &
299 Miyatake, 2007; Miyatake, Tabuchi, Sasaki, Okada, Katayama, & Moriya, 2008b;
300 Nakayama & Miyatake, 2010; Nakayama, Nishi, & Miyatake, 2010; Nakayama, Sasaki,
301 Matsumura, Lewis, & Miyatake, 2012; Matsumura, Fuchikawa, & Miyatake, 2017).
302 Therefore, we hypothesized that there would be a correlation among these behavioural
303 traits in wild populations. However, we did not find a significant correlation among

304 these behaviours. The results suggest that the intensity of the genetic correlations among
305 each behaviour may be relatively lower in *T. castaneum*. Previous studies reported that
306 moving ability and locomotor activity showed correlated responses to artificial selection
307 for death-feigning behaviour for over 10 generations (Ohno & Miyatake, 2007;
308 Miyatake, Tabuchi, Sasaki, Okada, Katayama, & Moriya, 2008b; Nakayama, Nishi, &
309 Miyatake, 2010). Moreover, death-feigning and locomotor activity showed correlated
310 responses to artificial selection for moving ability for more than 15 generations
311 (Matsumura, Fuchikawa, & Miyatake, 2016). If the intensity of the genetic correlations
312 among these behaviours is relatively low, these correlations may be difficult to observe
313 in the field. Alternatively, the artificial selection pressure may be of abnormal intensity
314 compared with the field. For example, although beetles from strains selected artificially
315 for longer death-feigning duration feigned death for up to 1,000 seconds (Miyatake,
316 Katayama, Takeda, Nakashima, Sugita, Mizumoto, & Miyatake, 2004; Matsumura &
317 Miyatake, 2018b), few beetles feigned death this long in the wild populations (Fig. 2a).
318 Another study reported that the duration of death feigning was correlated with flight
319 activity within and among wild populations in *C. chinensis* (Ohno & Miyatake, 2007).
320 Therefore, the intensity of the relationship between behaviours may differ among
321 behaviours or species. Similar investigations of other behaviours or species are required.

322 In conclusion, a genetic correlation seen in artificial selection experiments in the
323 laboratory among behavioural traits may not always be observed in the field. This is
324 because the selection pressure due to artificial selection may be abnormal. This suggests
325 that artificial selection may lead to overestimation of the results. Therefore, to study the
326 evolution of genetic correlations in behavioural traits, behavioural ecologists should
327 examine the relationships among behavioural traits using both artificial selection and
328 wild populations.

329

330 **Acknowledgement**

331 We thanks to Mr. Yusuke Tsushima and Kohei Nakao for collection of *T. castaneum* in
332 Aomori and Hyogo prefectures. This work was supported by a grant from the Japan
333 Society for the Promotion of Science KAKENHI 26291091, 16K14810, 17H05976 and
334 18H02510 to TM.

335

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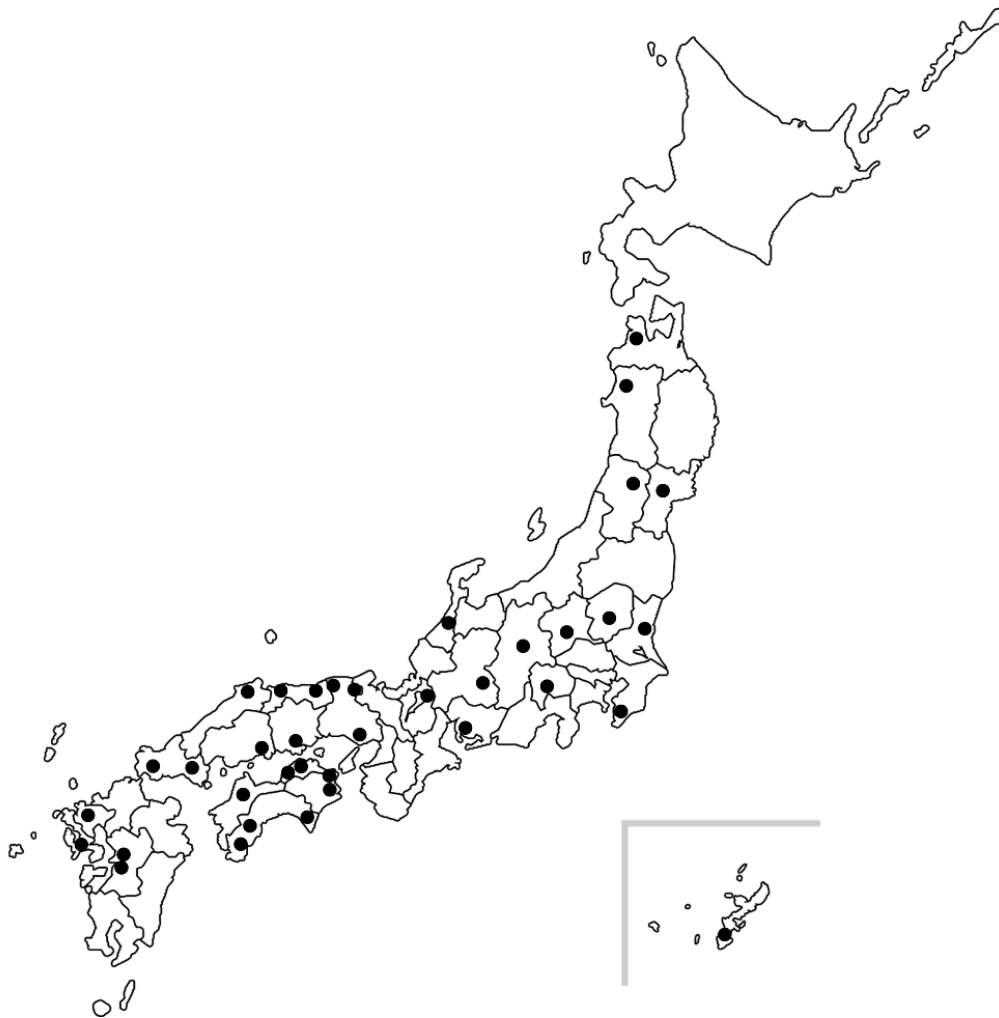
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485 **Figure and Table**

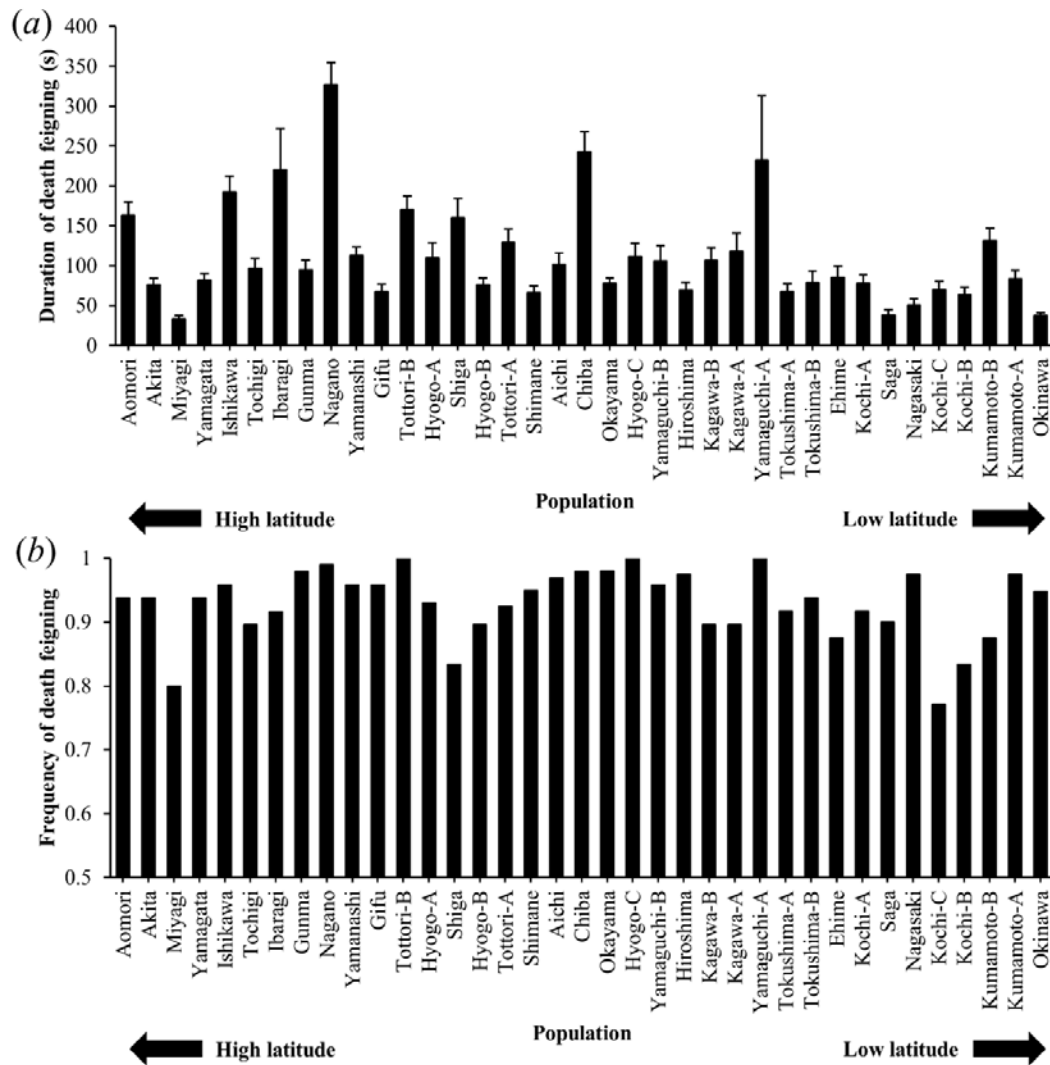
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487

488 **Figure 1.** Places that captured of wild population of *T. castaneum* in Japan.

489

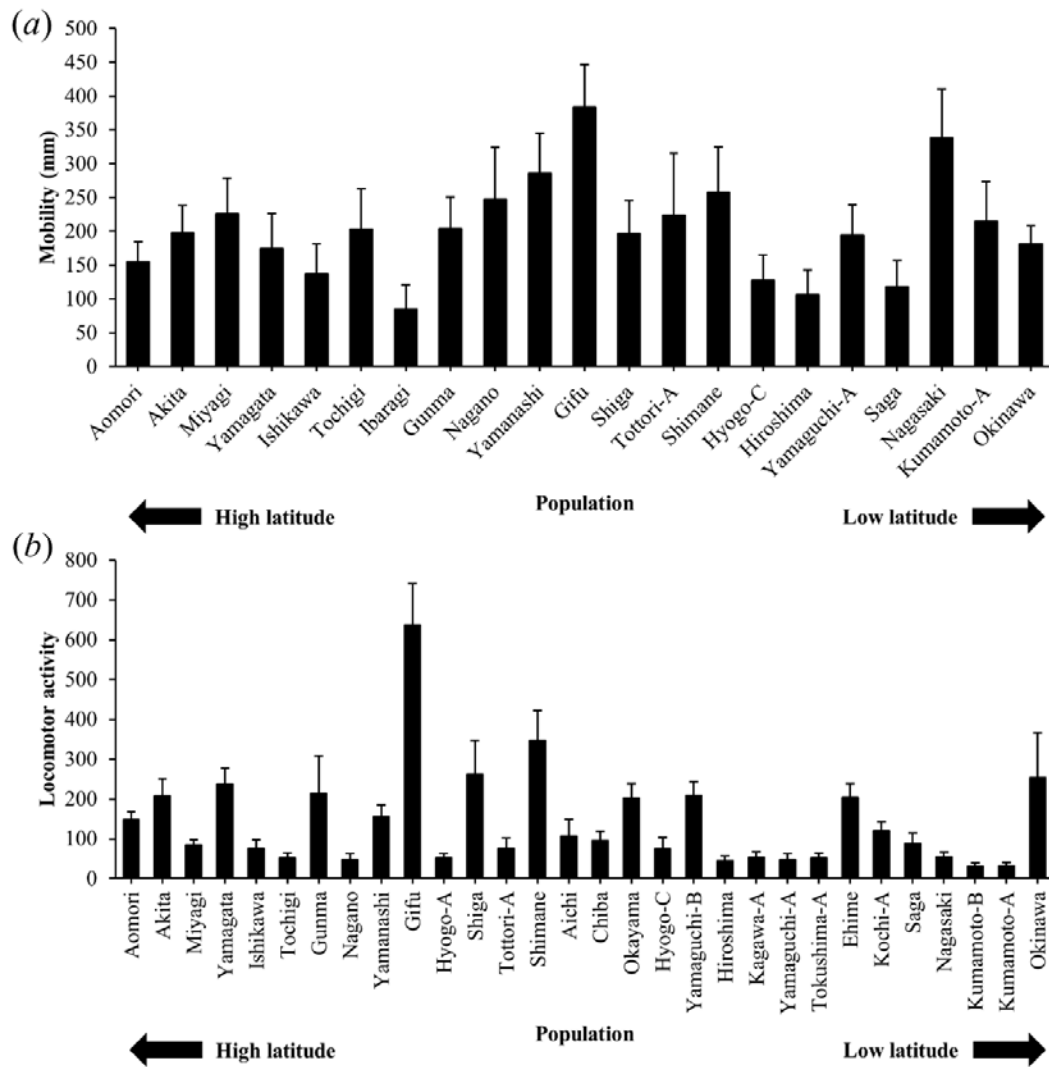


490

491 **Figure 2.** Duration (a) and frequency (b) of death-feigning behaviour in each population.

492 Error bars show SE.

493



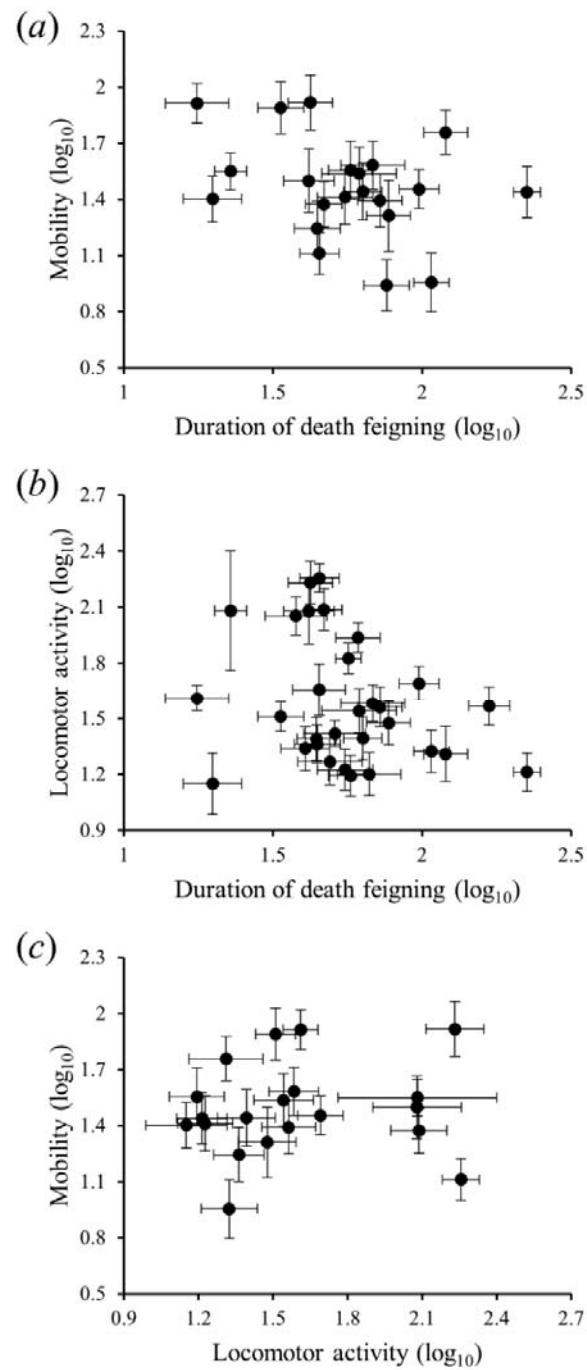
494

495 **Figure 3.** Mobility (a) and locomotor activity (b) in each population. Error bars show

496 SE.

497

498



499

500 **Figure 4.** Relationship between duration of death feigning and walking speed or

501 locomotor activity. Each point shows mean value of each population. Error bars show

502 SE.

504 **Table 1.** Mean and SE of each trait.

Trait	Male		Female	
	Mean \pm SE	<i>N</i>	Mean \pm SE	<i>N</i>
Duration of death feigning (s)	116.35 \pm 5.26	1064	120.81 \pm 6.31	1022
Frequency of death feigning	0.94 \pm 0.01	1064	0.92 \pm 0.01	1022
Walking distance (mm)	214.11 \pm 17.49	533	184.63 \pm 14.52	533
Locomotor activity	95.52 \pm 7.09	614	216.62 \pm 19.82	605

505

506

507 **Table 2.** Results of GLM for effects of population and sex on death feigning, walking
508 distance, and locomotor activity.

Trait	Factor	<i>d.f.</i>	χ^2	<i>p</i>
Duration of death feigning	Population	36	564.08	< 0.0001
	Sex	1	0.38	0.5368
	Population×sex	35	41.88	0.2308
	Error	2012		
Frequency of death feigning	Population	36	85.88	< 0.0001
	Sex	1	4.88	0.02713
	Population×sex	36	28.09	0.82391
	Error	2012		
Walking distanc	Population	20	31.92	0.04414
	Sex	1	1.39	0.2379
	Population×sex	20	20.37	0.43457
	Error	1025		
Locomotor activity	Population	29	255.60	< 0.0001
	Sex	1	59.84	< 0.0001
	Population×sex	27	30.53	0.2909

Error

1161

509

510

511 **Table 3.** Results of mixed ANOVA for effects of latitude, longitude and sex on each
512 behavioural traits.

Trait	Factor	<i>d.f.</i>	<i>F</i>	<i>P</i>
Death feigning	Latitude	1	0.05	0.8199
	Longitude	1	1.87	0.1803
	Sex	1	0.26	0.6128
	Error	2082		
Mobility	Latitude	1	0.01	0.9276
	Longitude	1	0.00	0.9674
	Sex	1	1.74	0.1880
	Error	1062		
Activity	Latitude	1	0.01	0.9143
	Longitude	1	0.15	0.6993
	Sex	1	40.80	< 0.0001
	Error	1217		

513

514

515 **Table 4.** Results of Spearman's rank correlation coefficient for mean values in each
516 behavioural trait.

Factors	<i>N</i>	ρ	<i>P</i>
Duration of death feigning & mobility	21	-0.21	0.3630
Duration of death feigning & activity	30	-0.27	0.1521
Mobility & activity	20	0.20	0.4015

517

518

519 **Appendix**

520

521 **Table A1.** Name, latitude, longitude, and sample size of each behavioural traits of each

522 place that captured of populations of *T. castaneum*.

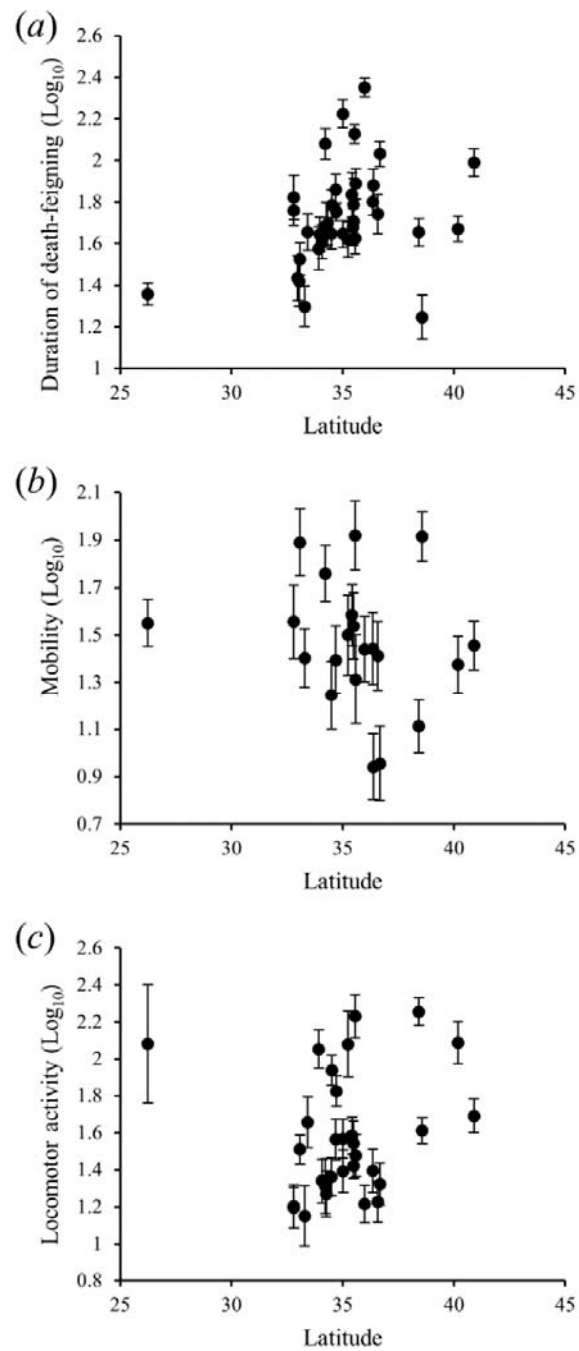
Population	Latitude	Longitude	Sample size		
			Death feigning	Walking speed	Locomotor activity
Aomori	40.8909	140.4552	80	77	80
Akita	40.1825	140.0518	80	79	20
Miyagi	38.5726	140.8723	40	40	64
Yamagata	38.4264	140.3856	80	78	20
Ishikawa	36.6707	136.7276	96	48	42
Tochigi	36.5747	139.8776	48	48	42
Ibaragi	36.3746	140.4169	95	48	-
Gunma	36.3546	139.1299	48	48	64
Nagano	35.9904	137.9795	96	47	44
Yamanashi	35.5952	138.5768	48	47	64
Gifu	35.5583	137.4489	48	48	64
Tottori-B	35.5357	134.3827	48	-	-

Hyogo-A	35.4942	134.5642	43	-	64
Shiga	35.4862	136.2289	48	48	64
Hyogo-B	35.4649	134.9447	48	-	-
Tottori-A	35.4216	133.7830	40	40	24
Shimane	35.2467	132.5565	40	38	22
Aichi	35.0232	136.9688	96	-	42
Chiba	35.0029	139.9148	48	-	43
Okayama	34.7247	133.6436	100	-	76
Hyogo-C	34.6903	134.9988	40	40	24
Yamaguchi-B	34.5033	131.4720	48	-	64
Hiroshima	34.5006	133.3735	40	40	30
Kagawa-B	34.3208	133.9018	48	-	-
Kagawa-A	34.2553	133.7846	48	-	32
Yamaguchi-A	34.2240	131.8132	40	40	20
Tokushima-A	34.0737	134.3950	48	-	32
Tokushima-B	33.9734	134.6358	48	-	-
Ehime	33.9224	133.0625	48	-	32
Kochi-A	33.4298	133.9903	48	-	32

Saga	33.2845	130.0928	40	40	32
Nagasaki	33.0817	129.8691	40	40	34
Kochi-C	33.0401	133.0547	48	-	-
Kochi-B	32.9847	132.9482	48	-	-
Kumamoto-B	32.8051	130.8083	48	-	20
Kumamoto-A	32.7974	130.7815	40	39	24
Okinawa	26.2459	127.6915	96	93	4

523

524

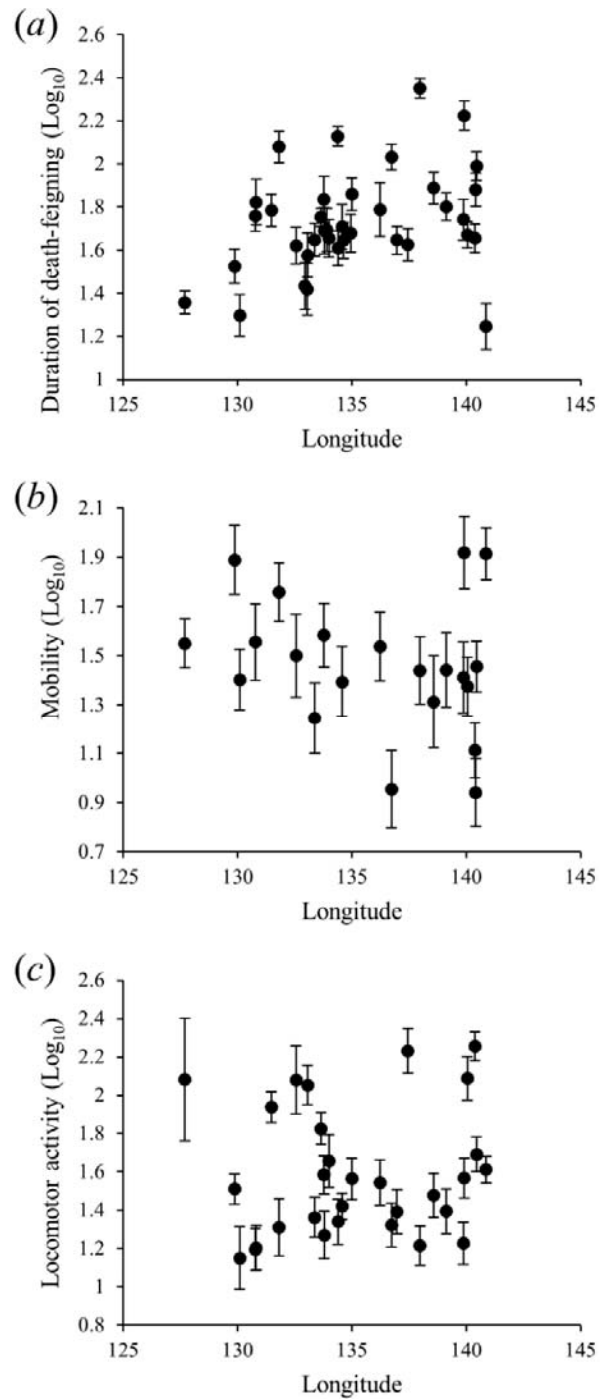


525

526 **Figure A1.** Relationship between latitude and each behavioural trait (a: death-feigning,

527 b: mobility, c: locomotor activity). Error bars show SE.

528



529

530 **Figure A2.** Relationship between longitude and each behavioural trait (a: death-feigning,

531 b: mobility, c: locomotor activity). Error bars show SE.