

1 **Type of manuscript:** Brief Report

2

3 **Title**

4 Mating behavior between alien Asian longhorned beetle *Anoplophora glabripennis*

5 (Coleoptera: Cerambycidae) and a native related species *Anoplophora chinensis* in

6 Japan

7

8 **Authors**

9 Eiriki Sunamura¹ · Shigeaki Tamura¹ · Hiromi Mukai¹ · Masahiko Tokoro¹ · Etsuko

10 Shoda-Kagaya¹

11

12 **Affiliation**

13 ¹ Department of Forest Entomology, Forestry and Forest Products Research Institute,

14 Forest Research and Management Organization, Matsunosato 1, Tsukuba, Ibaraki

15 305-8687, Japan

16

17 **Corresponding author**

18 Eiriki Sunamura

19 E-mail: esunamura@ffpri.affrc.go.jp

20 Tel.: +81 (0)29 829 8254

21 Fax: +81 (0)29 873 1543

22

23 **Abstract**

24 A highly invasive alien species in North America and Europe, the Asian longhorned

25 beetle *Anoplophora glabripennis* (Motschulsky) (Coleoptera: Cerambycidae), has been
26 detected in various parts of Japan since 2020. In addition to damaging host trees, *A.*
27 *glabripennis* may severely impact the Japanese ecosystem by interfering with *A.*
28 *chinensis* (Foster) (formerly called *A. malasiaca*), a common and related native species
29 with a similar niche. To assess the potential risk of reproductive interference between
30 the two species, mating behavior between interspecific females and males was
31 examined in the laboratory using field-collected sample individuals. Males of both
32 species mounted on female *A. glabripennis* and attempted to insert genitalia at similar
33 frequencies (approximately 50%), but *A. chinensis* males always failed to copulate. In
34 contrast, male *A. glabripennis* did not have sexual behavior with female *A. chinensis*.
35 The results show that mating between the two species is typically unsuccessful,
36 although recent research showed that the two species could produce a hybrid when
37 paired for four weeks. The erroneous interspecific mating attempts may reduce both
38 species' fitness. Therefore, the population-level impact of this reproductive interference
39 should be assessed. ~~Additionally, reproductive interference may be used to control the~~
40 ~~*Anoplophora* pests.~~

41

42 **Keywords** *Anoplophora malasiaca* · biological invasions · mate recognition ·
43 pheromone · wood-boring pest

44

45 **Introduction**

46

47 The Asian longhorned beetle *Anoplophora glabripennis* (Motschulsky) (Coleoptera:
48 Cerambycidae) is native to China and the Korean Peninsula and has become a highly
49 invasive alien species in North America and Europe since the late 1990s (Haack et al.
50 2010). This species uses maples of the genus *Acer* and various other hardwood trees as
51 its hosts and kills them through mass infestation. It is listed among 100 of the world's
52 worst invasive species owing to its severe damage (Lowe et al. 2000). Since 2020, *A.*
53 *glabripennis* establishment and associated tree damage have been detected in several
54 locations in Japan: Hyogo, Aichi, Toyama, Saitama, Ibaraki, Fukushima, and Miyagi
55 Prefectures (Akita et al. 2021; Iwata and Kiriyama 2022; Kanada and Kidono 2021;
56 Nishiura et al. 2021; Saitama Prefecture 2021; Sato and Nishiura 2021; Yanagi et al.
57 2021).

58 There are native *Anoplophora* species closely related to *A. glabripennis* in Japan,
59 unlike in North America and Europe (Makihara 2007). Among them, *A. chinensis*
60 (Foster) [formerly called *A. malasiaca* (Thomson); detailed later] is commonly found in
61 Japan except for some remote islands. Like *A. glabripennis*, it uses various hardwood
62 trees as its hosts, such as maple, citrus, and willow (Fukaya 2003). Additionally, the
63 following ecological traits of Japanese *A. chinensis* overlap those of *A. glabripennis*:
64 host tree, body size, and adult occurrence period (most abundant in June and July)
65 (Akita et al. 2021). Therefore, in addition to tree damage already reported from invaded
66 countries (Haack et al. 2010), *A. glabripennis* may severely impact the native Japanese
67 ecosystem by interacting with *A. chinensis*, a related species with a similar niche.

68 Reproductive interference, a possible interaction between the two *Anoplophora*

69 species, is defined as any interspecific interaction during reproduction that reduces the
70 fitness of one or both species (Gröning and Hochkirch 2008). It can strongly affect
71 species distribution, including the displacement of native species by alien species (e.g.,
72 Nishida et al. 2014). For example, misdirected courtship and mating attempts among
73 closely related insect species can lead to species exclusion (Fea et al. 2013; Kishi et al.
74 2009). If such reproductive interference occurs between *A. glabripennis* and Japanese *A.*
75 *chinensis*, it negatively affects *A. chinensis*. The displacement of *A. chinensis* concerns
76 the Japanese ecosystem, even though *A. chinensis* is an agricultural pest in Japan
77 (Fukaya 2003). Alternatively, if such reproductive interference adversely affects *A.*
78 *glabripennis*, *A. chinensis* may be used as a biological control agent of *A. glabripennis*.
79 (e.g., [Honma et al. 2018](#)). Therefore, investigating the reproductive interaction between
80 *A. chinensis* and *A. glabripennis* is crucial for assessing the potential impact of *A.*
81 *glabripennis* in Japan.

82 *Anoplophora* mating begins from the attraction of males and females to host trees
83 via host tree volatiles (Fukaya 2003; Xu and Teale 2021). In the host trees, males and
84 females seek mates, in which males are more active than females (Hoover et al. 2014;
85 Yasui 2009) using male/female volatile pheromones, female trail pheromones, and
86 visual cues (Fukaya 2003; Xu and Teale 2021). Primarily, males palpate around the tree
87 surface to detect and follow the female trail pheromone laid on the female's path
88 (Hoover et al. 2014). When the two sexes come together, the male, with his antennae,
89 tarsi, or palpi, senses the female contact pheromone on the female's body (Fukaya 2003;
90 Zhang et al. 2003). The male then quickly mounts the female without any prior
91 courtship behavior, rapidly and actively turning his abdomen to seek out the end of the
92 female abdomen and attempting to insert his genitalia (Fukaya 2003; Keena and

93 Sánchez 2018a; Zhang et al. 2003). The male may mount the female for a long time (1–
94 2 h) and copulate several times (5–10 min each) while protecting his mate (Lingafelter
95 and Hoebeke 2002). The females lay eggs during or after this mating, with lifetime
96 fecundity of >50 eggs (Adachi 1988; Keena 2002). In *A. glabripennis*, a single
97 pair-bond is sufficient to achieve approximately 60% fertility during the female’s
98 lifetime (Keena and Sánchez 2018a). Both sexes of *A. glabripennis* and Japanese *A.*
99 *chinensis* copulate multiple times (Fukaya 2003; Morewood et al. 2004). *Anoplophora*
100 *glabripennis* and Japanese *A. chinensis* have some common female and male volatile
101 pheromone components but no common contact pheromones (Fukaya 2003; Xu and
102 Teale 2021; Yasui et al. 2019) (Online Resource 1).

103 This study examined the interspecific mating behavior between *A. glabripennis*
104 and Japanese *A. chinensis* to assess the potential risk of reproductive interference. The
105 focus was placed on the last steps of mating behavior, a laboratory situation where a
106 male approaches a conspecific or heterospecific female on a branch was created, and the
107 male responses to females between the two *Anoplophora* species were compared. The
108 hybridization potential between *A. glabripennis* and Japanese *A. chinensis* was recently
109 studied by four-week laboratory crossing experiments (Wang and Keena 2021). Egg
110 laying, hatching, and development were observed by crossing *A. glabripennis* and
111 Japanese *A. chinensis*, although less than conspecific pairings. However, mating
112 behavior was not recorded in detail. Thus, the likelihood of mating at brief encounters in
113 the field is unclear. Additionally, reproductive interference during the mating behavior
114 might have been overlooked. Furthermore, Wang and Keena (2021) used laboratory
115 strains of *Anoplophora* maintained for multiple generations. Using individuals collected
116 in the field could produce results closer to what is in nature. *Anoplophora glabripennis*

117 and *A. malasiaca* collected in the field were observed in detail to determine
118 reproductive interference in this study.

119 Importantly, researchers have not agreed on the taxonomy of Japanese *A. chinensis*.
120 Previously, the Japanese and South Korean populations of *A. chinensis* were called *A.*
121 *malasiaca* (Thomson) and considered an independent species (Makihara 2000).
122 Meanwhile, *A. chinensis* (~~Thompson~~Foster) naturally occurred in China, North Korea,
123 and Myanmar but was not found in Japan (Makihara 2000). They are distinguished by
124 their external morphology (Makihara 2000) and are genetically different (Muraji et al.
125 2011; Ohbayashi and Ogawa 2009). Lingafelter and Hoebeke (2002) placed *A.*
126 *malasiaca* as a synonym of *A. chinensis* because they found no consistent differences in
127 the genitalia of the two species. However, Makihara (2007) stated that there was
128 confusion in the specimens Lingafelter and Hoebeke (2002) examined and did not adopt
129 synonymization. Recently, Wang and Keena (2021) showed that *A. chinensis* and *A.*
130 *malasiaca* are reproductively compatible and have been suggested to be subspecies.
131 Therefore, following Lingafelter and Hoebeke (2002) and Wang ~~&~~and Keena (2021),
132 this study regards *A. malasiaca* as a junior synonym of *A. chinensis*, and refers to the
133 former as Japanese *A. chinensis*.

134

135

136 **Materials and methods**

137

138 **Insects**

139

140 The tested insects were collected in July and August 2021. Adults of *A. glabripennis*

141 were collected from elm *Ulmus parvifolia*, katsura *Cercidiphyllum japonicum*, and
142 willow *Salix chaenomeloides* trees in Tsukuba City, Ibaraki Prefecture (17 males and 20
143 females; mean \pm SD body length was 25.1 ± 2.1 mm for males and 27.2 ± 4.2 mm for
144 females). In Tsukuba, *A. glabripennis* establishment was detected in June 2021
145 (Nishiura et al. 2021). Adults of Japanese *A. chinensis* were collected from maple *Acer*
146 *buergerianum* and *Acer palmatum* trees (11 males and four females) in Tsukuba City,
147 Yotsukaido City, Chiba Prefecture (one female, host tree not recorded), Asaka City,
148 Saitama Prefecture (one female, host tree not recorded), and Hachijo Town, Tokyo
149 Metropolis (four males and two females from alder *Alnus sieboldiana* trees) (body
150 length 28.0 ± 2.4 mm for males and 30.0 ± 3.5 mm for females). The adults were
151 individually maintained in plastic containers (10 cm in diameter and 5.5 cm in height) at
152 25°C and 14L:10D in the laboratory. They were fed cupped jelly for beetle rearing (Pro
153 Jelly, Wraios Corporation, Japan) *ad libitum*. All individuals were used for testing
154 within 30 d after collection.

155

156 **Laboratory mating experiments**

157

158 The behavioral tests were carried out following the procedure of Keena and
159 Sánchez (2018a, b). A fresh branch of cherry tree *Cerasus* \times *yedoensis* ‘Somei-yoshino’
160 (2–3 cm in diameter; 20–25 cm in length) was placed in the center of the bottom of the
161 plastic cover (22 \times 30 \times 6 cm). Cherry trees attract both *A. glabripennis* and Japanese *A.*
162 *chinensis* in the field (Yanagi et al. 2021). First, a female of *A. glabripennis* or *A.*
163 *chinensis* was released on the branch. One minute later, a male of *A. glabripennis* or *A.*
164 *chinensis* was released on the branch, and the actions of the two were recorded for 15

165 min. In particular, the presence or absence of 1) male mounting on female, 2) male
166 genital insertion attempt into the female, and 3) successful copulation (connection of
167 male and female genitalia) were recorded as the indicators of mating behavior. The
168 plastic cover and branch were renewed for each trial. The experiments were performed
169 at 25°C–28°C during the daytime (around 11 am to 5 pm).

170 The number of tested pairs was 10 for *Ag* ♀ × *Ag* ♂, 10 for *Ag* ♀ × *Ac* ♂, eight for
171 *Ac* ♀ × *Ag* ♂, and five for *Ac* ♀ × *Ac* ♂ (*Ag*: *A. glabripennis*, *Ac*: Japanese *A.*
172 *chinensis*). Mean ± SD female/male body length ratio was 1.1 ± 0.18 , 0.99 ± 0.13 , $1.2 \pm$
173 0.20 , and 1.2 ± 0.23 for the respective combinations. Because of the small number of
174 insects tested, some individuals were used for more than one experiment (5/60
175 individuals).

176 Fisher's exact tests were conducted for *A. glabripennis* and *A. chinensis* females
177 separately, using the occurrence and absence of male mounting on the female as the
178 variables to test whether the frequency of mating attempts toward females differed
179 between conspecific and heterospecific males.

180

181

182 **Results**

183

184 The mounting frequency on *A. glabripennis* females did not differ between *A.*
185 *glabripennis* and *A. chinensis* males (Fisher's exact test, $p = 1$) and was approximately
186 50% (Fig. 1). Mounting occurred at first contact between *A. glabripennis* females and *A.*
187 *glabripennis* males in 5/10 observed trials. Similarly, *A. chinensis* males mounted *A.*
188 *glabripennis* females at the first or second contact in 4/10 trials. Where mounting did

189 not occur, the number of contacts was 8.4 ± 10.7 (1–27 times) in *Ag* ♀ × *Ag* ♂
190 experiments, and 3.2 ± 1.7 (1–6 times) in *Ag* ♀ × *Ac* ♂ experiments. After mounting,
191 males of both species attempted to insert their genitalia into *A. glabripennis* females.
192 The males continued to mount until the end of the 15-min trials, spending most of the
193 time bending their abdomen and trying to insert their genitalia into females, and
194 copulating (Fig. 2). Time spent by males just resting on females was short. In the
195 longest case, it was 92 s of 892 s mounting. *Anoplophora glabripennis* males achieved
196 copulation in many cases (Online Resource 2). It took 366 ± 341 s (range: 115–860 s)
197 for *A. glabripennis* males to achieve copulation after mounting (Fig. 2). In contrast, *A.*
198 *chinensis* males always failed to copulate (Online Resource 2, 3).

Fig. 1

199 The mounting frequency on *A. chinensis* females was higher in *A. chinensis* males
200 than in *A. glabripennis* males (Fisher's exact test, $p < 0.001$). *Anoplophora glabripennis*
201 males showed no mounting or mating behavior toward *A. chinensis* females [number of
202 contacts was 3.4 ± 2.6 (1–9 times)] (Fig. 1). *Anoplophora glabripennis* males tried to
203 repel *A. chinensis* females by opening mandibles and menacing them in three trials (Fig.
204 2). In contrast, *A. chinensis* males mounted on *A. chinensis* females in all five trials.
205 Mounting occurred at the first contact in four trials, and the second contact in one trial.
206 Males copulated successfully in two trials (Figs. 1 and 2; Online Resource 2). The time
207 required from mounting to successful copulation was 163 and 671 s, respectively.

208 Generally, individuals showed no interest in other conspecific or heterospecific
209 individuals unless they came in direct contact. In one *Ag* ♀ × *Ag* ♂ trial and two *Ag* ♀ ×
210 *Ac* ♂ experiments, the males approached the females by following the female paths (Fig.
211 2). When doing so, the males palpated the branch and container surface with their
212 mouthparts. This behavior is known as trail pheromone following (Hoover et al. 2014).

Fig. 2

213 Another notable behavior observed was that some females resisted male mounting by
214 kicking the males by hind legs or trying to run away, as reported by Keena and Sánchez
215 (2018a, b). This behavior was observed in both conspecific and heterospecific pairs (Fig.
216 2).

217

218

219 **Discussion**

220

221 *Anoplophora glabripennis* females attracted *A. glabripennis* males and Japanese *A.*
222 *chinensis* males at similar frequencies on contact. This finding contradicts the previous
223 findings that *A. glabripennis* female contact pheromone does not have a common
224 component to Japanese *A. chinensis* female contact pheromone (Fukaya 2003; Zhang et
225 al. 2003) (Online Resource 1). There may be unidentified common or similar
226 components. Another possibility is that female visual cues play a significant role in
227 mate recognition by Japanese *A. chinensis* males, and *A. glabripennis* and *A. chinensis*
228 females have similar visual cues. *Anoplophora glabripennis* males' indifference toward
229 Japanese *A. chinensis* females suggests that species recognition ability is more accurate
230 in *A. glabripennis* males than in Japanese *A. chinensis* males.

231 Wang and Keena (2021) briefly reported the results of 30-min observation of their
232 cross-breeding experiments between *A. glabripennis* and Japanese *A. chinensis*; Mating
233 behavior was observed in 4/25 *Ag* ♀ × *Ac* ♂ pairs (16%) and none of the 25 *Ac* ♀ × *Ag*
234 ♂ pairs. In this study, however, mating behavior was observed more frequently in *Ag* ♀
235 × *Ac* ♂ pairs (40%). The experimental settings were similar between the two studies,
236 including the container size. However, two factors could have reduced the possibility of

237 detecting interspecific mating attempts in this study. First, the duration of observation
238 was shorter (15 min). Second, *A. glabripennis* and Japanese *A. chinensis* used in this
239 study were collected from different host plants. Prior host usage by *Anoplophora* beetles
240 may alter their sexual attractiveness because some host compounds are acquired in their
241 elytra as attractants or repellents (Fujiwara-Tsujii et al. 2013; Yasui et al. 2007; Yasui
242 and Fujiwara-Tsujii 2016). Therefore, if the beetles feed on different hosts, they may not
243 recognize each other. Despite these factors, Japanese *A. chinensis* males' mating
244 attempts on *A. glabripennis* females were more frequent than those in Wang and Keena
245 (2021). The results suggested that field populations of Japanese *A. chinensis* males
246 attempt copulation with *A. glabripennis* females more often than reported from
247 laboratory strains. Therefore, field-collected individuals may be useful in predicting the
248 reproductive interference level between the two species on the field.

249 The observation that copulation between *A. glabripennis* females and Japanese *A.*
250 *chinensis* males failed suggests that the morphology of their genitalia does not fit each
251 other. Lingafelter and Hoebeke (2002) recognized qualitative differences between the
252 genital morphology of the *A. chinensis* group (including Japanese samples) and *A.*
253 *glabripennis*. However, the morphological differences may not be sufficient to achieve
254 complete reproductive isolation, because the females produced eggs in the four-week
255 crossing experiments between *A. glabripennis* and Japanese *A. chinensis* in Wang and
256 Keena (2021).

257 Hybridization is a harmful form of reproductive interference that native species can
258 receive from related invasive species (Gröning and Hochkirch 2008). Wang and Keena
259 (2021) showed that hybridization could occur between *A. glabripennis* and Japanese *A.*
260 *chinensis* by pairing them for four weeks. However, this research showed that Japanese

261 *A. chinensis* males' mating attempts on *A. glabripennis* females are typically
262 unsuccessful. Besides, *A. glabripennis* males are usually indifferent to Japanese *A.*
263 *chinensis* females. Furthermore, the hybrid's fitness appears low (Wang and Keena
264 2021). Therefore, the risk of hybridization between the two *Anoplophora* species may
265 not be high in the field, although further field survey is necessary.

266 However, Japanese *A. chinensis* males' mating attempts on *A. glabripennis* females
267 may reduce the fitness of both species. In particular, both species may waste their time
268 and energy in attempting or refusing copulation and may lose chances to mate normally
269 with conspecific partners. Therefore, erroneous interspecific mating can be regarded as
270 reproductive interference. The population-wide impact of this reproductive interference
271 deserves further investigation. Additionally, the possibility of interspecific attraction on
272 a medium to long-distance scale should be evaluated because the two *Anoplophora*
273 species have several common volatile attractants (Xu et al. 2020; Yasui et al. 2007)
274 (Online Resource 1).

275 Reproductive interference found in this research may be used to control *A.*
276 *glabripennis* and Japanese *A. chinensis*. Recently, a new pest management technique
277 incorporating reproductive interference into a sterile insect was proposed by Honma et
278 al. (2018): mass release of a sterile insect pest can control field populations of the pest
279 species and related pest species through reproductive interference. In this case, mass
280 release of sterile Japanese *A. chinensis* males may effectively suppress the reproduction
281 of the alien *A. glabripennis*. In *A. glabripennis*, female fertility decreases with age
282 (Keena and Sánchez 2018a). Therefore, delayed mating and reduced fertility in *A.*
283 *glabripennis* females can be expected in the reproductive interference from sterile
284 beetles. Additionally, sterile *A. chinensis* males can effectively suppress wild *A.*

285 *chinensis* in orchards where it is a serious pest (Fukaya 2003).

286

287 **Supplementary Information** The online version contains supplementary material

288 available at XXX.

289

290 **Acknowledgements**

291 This study could not have been completed without *A. chinensis* individuals kindly
292 provided by Hitoshi Tojo. Mamoru Terayama and Katsuyuki Eguchi gave us helpful
293 comments on the taxonomy of *A. chinensis*. This study was supported by grants from
294 the NARO Bio-oriented Technology Research Advancement Institution (research
295 program on development of innovative technology: 30023C) and the Support program
296 of FFPRI for researchers having family obligations.

297

298

299 **References**

300

301 Adachi I (1988) Reproductive biology of the white-spotted longicorn beetle,

302 *Anoplophora malasiaca* THOMSON (Coleoptera: Cerambycidae), in citrus trees.

303 Appl Entomol Zool 23:256–264. <https://doi.org/10.1303/aez.23.256>

304 Akita A, Katô T, Yanagi T, Kubota K (2021) Reports of the alien species *Anoplophora*

305 *glabripennis* (Motschulsky, 1853) (Coleoptera, Cerambycidae) found in Hyogo

306 pref., Japan. GEKKAN–MUSHI 601:41–45. **(in Japanese)**

307 Fea MP, Stanley MC, Holwell GI (2013) Fatal attraction: sexually cannibalistic invaders

308 attract naive native mantids. Biol Lett 9:20130746.

309 <https://doi.org/10.1098/rsbl.2013.0746>

310 Fujiwara-Tsujii N, Yasui H, Wakamura S (2013) Population differences in male
311 responses to chemical mating cues in the white-spotted longicorn beetle,
312 *Anoplophora malasiaca*. *Chemoecol* 23:113–120.
313 <https://doi.org/10.1007/s00049-013-0126-1>

314 Fukaya M (2003) Recent advances in sex pheromone studies on the white-spotted
315 longicorn beetle, *Anoplophora malasiaca*. *Jpn Agric Res Q* 37:83–88.
316 <https://doi.org/10.6090/jarq.37.83>

317 Gröning J, Hochkirch A (2008) Reproductive interference between animal species. *Q*
318 *Rev Biol* 83:257–282. <https://doi.org/10.1086/590510>

319 Haack RA, Herard F, Sun JH, Turgeon JJ (2010) Managing invasive populations of
320 Asian longhorned beetle and citrus longhorned beetle: a worldwide perspective.
321 *Annu Rev Entomol* 55:521–546.
322 <https://doi.org/10.1146/annurev-ento-112408-085427>

323 Honma A, Kumano N, Noriyuki S (2018) Killing two bugs with one stone: a
324 perspective for targeting multiple pest species by incorporating reproductive
325 interference into sterile insect technique. *Pest Manag Sci* 75:571–577.
326 <https://doi.org/10.1002/ps.5202>

327 Hoover K, Keena M, Nehme M, Wang S, Meng P, Zhang A (2014) Sex-specific trail
328 pheromone mediates complex mate finding behavior in *Anoplophora glabripennis*.
329 *J Chem Ecol* 40:169–180. <https://doi.org/10.1007/s10886-014-0385-5>

330 Iwata T, Kiriya S (2022) First records of *Anoplophora glabripennis* (Motschulsky,
331 1853) in Toyama prefecture, including a collected record of 2010. *GEKKAN–*
332 *MUSHI* 611:34–36. **(in Japanese)**

- 333 Kanada Y, Kidono H (2021) The Asian longhorned beetle of Aichi prefecture.
334 GEKKAN–MUSHI 608:53–54. **(in Japanese)**
- 335 Keena M (2002) *Anoplophora glabripennis* (Coleoptera: Cerambycidae) fecundity and
336 longevity under laboratory conditions: comparison of populations from New York
337 and Illinois on *Acer saccharum*. Environ Entomol 31:490–98.
338 <https://doi.org/10.1603/0046-225X-31.3.490>
- 339 Keena M, Sánchez V (2018a) Reproductive behaviors of *Anoplophora glabripennis*
340 (Coleoptera: Cerambycidae) in the laboratory. J Econ Entomol 111:620–628.
341 <https://doi.org/10.1093/jee/tox355>
- 342 Keena M, Sánchez V (2018b) Inter- and intrasexual interactions in *Anoplophora*
343 *glabripennis* (Coleoptera: Cerambycidae) and the impact of different sex ratios. J
344 Econ Entomol 111:2163–2171. <https://doi.org/10.1093/jee/toy207>
- 345 Kishi S, Nishida T, Tsubaki Y (2009) Reproductive interference determines persistence
346 and exclusion in species interactions. J Anim Ecol 78:1043–1049.
347 <https://doi.org/10.1111/j.1365-2656.2009.01560.x>
- 348 Lingafelter SW, Hoebeke ER (2002) Revision of the genus *Anoplophora* (Coleoptera:
349 Cerambycidae). Entomological Society of Washington, Washington
- 350 Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the World’s Worst
351 Invasive Alien Species. A Selection from the Global Invasive Species Database.
352 The Invasive Species Specialist Group, International Union for Conservation of
353 Nature, Gland
- 354 Makihara H (2007) Genus *Anoplophora* Hope, 1839. In: Ohbayashi N and Niisato T
355 (eds.) Longicorn Beetles of Japan. Tokai University Press, Hadano, pp 583–585 **(in**
356 **Japanese)**

357 Makihara H (2000) True taxonomy and distribution of *Anoplophora* cerambycid beetles
358 in East Asia. *Forest Pests* 49:180–194 **(in Japanese)**

359 Morewood WD, Neiner PR, Sellmer JC, Hoover K (2004) Behavior of adult
360 *Anoplophora glabripennis* on different tree species under greenhouse conditions. *J*
361 *Insect Behav* 17:215–226. <https://doi.org/10.1023/B:JOIR.0000028571.52739.50>

362 Muraji M, Wakamura S, Yasui H, Arakaki N, Sadoyama Y, Ohno S, Matsuhira K (2011)
363 Genetic variation of the white-spotted longicorn beetle *Anoplophora* spp.
364 (Coleoptera: Cerambycidae) in Japan detected by mitochondrial DNA sequence.
365 *Appl Entomol Zool* 46:363–373. <https://doi.org/10.1007/s13355-011-0056-8>

366 Nishida S, Kanaoka M, Hashimoto K, Takakura K, Nishida T (2014) Pollen–pistil
367 interactions in reproductive interference: comparisons of heterospecific pollen tube
368 growth from alien species between two native *Taraxacum* species. *Funct Ecol*
369 28:450–457. <https://doi.org/10.1111/1365-2435.12165>

370 Nishiura K, Shinozaki S, Suzuki H, Fuda S (2021) Collection of the Asian longhorned
371 beetle in Tsukuba City, Ibaraki Prefecture. *Gekkan-Mushi* 608:53. **(in Japanese)**

372 Ohbayashi N, Ogawa J (2009) Phylogenetic analysis of the Lamiine genus *Anoplophora*
373 and its relatives (Coleoptera, Cerambycidae) based on the mitochondrial COI gene.
374 *Spec. Bull Jpn Soc Coleopterol* 7:309–324.

375 Saitama Prefecture (2021) Beware of the Asian longhorned beetle.
376 <https://www.pref.saitama.lg.jp/a0907/tuyahadakamikiri.html> Accessed 12 February
377 2022.

378 Sato H, Nishiura K (2021) Collection of the Asian longhorned beetle in Shirakawa City,
379 Fukushima Prefecture. *Gekkan-Mushi* 608:54. **(in Japanese)**

380 Wang X, Keena MA (2021) Hybridization potential of two invasive Asian longhorn

381 beetles. *Insects* 12:1139. <https://doi.org/10.3390/insects12121139>

382 Xu T, Hansen L, Cha DH, Hao D, Zhang L, Teale SA (2020) Identification of a
383 female-produced pheromone in a destructive invasive species: Asian longhorn
384 beetle, *Anoplophora glabripennis*. *J Pest Sci* 93:1321–1332.
385 <https://doi.org/10.1007/s10340-020-01229-3>

386 Xu T, Teale SA (2021) Chemical ecology of the Asian longhorn beetle, *Anoplophora*
387 *glabripennis*. *J Chem Ecol* 47:489–503.
388 <https://doi.org/10.1007/s10886-021-01280-z>

389 Yanagi T, Nagahata Y, Yoshino K, Akita K (2021) A new record of *Anoplophora*
390 *glabripennis* (Motschulsky, 1853) (Coleoptera, Cerambycidae), with newly host
391 plants recorded *Cercidiphyllum japonica* and *Cerasus* × *yedoensis* from Miyagi
392 pref., Japan. *Gekkan-Mushi* 609:23–27. **(in Japanese)**

393 Yasui H (2009) Chemical communication in mate location and recognition in the
394 white-spotted longicorn beetle, *Anoplophora malasiaca* (Coleoptera:
395 Cerambycidae). *Appl Entomol Zool* 44:183–194.
396 <https://doi.org/10.1303/aez.2007.255>

397 Yasui H, Fujiwara-Tsujii N (2016) Host plant affects the sexual attractiveness of the
398 female white-spotted longicorn beetle, *Anoplophora malasiaca*. *Sci Rep* 6:29526.
399 <https://doi.org/10.1038/srep29526>

400 Yasui H, Yasuda T, Fukaya M, Akino T, Wakamura S, Hirai Y, Kawasaki K, Ono H,
401 Narahara M, Kousa K, Fukuda T (2007) Host plant chemicals serve intraspecific
402 communication in the white-spotted longicorn beetle, *Anoplophora malasiaca*
403 (Thomson) (Coleoptera: Cerambycidae). *Appl Entomol Zool* 42:255–268.
404 <https://doi.org/10.1303/aez.2007.255>

405 Yasui H, Fujiwara–Tsuji N, Yasuda T (2019) Detection of volatile pheromone
406 candidates from the white-spotted longicorn beetle, *Anoplophora malasiaca*
407 (Coleoptera: Cerambycidae). Appl Entomol Zool 54:203–211.
408 <https://doi.org/10.1007/s13355-019-00614-4>

409 Zhang A, Oliver JE, Chauhan K, Zhao B, Xia L, Xu Z (2003) Evidence for contact sex
410 recognition pheromone of the Asian longhorned beetle, *Anoplophora glabripennis*
411 (Coleoptera: Cerambycidae). Sci Nat 90:410–413.
412 <https://doi.org/10.1007/s00114-003-0452-1>
413

414 **Figure legends**

415

416 **Fig. 1** Results of intraspecific and interspecific mating experiments using *Anoplophora*
417 *glabripennis* (*Ag*) and Japanese *A. chinensis* (*Ac*). Shown are the occurrence ratios of
418 mounting, genital insertion attempt, and copulation success (connection of genitalia) for
419 female *A. glabripennis* (left) and female *A. chinensis* (right)

420

421 **Fig. 2** Sexual interaction between different combinations of *Anoplophora glabripennis*
422 (*Ag*) and Japanese *A. chinensis* (*Ac*). The behaviors observed between each individual
423 pair over 15 min are shown as follows. Light gray column: male mounting on female,
424 dark gray column: copulation success (connection of genitalia), diagonal line column:
425 male following of female path, black diamond: contact, white diamond with M: male
426 menacing female with open mandibles, two-headed arrow with R: female resisting to
427 male by trying to shake off mounting male or escape. n = 10 for *Ag* ♀ × *Ag* ♂, 10 for *Ag*
428 ♀ × *Ac* ♂, 8 for *Ac* ♀ × *Ag* ♂, and 5 for *Ac* ♀ × *Ac* ♂

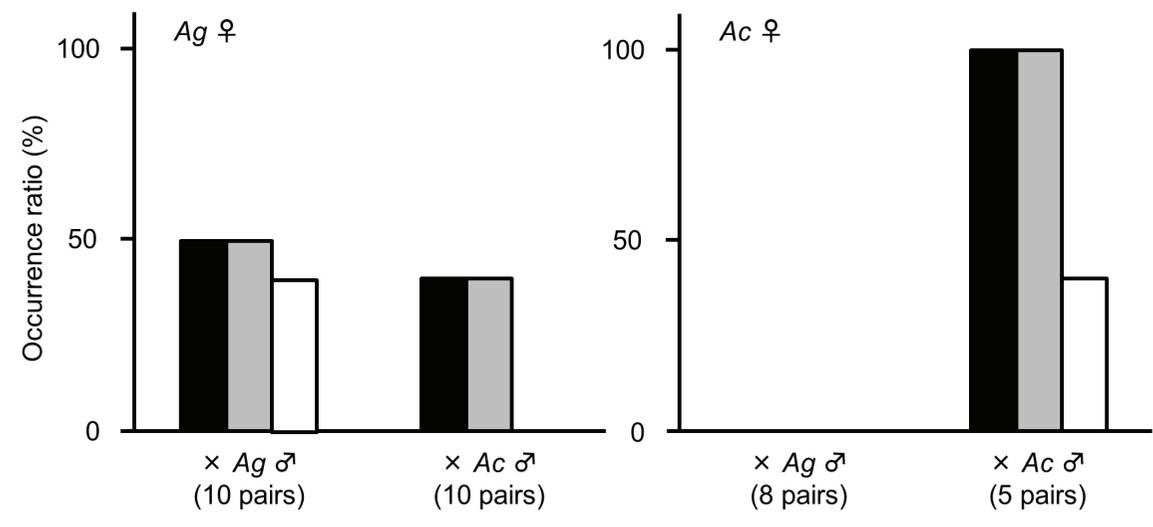
429

430 **Legends for Electronic Supplementary Material**

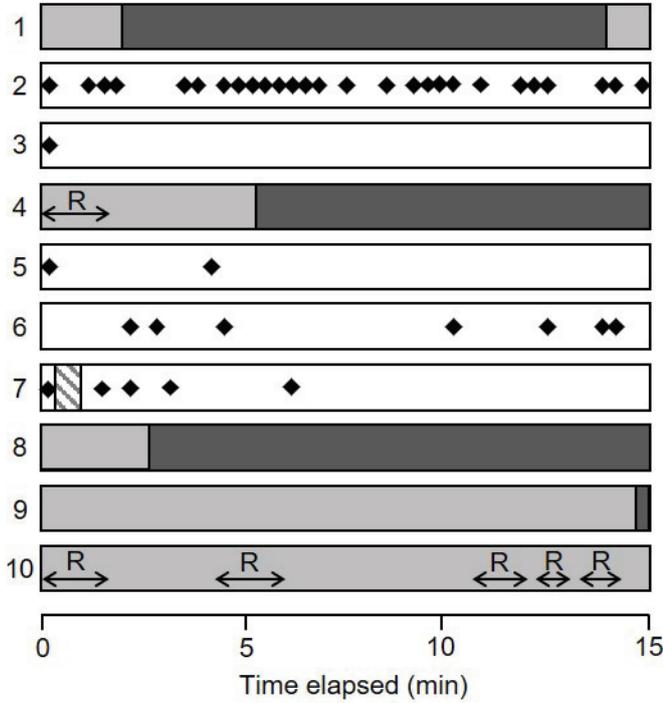
431

432 **Online Resource 3** A Japanese *Anoplophora chinensis* male failing to insert genitalia
433 into an female *A. glabripennis*. Here the male individual failed to open the female
434 terminal abdominal tergite and sternite. However, in some other cases, the tergite and
435 sternite were open, but males still failed to copulate

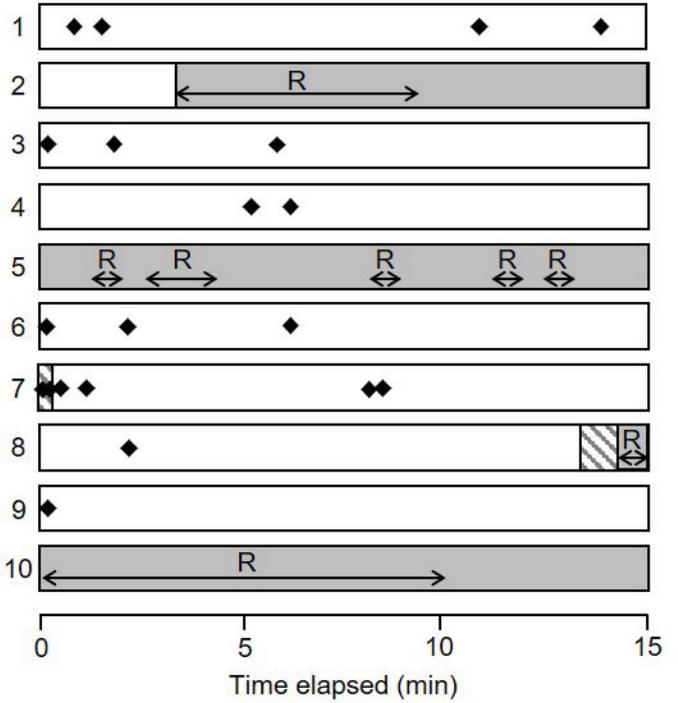
■ Mounting ■ Insertion attempt □ Copulation success



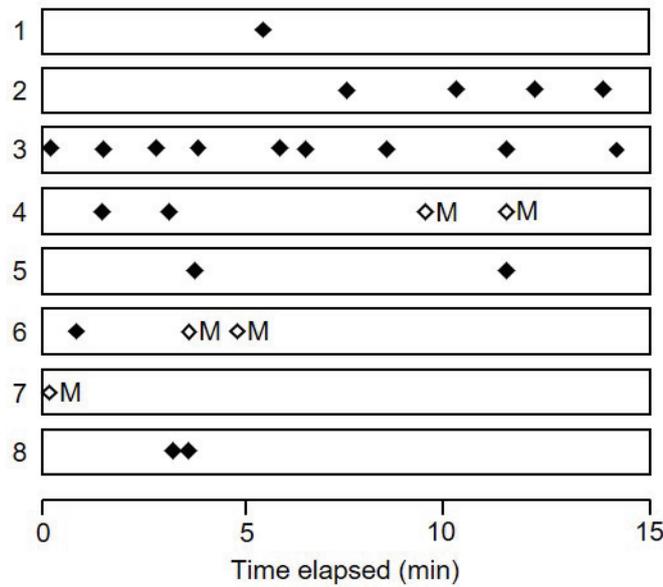
$Ag \text{ } \text{♀} \times Ag \text{ } \text{♂}$



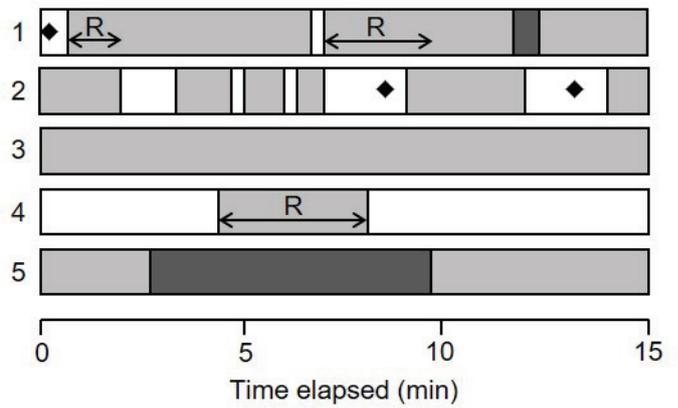
$Ag \text{ } \text{♀} \times Ac \text{ } \text{♂}$



$Ac \text{ } \text{♀} \times Ag \text{ } \text{♂}$



$Ac \text{ } \text{♀} \times Ac \text{ } \text{♂}$



Online Resource 1 List of known pheromone compounds [and attractants](#) associated with mating behavior of *Anoplophora glabripennis* and [Japanese *A. chinensis*](#)~~*malasiaea*~~

	<i>Anoplophora glabripennis</i>	Japanese <i>Anoplophora chinensis</i> <i>malasiaea</i>
Female volatile sex pheromone and attractants *	hexadecanal ¹	β -elemene ^{7*}
	heptanal ¹	β -caryophyllene ^{7*}
	nonanal ¹	α -humulene ^{7*}
	α -longipinene ²	α -farnesene ^{7*}
	α -cubebene ²	
	α -ylangene ²	
	(-)- α -copaene ²	
	α -bergamotene ²	
	<u>β-caryophyllene²</u>	
	<u>α-farnesene²</u>	
Male volatile sex pheromone and attractants *	<u>4-(<i>n</i>-heptyloxy)butanal³</u>	β -elemene ^{7*}
	<u>4-(<i>n</i>-heptyloxy)butan-1-ol³</u>	β -caryophyllene ^{7*}
	<u>(3<i>E</i>,6<i>E</i>)-α-farnesene⁴</u>	α -humulene ^{7*}
		<u>α-farnesene^{7*}</u>
		<u>4-(<i>n</i>-heptyloxy)butan-1-ol⁸</u>
		<u>4-(<i>n</i>-heptyloxy)butanal⁸</u>
	nonanal ⁸	
Female trail pheromone	2-methyldocosane ⁵	No study
	(<i>Z</i>)-9-tricosene ⁵	
	(<i>Z</i>)-9-pentacosene ⁵	
	(<i>Z</i>)-7-pentacosene ⁵	
Female contact sex pheromone	(<i>Z</i>)-9-tricosene ⁶	<i>n</i> -heptacosane ⁹
	(<i>Z</i>)-7-pentacosene ⁶	<i>n</i> -nonacosane ⁹
	(<i>Z</i>)-9-pentacosene ⁶	4-methylhexacosane ⁹
	(<i>Z</i>)-7-heptacosene ⁶	4-methyloctacosane ⁹
	(<i>Z</i>)-9-heptacosene ⁶	9-methylheptacosane ⁹
		9-methylnonacosane ⁹
		15-methylhentriacontane ⁹
		15-methyltrtriacontane ⁹
		10-heptacosanone ¹⁰
		(<i>Z</i>)-18-heptacosen-10-one ¹⁰
		(18 <i>Z</i> ,21 <i>Z</i>)-heptacosan-18,21-dien-10-one ¹⁰
	(18 <i>Z</i> ,21 <i>Z</i> ,24 <i>Z</i>)-heptacosan-18,21,24-trien-10-one ¹⁰	
	gomadalactones A, B, and C ¹¹	

Compounds commonly used by the two species are underlined.

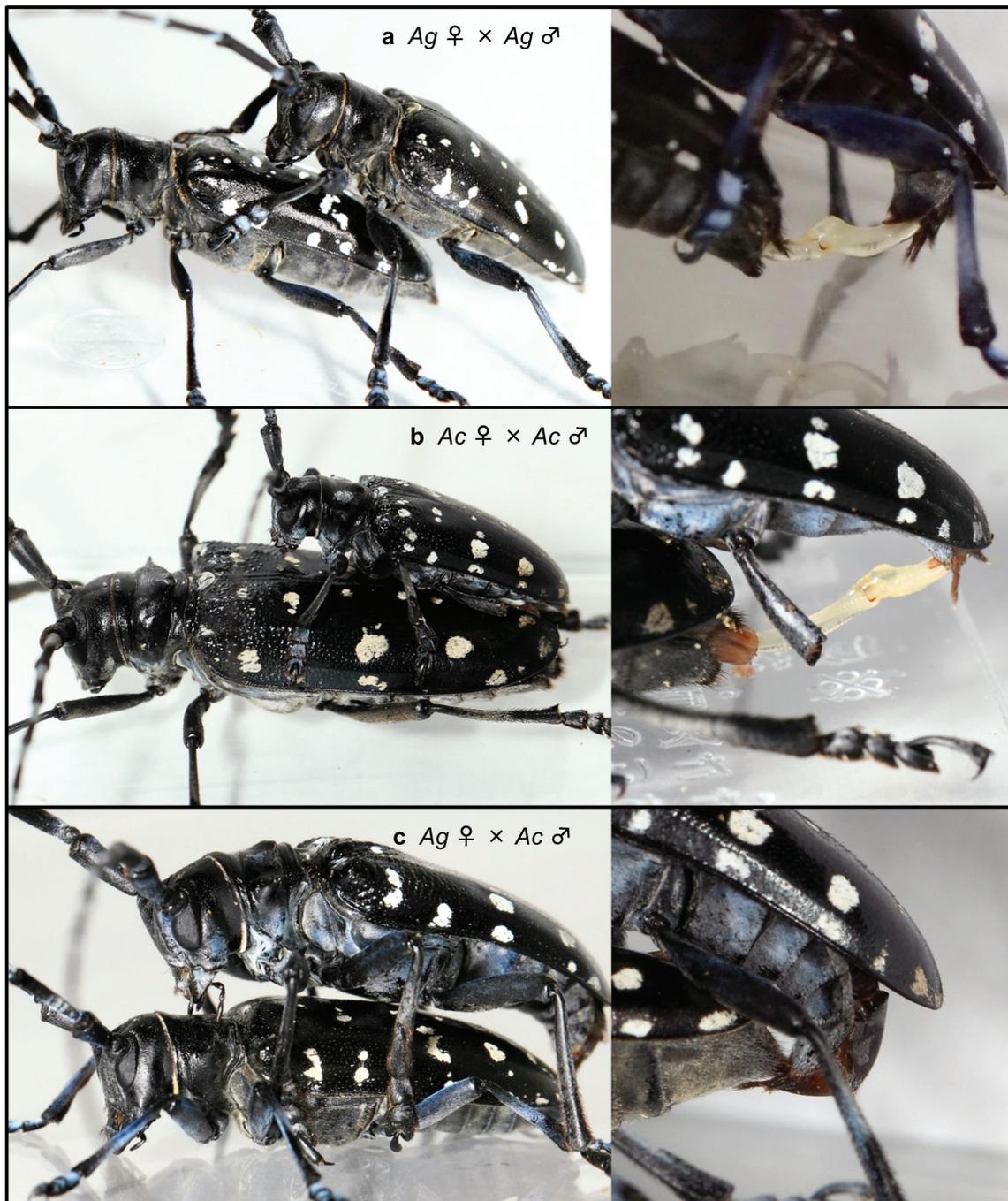
References which identified respective compounds are:

¹Wickham et al. 2012, ²Xu et al. 2020, ³Zhang et al. 2002, ⁴Crook et al. 2014, ⁵Hoover et al. 2014, ⁶Zhang et al. 2003, ⁷Yasui et al. 2007a, ⁸Yasui et al. 2019, ⁹Fukaya et al. 2000, ¹⁰Yasui et al. 2003, ¹¹Yasui et al. 2007b

[*Attractants: unlike pheromones, the attractants are not biosynthesized by the insects but are acquired from their host plants](#)

References

- Crook DJ, Lance DR, Mastro VC (2014) Identification of a potential third component of the male-produced pheromone of *Anoplophora glabripennis* and its effect on behavior. *J Chem Ecol* 40: 241–1250. <https://doi.org/10.1007/s10886-014-0520-3>
- Fukaya M, Akino T, Yasuda T, Wakamura S, Satoda S, Senda S (2000) Hydrocarbon components in contact sex pheromone of the white-spotted longicorn beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae) and pheromonal activity of synthetic hydrocarbons. *Entomol Sci* 3:211–218
- Hoover, K., M. Keena, M. Nehme, S. Wang, P. Meng, and A. Zhang. 2014. Sex-specific trail pheromone mediates complex mate finding behavior in *Anoplophora glabripennis*. *J. Chem. Ecol.* 40: 169–180.
- Wickham JD, Xu Z, Teale SA (2012) Evidence for a female-produced, long range pheromone of *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *Insect Sci* 19:355–371. <https://doi.org/10.1111/j.1744-7917.2012.01504.x>
- Xu T, Hansen L, Cha DH, Hao D, Zhang L, Teale SA (2020) Identification of a female-produced pheromone in a destructive invasive species: Asian longhorn beetle, *Anoplophora glabripennis*. *J Pest Sci* 93:1321–1332. <https://doi.org/10.1007/s10340-020-01229-3>
- Yasui H., Akino T, Yasuda T, Fukaya M, Ono H, Wakamura S (2003) Ketone components in the contact sex pheromone of the white-spotted longicorn beetle, *Anoplophora malasiaca*, and pheromonal activity of synthetic ketones. *Entomol Exp Appl* 107:167–176. <https://doi.org/10.1046/j.1570-7458.2003.00053.x>
- Yasui H, Yasuda T, Fukaya M, Akino T, Wakamura S, Hirai Y, Kawasaki K, Ono H, Narahara M, Kousa K, Fukuda T (2007a) Host plant chemicals serve intraspecific communication in the white-spotted longicorn beetle, *Anoplophora malasiaca* (Thomson) (Coleoptera: Cerambycidae). *Appl Entomol Zool* 42:255–268. <https://doi.org/10.1303/aez.2007.255>
- Yasui H, Akino T, Yasuda T, Fukaya M, Wakamura S, Ono H (2007b) Gomadalactones A, B, and C: novel 3-oxabicyclo[3.3.0]octane compounds in the contact sex pheromone of the white-spotted longicorn beetle, *Anoplophora malasiaca*. *Tetrahedron Lett* 48:2395–2400. <https://doi.org/10.1016/j.tetlet.2007.01.101>
- Yasui H, Fujiwara–Tsuji N, Yasuda T (2019) Detection of volatile pheromone candidates from the white-spotted longicorn beetle, *Anoplophora malasiaca* (Coleoptera: Cerambycidae). *Appl Entomol Zool* 54:203–211. <https://doi.org/10.1007/s13355-019-00614-4>
- Zhang A, Oliver JE, Aldrich JR, Wang B, Mastro VC (2002) Stimulatory beetle volatiles for the Asian longhorned beetle, *Anoplophora glabripennis* (Motschulsky). *Z Naturforsch C* 57:553–558. <https://doi.org/10.1515/znc-2002-5-626>
- Zhang A, Oliver JE, Chauhan K, Zhao B, Xia L, Xu Z (2003) Evidence for contact sex recognition pheromone of the Asian longhorned beetle, *Anoplophora glabripennis* (Coleoptera: Cerambycidae). *Sci Nat* 90:410–413. <https://doi.org/10.1007/s00114-003-0452-1>



Online Resource 2 Intraspecific and interspecific mating behavior of *Anoplophora glabripennis* (Ag) and Japanese *A. chinensis* (Ac). Male mounting on female was observed in pairs of: **a** Ag ♀ × Ag ♂; **b** Ac ♀ × Ac ♂; and **c** Ag ♀ × Ac ♂. Conspecific pairs achieved copulation (**a** and **b**). In contrast, heterospecific pairs always failed, although Ac ♂ bent their abdomens, searched for Ag ♀ abdominal tip, and tried to insert genitalia repeatedly (**c**). Ac can be distinguished from Ag by its granulated elytra base, blue-white spots on pronotum, and blue hairs on the side