1 **The article category:** Original Article

2 **Title:** Genetic variation among populations in parental exclusion of heterospecific larvae in

3 burying beetles

4 Japanese title: モンシデムシにおける異種幼虫排除行動の個体群間での遺伝的変異

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15

16 **Abstract**

17 The family is the smallest unit of society, where parents invest significant time and resources in

18 their offspring, providing parental care through defense and feeding. These parental investments,

19 which benefit the offspring by increasing their survival rates and growth, are constantly at risk of

20 exploitation by both conspecifics and heterospecifics. As a result, parents are known to employ

21 various mechanisms that prevent the inclusion of unrelated young in the family unit. In this study,

22 we investigated burying beetle *Nicrophorus quadripunctatus*, which exhibits elaborate parental

23 care, and found that the rate of exclusion of heterospecific larvae varies genetically across

24 different populations. Two populations were identified: one where competing congeners were

25 present and one where they were absent. We bred individuals from these populations under

26 laboratory conditions, isolated from competitors, and tested the exclusion rates of unrelated larvae.

27 Our results revealed that parents from the population coexisting with competitors had a

28 significantly higher exclusion rate of heterospecific larvae. In contrast, no significant differences

29 were observed between the populations regarding the exclusion of unrelated conspecific larvae.

30 These findings suggest that the exclusion of heterospecific larvae is genetically higher in

31 populations where competitors are present. This study represents an important step toward

32 understanding the genetic basis underlying parental acceptance or rejection of unrelated young,

33 offering new insights into the evolution of family life and parental care strategies.

34 **Keywords:** parental care, genetic variation, burying beetles, local adaptation, interspecific

35 competition

36 **Short abstract**

37 This study reveals genetic variation in the exclusion of heterospecific larvae in *Nicrophorus*

38 *quadripunctatus*. Populations coexisting with competitors exhibit higher exclusion rates,

39 suggesting local adaptation in parental care strategies.

40

41 **Graphical abstract**

Genetic variation in the exclusion of heterospecific larvae

42

43
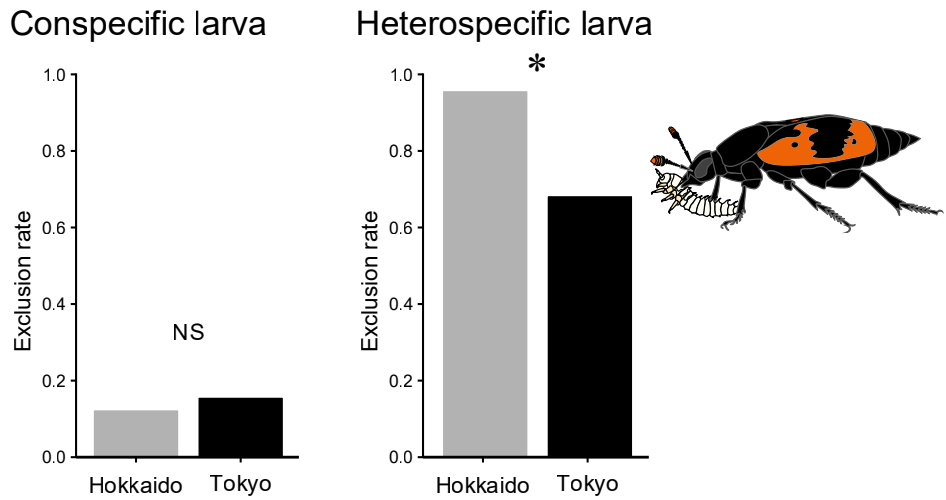
44 **Main text**

45 **Introduction**

46 Parental care, a critical component of reproductive strategies, plays a pivotal role in enhancing

47 offspring survival and reproductive success across various species (Clutton-Brock, 1991; Royle

48 et al., 2012). This investment, often in the form of protection, provisioning, and defense, incurs

49 inherent costs. One of the main challenges faced by parents is the risk of exploitation by non-

50 offspring, which can include both conspecific and heterospecific young. When care is exploited

51 by non-offspring, the cost remains fixed, but it fails to yield the fitness benefits associated with

52 investing in one's own offspring. Evolutionary pressures have, therefore, favored the development

53 of mechanisms that enable parents to recognize and preferentially care for their own offspring,

54 thereby maximizing the benefits of their parental investment while minimizing losses to unrelated

55 individuals (Hamilton, 1964a, 1964b; Maynard Smith, 1964; West & Gardner, 2013). These

56 strategies are particularly crucial in competitive environments where resources are limited and

57 the risk of exploitation is high.

58   Ecological variation across populations can lead to local adaptation, where genetic

59 differences arise due to selective pressures that vary across environments (Hereford, 2009;

60 Savolainen et al., 2013). Local adaptation occurs when populations evolve traits that are

61 optimized for survival and reproduction in specific environments, leading to genetic divergence

62 between populations (Kawecki & Ebert, 2004). In competitive or resource-scarce environments,

63 such adaptations can significantly influence reproductive behaviors, including parental care

64 strategies (Royle et al., 2014). For instance, populations that experience high levels of competition

65 may evolve stronger mechanisms for offspring recognition to reduce the risk of providing care to

66 unrelated offspring. These genetic differences reflect the intricate relationship between ecological

67 pressures and reproductive strategies, shedding light on how local environmental factors drive the

68 evolution of parental care behaviors.

69   Burying beetles (*Nicrophorus*) are particularly well known for their sophisticated parental

70 care, with both males and females contributing to the care of offspring, including feeding and

71 protecting the larvae (Eggert & Müller, 1997; Potticary et al., 2024; Scott, 1998). Burying beetles

72 of *Nicrophorus* species use the carcasses of small vertebrates as a food resource for their larvae.

73 Due to the intense competition for carcasses, males and females cooperate in burying the carcass

74 and actively defend it against conspecific intruders and members of other *Nicrophorus* species

75 (Pukowski, 1933; Scott, 1990; Trumbo, 1990, 2007). Larger species tend to have a competitive

76 advantage in securing carcasses (Otronen, 1988; Trumbo, 1990). Once the carcass is secured,

77 females lay eggs in the soil nearby. After hatching, the larvae crawl to the carcass and feed, partly

78 by begging for pre-digested carrion from their parents, and partly by self-feeding (Capodeanu-

79 Nägler et al., 2016). Individuals that fail to secure a carcass often engage in brood parasitism by

80   laying eggs in the surrounding soil and rely on others to raise their offspring (Müller et al., 1990;

81   Trumbo & Valletta, 2007). One of the key mechanisms that enables these beetles to avoid caring

82   for unrelated larvae is temporal kin recognition, where parents time their behavioral switch from

83   infanticide to caregiving based on the expected hatching time of their own eggs (Müller & Eggert,

84   1990; Oldekop & Smiseth, 2007). This timing allows them to care only for their own offspring

85   and eliminate unrelated larvae prior to the caregiving phase, minimizing the risk of exploitation.

86   Consequently, brood parasitism is successful only if the parasitic larvae hatch either later than or

87   at the same time as the host's own larvae (Trumbo, 1994). All *Nicrophorus* species studied to date

88   attempt facultative brood parasitism when they are unable to secure control of a carcass. Brood

89   parasitism occurs both within and between species (Trumbo, 1994). No *Nicrophorus* species are

90   known to be specialist brood parasites. The only potential specialist brood parasite within the

91   group of burying beetles is *Ptomascopus morio*, a sister genus of *Nicrophorus*, which prefers

92   *Nicrophorus concolor* as its host (Trumbo et al., 2001). Recent studies have also demonstrated

93   local adaptations in reproductive strategies across *Nicrophorus* populations, highlighting the

94   influence of ecological factors, such as competition and resource availability, on parental care

95   behaviors (Sun et al., 2020). These adaptations underscore the significance of local environmental

96   pressures in shaping reproductive traits and behavioral strategies in burying beetles.

97   In this study, we investigate two populations of *Nicrophorus quadripunctatus* from distinct

98   ecological regions—one from Hokkaido, where competition with other *Nicrophorus* species is

99   high, and one from Tokyo, where competition is lower (Nagano & Suzuki, 2003; Ohkawara et al.,

100   1998). By comparing these populations under controlled laboratory conditions, we aim to

101   determine whether interspecific competition drives local adaptations in parental care strategies,

102   specifically the exclusion of heterospecific larvae.

103

104   **Materials and methods**

105   **Collection of burying beetles in the field**

106   To investigate the burying beetle guild, plastic cup traps (122 mm diameter, 111 mm depth), baited

107   with a 40 g piece of chicken, were set approximately 1.5 m above the ground on the trunks of

108   trees. Ten traps were set about 300 m apart in forests in Hokkaido and Tokyo, Japan, from June

109   to September between 2014 and 2018. After 1 day, all trapped adult burying beetles were collected.

110   For each individual, species, sex, and pronotum width (measured to the nearest 0.1 mm) were

111   recorded.

112

113   **Burying beetle husbandry in the laboratory**

114   Adult *N. quadripunctatus* and *N. vespilloides* were maintained individually in transparent plastic

115   cups (height 4 cm, diameter 6 cm) containing 2 cm of moist peat. The beetles were kept under a

116   14:10 h light: dark cycle and fed approximately 200 mg of freshly killed mealworms (*Zophobas*
117   *atratus*) three times per week. To ensure sexual maturity, the beetles were reared under these
118   conditions for 2 weeks.
119   For breeding, a male-female pair was housed in a breeding cage (height 80 mm, diameter
120   115 mm) containing 2 cm of moist peat and a whole-body mouse carcass (4.0 ± 0.5 g; Cyber
121   Cricket, Shiga, Japan). The pair was kept in a dark incubator until their larvae reached the
122   wandering stage. At this point, the larvae were transferred individually into transparent plastic
123   cups (height: 4 cm, diameter: 6 cm) containing 2 cm of moist peat and maintained under a 14:10
124   h light: dark cycle until adult eclosion. The rearing and breeding conditions for adults after
125   eclosion followed the same protocol as described above. All life stages of the burying beetles
126   were maintained at 20 ± 1°C.
127
128   **Exclusion assay of unrelated larvae**
129   (i) Preparation of foster female parents and larvae
130   Non-sibling male and female pairs of *N. quadripunctatus*, aged 2–3 weeks post-eclosion and
131   without previous breeding experience, were placed in breeding cages for 72 h in a dark incubator
132   to prepare carcasses and initiate egg laying. A total of 24 pairs from the Hokkaido population and
133   19 pairs from the Tokyo population were set up to provide female parents and larvae for the
134   experiment. To supply *N. vespilloides* larvae, 20 pairs of *N. vespilloides* were set up. First- and
135   second-generation adults, bred in the laboratory, were used for the experiment. After 72 h, the
136   female beetles and the carcasses were transferred to new breeding cages containing 2 cm of moist
137   peat. The male beetles were removed at this stage, as previous research indicates that male
138   parental care does not affect larval growth or survival under laboratory conditions (Smiseth et al.,
139   2005). The eggs were left to hatch in the original breeding cage. Hatching was monitored at 2-h
140   intervals, and newly hatched larvae were used for the experiments. Females were selected as
141   foster parents for the experiment only after their own eggs had begun to hatch, as burying beetles
142   typically kill any larvae that arrive before their own eggs hatch (Müller & Eggert, 1990).
143
144   (ii) Experimental procedure
145   The aim of this experiment was to investigate whether there is a difference in the exclusion rate
146   of larvae between *N. quadripunctatus* female parents from the Hokkaido and Tokyo populations.
147   One larva from the Hokkaido population of *N. quadripunctatus* and one larva from *N. vespilloides*
148   were introduced into the breeding cages of unrelated *N. quadripunctatus* female parents from both
149   populations. To ensure individual identification, each larva was anesthetized on ice, and one of
150   its hind leg tarsi (randomly selected as either the right or left) was clipped. The larvae were then
151   introduced into the new breeding cage containing the unrelated female. After 24 h, the survival of

152 the larvae was recorded.

153

Measurement of larval head width in *N. quadripunctatus* and *N. vespilloides*

155 To compare larval body size between *N. quadripunctatus* and *N. vespilloides*, the head widths of
156 10 first instar larvae, hatched within 2 h, were measured for *N. quadripunctatus* from both the
157 Hokkaido and Tokyo populations, as well as for *N. vespilloides*. Measurements were taken using
158 light microscopy (cellSens Standard Olympus software version 1.6, Olympus Optical Co., Tokyo,
159 Japan) with an accuracy of 1 μm.

160

Data analysis

162 Body size comparisons between species within the Hokkaido and Tokyo populations were
163 analyzed using generalized linear mixed models (GLMMs) with a Gaussian error distribution and
164 identity link function. The response variable was pronotum width, and the explanatory variable
165 was species. Sampling year was included as a random factor. Tukey's post-hoc pairwise
166 comparisons were performed using the "glht" function from the "multcomp" package.

167 For *N. quadripunctatus*, body size comparisons between the Hokkaido and Tokyo
168 populations were also analyzed using GLMM with a Gaussian error distribution and an identity
169 link function. The response variable was pronotum width, and the explanatory variables were
170 population and sex. Sampling year was included as a random factor.

171 The mortality rate of unrelated larvae was compared between populations using a GLMM
172 with a binomial error distribution and logit link function. The response variable was larval
173 survival (survived or dead), and the explanatory variables were parental origin (Hokkaido or
174 Tokyo), larval species, and their interaction. Female parent ID was included as a random factor.
175 Since the interaction term was significant, the data were separated by larval species (*N.*
176 *quadripunctatus* and *N. vespilloides*). For each dataset, the effect of parental origin on larval
177 survival was tested using generalized linear models (GLMs) with a Gaussian error distribution
178 and an identity link function. The response variable was larval survival, and the explanatory
179 variable was parental origin.

180 The head width of first instar larvae was compared among *N. quadripunctatus* from the
181 Hokkaido and Tokyo populations and *N. vespilloides* using a GLM with a Gaussian error
182 distribution and an identity link function. The response variable was larval head width, and the
183 explanatory variable was either population (Hokkaido or Tokyo) or species.

184 All analyses were conducted in R version 4.2.3 (R Core Team, 2022), using the "lme4" and
185 "multcomp" packages. For both GLMMs and GLMs, likelihood ratio tests (LRTs) were used to
186 determine the statistical significance of explanatory variables. A p-value of less than 0.05 was
187 considered statistically significant.

188

189 **Results**

190 **The guild of burying beetles in Hokkaido and Tokyo populations**

191 *Nicrophorus quadripunctatus* was captured in both populations, while other species were

192 captured exclusively in one population. In the Hokkaido population, five species were captured:

193 *N. tenuipes*, *N. investigator*, *N. maculifrons*, *N. vespilloides*, and *N. quadripunctatus*. The body

194 size of *N. tenuipes* was the largest (6.18 ± 0.49 mm, mean $\pm$ SD, range 4.3–7.2 mm), followed by

195 *N. investigator* (5.47 ± 0.49 mm, mean $\pm$ SD, range 4.0–7.0 mm). The body sizes of *N. maculifrons*

196 (5.30 ± 0.69 mm, mean $\pm$ SD, range 3.8–7.0 mm) and *N. vespilloides* (5.22 ± 0.43 mm, mean $\pm$

197 SD, range 4.2–6.3 mm) were statistically similar, and *N. quadripunctatus* was the smallest (4.90

198 ± 0.42 mm, mean $\pm$ SD, range 3.8–6.0 mm) (Tukey's HSD tests, p < 0.05; Fig. 1a). The body sizes

199 of the four larger species overlapped with that of *N. quadripunctatus*. In the Tokyo population,

200 three species were captured: *N. concolor*, *N. quadripunctatus*, and *P. morio*. The body size of

201 these species in descending order was *N. concolor* (10.84 ± 1.00 mm, mean $\pm$ SD, range 8.9–13.0

202 mm), *N. quadripunctatus* (5.05 ± 0.44 mm, mean $\pm$ SD, range 3.5–6.2 mm), and *P. morio* ($4.16 \pm$

203 0.38 mm, mean $\pm$ SD, range 3.3–4.7 mm) (Tukey's HSD, $p < 0.05$; Fig. 1b). In the Tokyo

204 population, no larger burying beetle species with body sizes overlapping with *N. quadripunctatus*.

205 The body size of *N. quadripunctatus* in the Tokyo population was significantly larger compared

206 to that in the Hokkaido population (GLMM, LRT: $\chi^2 = 5.646$, df = 1, $p = 0.017$; Fig. 2). A

207 significant sexual dimorphism in body size was also detected, with females being larger than

208 males (GLMM, LRT: $\chi^2 = 7.073$, df = 1, $p = 0.008$; Fig. 2).

209

210 **Comparison of exclusion rate of unrelated larvae between *N. quadripunctatus* populations**

211 The interaction term between parental origin (Hokkaido or Tokyo) and larval species was

212 significant (GLMM, LRT: $\chi^2 = 4.117$, df = 1, $p = 0.042$), indicating that the mortality rate of larvae

213 varied depending on the combination of the *N. quadripunctatus* population of foster parents and

214 the larval species (Fig. 3). While the effect of the parental population on the survival rate of *N.*

215 *quadripunctatus* larvae was not significant (GLMM, LRT: $\chi^2 = 0.091$, df = 1, $p = 0.763$), the

216 mortality rate of *N. vespilloides* larvae was significantly higher when introduced to foster parents

217 from the Hokkaido population compared to those introduced to parents from the Tokyo population

218 (GLMM, LRT: $\chi^2 = 6.452$, df = 1, $p = 0.011$).

219

220 **Comparison of larval body size in *N. quadripunctatus* and *N. vespilloides***

221 The larval body size (head width) was largest in *N. vespilloides* (968 ± 18 μm, mean $\pm$ SD, range

222 944–993 μm), followed by *N. quadripunctatus* from the Tokyo population (945 ± 19 μm, mean $\pm$

223 SD, range 901–961 μm), and *N. quadripunctatus* from the Hokkaido population (886 ± 18 μm,

224   mean ± SD, range 861–922 μm) (Tukey's HSD, $p < 0.05$).
225
226   **Discussion**
227   The present study demonstrates significant genetic variation in the exclusion behavior of *N.*
228   *quadripunctatus* females toward unrelated larvae, particularly in populations exposed to different
229   levels of interspecific competition. The results indicate that female parents from the Hokkaido
230   population, where *Nicrophorus* species with overlapping body sizes coexist with *N.*
231   *quadripunctatus* at high densities, have evolved stronger exclusion mechanisms for heterospecific
232   larvae compared to females from the Tokyo population, where fewer *Nicrophorus* species occur
233   and population densities are lower. In the Tokyo population, *N. concolor* coexists with *N.*
234   *quadripunctatus* but is more than twice its body size and can easily monopolize carcasses. In the
235   previous study on interspecific brood parasitism among burying beetles, cases where smaller
236   species parasitized larger species have been observed, but no cases of larger species parasitizing
237   smaller species have been confirmed (Trumbo, 1994). Therefore, *N. quadripunctatus* in the Tokyo
238   population is likely to face little to no threat of brood parasitism from other *Nicrophorus* species.
239   Our findings highlight the role of ecological pressures, such as competition, in shaping parental
240   care strategies, driving local adaptation that optimizes reproductive success by minimizing the
241   risk of investing in unrelated offspring.
242   This study provides new insights into an additional layer of selectivity, beyond temporal cues,
243   in the exclusion of heterospecific larvae. Temporal kin recognition, the ability of parents to switch
244   from infanticide to caregiving based on the expected timing of their own offspring's hatching, has
245   been well documented as a primary mechanism for offspring recognition in burying beetles
246   (Müller & Eggert, 1990; Oldekop & Smiseth, 2007). We observed that even after their own larvae
247   had hatched, *N. quadripunctatus* mothers from both Hokkaido and Tokyo were more likely to
248   exclude *N. vespilloides* larvae than unrelated conspecific larvae, suggesting that hatching time
249   alone is not sufficient to distinguish between offspring and non-offspring. The cues used by *N.*
250   *quadripunctatus* to detect and exclude *N. vespilloides* larvae require further investigation. Since
251   the larvae of *N. vespilloides* are larger than those of *N. quadripunctatus*, size differences may
252   serve as a basis for discrimination. Alternatively, the well-developed chemical communication
253   observed in burying beetles (Chemnitz et al., 2015; Engel et al., 2016; Haberer et al., 2008;
254   Smiseth et al., 2010; Takata et al., 2019) suggests that differences in the chemical profiles of the
255   larvae could also play a role in the recognition process.
256   This additional mechanism for the exclusion of heterospecific larvae appears to be locally
257   adapted in response to the competitive pressures in Hokkaido, where interspecific competition
258   among *Nicrophorus* species is more severe than in Tokyo. In environments with high competition
259   for carcasses, such as Hokkaido (Nagano & Suzuki, 2003; Ohkawara et al., 1998), natural

260 selection would favor individuals that are able to exclude heterospecific larvae more effectively,
261 as caring for unrelated offspring could reduce the reproductive success of the parents. This
262 supports the broader concept that local ecological conditions can lead to genetic divergence in
263 reproductive behaviors (Hereford, 2009; Kawecki & Ebert, 2004). In general, the strength of
264 defense mechanisms against brood parasitism is thought to depend on the cost imposed by
265 parasites on the host and the duration of the co-evolutionary history between hosts and parasites.
266 Studies on cuckoos and their hosts have shown that the greater the cost imposed by brood
267 parasitism, the more rigorous the defense mechanisms become (Kilner, 2005). In cases where
268 parasitism is thought to have emerged recently, host defenses are often poorly developed or
269 entirely absent (Begum et al., 2012; Brooke & Davies, 1988; Winfree, 1999). Our findings align
270 with previous studies on the role of local adaptation in shaping behaviors related to parental care
271 (Sun et al., 2020), highlighting the complex interplay between ecological context and
272 reproductive strategies in shaping evolutionary outcomes.

273   In conclusion, our results demonstrate that the exclusion of heterospecific larvae by *N.*
274 *quadripunctatus* female parents genetically differs between populations, with females from the
275 Hokkaido population showing a higher exclusion rate than those from the Tokyo population. This
276 suggests that the presence of competing congeners has led to the evolution of more selective
277 parental care behaviors in populations facing higher levels of competition. These behaviors likely
278 represent a local adaptation that optimizes reproductive success by ensuring that parental care is
279 directed primarily toward their own offspring. These findings contribute to our understanding of
280 how genetic and ecological factors interact to shape parental care behaviors, providing a
281 foundation for future research into the genetic basis of offspring discrimination and local
282 adaptation in reproductive strategies.

283

284 **Significance statement**
285 This study reveals genetic variation in parental care behaviors, particularly the exclusion of
286 heterospecific larvae, in *N. quadripunctatus*. By investigating populations with different
287 ecological backgrounds, we identified that the exclusion behavior toward heterospecific larvae
288 varies genetically between populations. Parents from populations coexisting with competitors
289 exhibited a higher rate of excluding unrelated young compared to those from competitor-free
290 environments. These findings provide new insights into the genetic basis influencing parental care
291 strategies, specifically the ability to discriminate between their own offspring and unrelated young.
292 This research contributes to a deeper understanding of how genetic factors shape family dynamics
293 and parental behaviors.

294

295 **AUTHOR CONTRIBUTIONS**

309
310 **COMFLICT OF INTEREST STATEMENT**
311 The authors declare no conflict of interest.
312
313 **DATA AVAILABILITY STATEMENT**
314 Data and code for statistical analyses are available in Supporting Information.
315
316 **ETHICS APPROVAL**
317 This work did not require ethical approval from a human subject or animal welfare committee.
318

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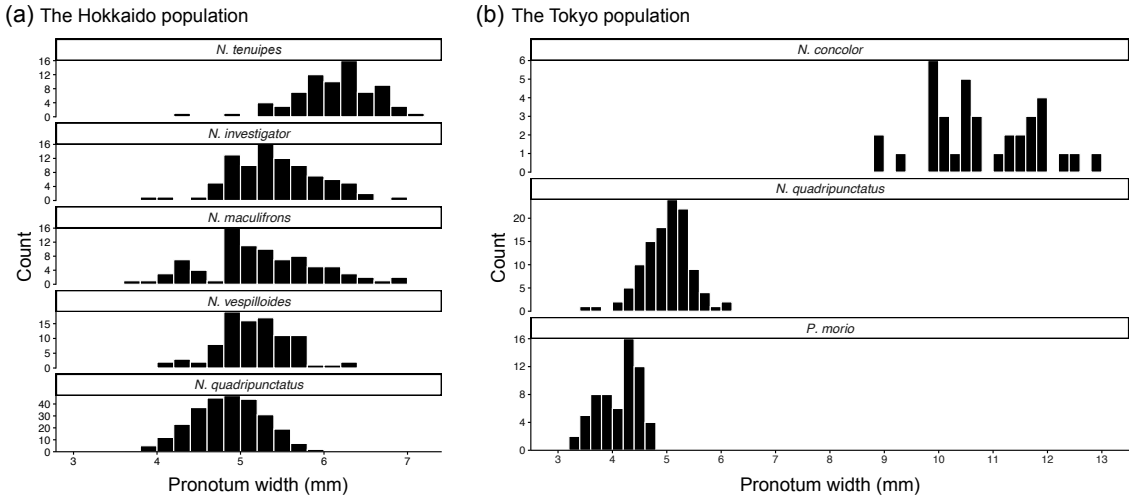421   *& Evolution*, *14*(9), 338–343.

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Figure 1. Body size comparison of burying beetles in the Hokkaido and Tokyo populations. (a) Body size (pronotum width) of burying beetle species captured in the Hokkaido population, showing the size differences among *Nicrophorus tenuipes* ($n = 74$), *N. investigator* ($n = 90$), *N. maculifrons* ($n = 87$), *N. vespilloides* ($n = 93$), and *N. quadripunctatus* ($n = 273$). *N. quadripunctatus* was the smallest species in this population. (b) Body size of burying beetle species captured in the Tokyo population, showing the size differences among *N. concolor* ($n = 36$), *N. quadripunctatus* ($n = 114$), and *Ptomascopus morio* ($n = 61$).

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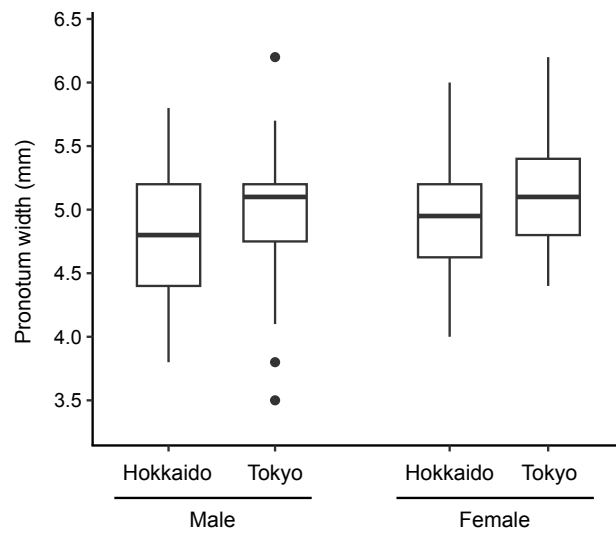
Figure 2. Body size comparison of *Nicrophorus quadripunctatus* between the Hokkaido and
Tokyo populations. Pronotum width of *N. quadripunctatus* individuals from the Hokkaido (male
= 95, female = 178) and Tokyo (male = 55, female = 59) populations. Boxes indicate interquartile
range, solid horizontal lines medians, whiskers minimum and maximum values, and points
indicate outliers. The Tokyo population exhibited significantly larger body sizes compared to the
Hokkaido population (GLMM, LRT, p = 0.017). A significant sexual dimorphism in body size
was also detected, with females being larger than males (GLMM, LRT, p = 0.008).

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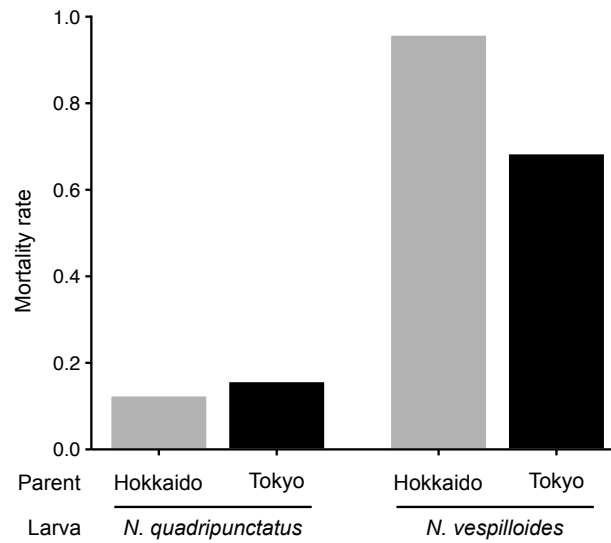
Figure 3. Exclusion rate of unrelated larvae by *Nicrophorus quadripunctatus* female parents from the Hokkaido and Tokyo populations. Survival rates of unrelated larvae (*N. quadripunctatus* and *N. vespilloides*) introduced to foster *N. quadripunctatus* parents from the Hokkaido (*n* = 24) and Tokyo (*n* = 19) populations are shown. While no significant difference was observed in the survival rate of *N. quadripunctatus* larvae between the two populations (GLMM, LRT, *p* = 0.763), *N. vespilloides* larvae experienced significantly higher exclusion by parents from the Hokkaido population compared to those from the Tokyo population (GLMM, LRT, *p* = 0.011). Gray and black bars represent the exclusion rate of unrelated larvae by Hokkaido and Tokyo parents, respectively.

1 **Supporting Information for**
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3 Genetic variation among populations in parental exclusion of heterospecific larvae in burying beetles
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14 **Supplementary Information for this manuscript includes the following:**

15 ● code for exclusion rate.Rmd

16 ● field beetle size.csv

17 ● exclusion rate.csv

18 ● larval size.csv

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Description of the data and file structure

"**code for exclusion rate.Rmd**": This file contains the R code used for conducting the statistical analyses presented in the study. It includes scripts for data processing, analysis, and visualization.

"**field beetle size.csv**":

This file provides the body size data for burying beetles collected from two populations: Hokkaido and Tokyo. The data includes measurements of individual beetles, along with relevant collection details.

Columns descriptions:

- "year" = The year in which the individual was sampled.
- "month" = The month in which the individual was sampled.
- "population" = The population (Hokkaido or Tokyo) from which the individual was collected.
- "species" = The species name of the individual beetle.
- "sex" = The sex of the individual beetle (male or female).
- "pronotum_width" = The pronotum width of the individual beetle, measured in millimeters (mm).

"**exclusion rate.csv**":

This file contains the data used to analyze the exclusion rate of unrelated larvae by *Nicrophorus quadripunctatus* female parents from the Hokkaido and Tokyo populations. The dataset includes survival outcomes of larvae from both *N. quadripunctatus* and *N. vespilloides* when introduced to unrelated female parents.

Column descriptions:

- "female_parent_ID" = A unique identifier for each female parent used in the experiment.
- "population_of_female_parent" = The population origin of the female parent (Hokkaido or Tokyo).
- "survival_of_quadripunctatus_larva" = The survival status of *N. quadripunctatus* larvae introduced to unrelated female parents (values: "survived" or "dead")
- "survival_of_vespilloides_larva" = The survival status of *N. vespilloides* larvae introduced to unrelated female parents (values: "survived" or "dead").

"**larval size.csv**":

This file provides body size data for burying beetle larvae, including *Nicrophorus quadripunctatus* from the Hokkaido and Tokyo populations, and *N. vespilloides*. The data include measurements of individual beetles along with relevant collection details.

Column descriptions:

- "population" = The population (Hokkaido or Tokyo) from which the beetle was collected.
- "species" = The species name of the individual beetle.
- "head_width" = The head width of the first instar larva, measured in micrometers (μm).