

Factors Related to Altitudinal Body Size Variation in the Earthworm-eating Ground Beetle *Carabus japonicus*

Yutaka Okuzaki^{1*} and Teiji Sota²

¹Field Science Center for Northern Biosphere, Hokkaido University, Kita, Sapporo 060-0811, Japan

²Department of Zoology, Graduate School of Science, Kyoto University, Sakyo, Kyoto 606-8502, Japan

To understand geographic body size variation in an insect species, various factors including habitat temperature, correlation between life history traits, and food availability must be examined. *Carabus (Ohomopterus) japonicus* is univoltine, feeds exclusively on earthworms during its larval stage, and shows a clinal body size variation along the habitat temperature gradient in northern Kyushu, Japan. *Carabus japonicus* occurs at both high and low altitudes; at high altitudes it coexists with a larger species, *C. (O.) dehaanii*. At low altitudes, *C. japonicus* shows larger body sizes. We sought to determine whether this increase in body size is only an adaptation to high habitat temperatures in the absence of *C. dehaanii*, and examined the life history of *C. japonicus* and seasonal trends in prey earthworms at six sites between altitudes of 30 and 980 m. While high-altitude populations used the entire warm season for reproduction and larval development, low-altitude populations showed shorter periods of reproductive activity and larval incidence coincided with seasonal trends in earthworm abundance. Thus, *C. japonicus* attained larger body sizes at lower altitudes without a notable extension of their juvenile period. At lower altitudes, earthworms grew faster and reached sizes, which may be too large for predation by small carabid larvae. Large females had higher fecundity, and laid larger eggs, from which larger first instar larvae hatch. The large body size of *C. japonicus* in warm habitats may thus be an adaptation not only for high fecundity, but also for producing large first instar larvae to more efficiently prey on large earthworms.

Key words: body size evolution, character release, converse Bergmann cline, earthworm, genus *Carabus*, reproductive interference, subgenus *Ohomopterus*

INTRODUCTION

Positive correlation between body size and habitat temperature, what might be called a converse Bergmann cline, is common in univoltine insects (Masaki, 1967; Mousseau, 1997; Blanckenhorn and Demont, 2004). This body size cline can be interpreted as a result of life-history optimization at locations along the habitat temperature gradient (Roff, 1980). Roff's (1980) model is based on the assumption that oviposition occurs simultaneously at the beginning of a reproductive season (big-bang reproduction), and that juvenile (pre-reproductive) survival and fecundity (via achieved body size) are functions of juvenile development time (e.g., in day-degree units). In fact, adult body size is generally correlated with juvenile development time (Roff, 1980; Blanckenhorn, 2000; Kingsolver and Pfennig, 2004), and fecundity increases with body size in many insects (Honěk, 1993). When fecundity and survival functions are constant across habitats, body size is predicted to be larger in warm habitats because of optimization of development time. However, the simple assumptions of the Roff's (1980) model can be violated in a number of ways. For example, juvenile

development is restricted not only by temperature but also by seasonal food availability (Blanckenhorn, 1999; Davidowitz et al., 2004; Stillwell et al., 2007). Moreover, although this model assumes both constant egg size and an increase in fecundity with adult body size, this may not be true when egg size increases with body size. A large larva hatched from a large egg may show high performance for resource utilization and against environmental stress (Dixon and Hemptinne, 2001; Smith, 2002; Blanckenhorn et al., 2007; Chown and Gaston, 2010). Thus, large adult body size may be selected not because of large fecundity, but for improved larval survival.

In Japan, ground beetles of the subgenus *Ohomopterus* belonging to the genus *Carabus* are univoltine spring breeders without larval overwintering (Sota, 1985a; Sota and Nagata, 2008). Overwintered adults begin reproducing from spring; larvae feed on earthworms and pupate in the soil, and new adults overwinter without reproduction. Most species occur in a wide range of altitudes and show positive correlations between adult body length and habitat annual mean temperature (Sota et al., 2000a). The actual factors causing the body size cline have not been completely resolved for *Ohomopterus* beetles. Larvae of *Ohomopterus* feed exclusively on earthworms, whereas adults are polyphagous (Sota, 1985a; Okuzaki et al., 2010a). Low habitat tem-

* Corresponding author. E-mail: yutaka@fsc.hokudai.ac.jp
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perature and limited availability of prey earthworms could both be selective factors resulting in small adult body sizes at high altitudes (Ikeda et al., 2012). Adult body size is positively related to development time from egg deposition to eclosion in *Ohomopterus* (Sota, 1985b; Tsuchiya et al., 2012), but the relationship between adult body size and fecundity has not been resolved.

Among *Ohomopterus* species, *Carabus japonicus* Motschulsky, 1857 shows a distinct pattern of body size variation related to character release along the habitat temperature gradient in Kyushu District, southwestern Japan (Sota et al., 2000a; Okuzaki et al., 2015). *Carabus japonicus* coexists with the larger congener, *C. dehaanii* Chaudoir, 1848, in most parts of Kyushu, and occurs solely in small islands and the peninsula of northern Kyushu. Both species show positive correlation between body size and habitat temperature with a constant interspecific body size difference in their sympatric area; however, allopatric populations of *C. japonicus* occurring at low altitudes show a large variation in body size at similar habitat temperature conditions (Sota et al., 2000a; Okuzaki et al., 2015). This suggests that some ecological factors other than habitat temperature affect the body size evolution of *C. japonicus* at low altitudes. Geographic and seasonal variations in available prey (earthworms) size may be important factors, as prey size affects foraging success (Okuzaki et al., 2010b). In addition, larger females of *C. japonicus* produce larger eggs (thereby large first-instar larvae), which result in large adult body sizes (Okuzaki et al., 2015). This may imply that fecundity does not increase with body size.

In the present study, we examined the seasonal life history of *C. japonicus* populations together with *C. dehaanii* populations, including an assessment of the relationship between female body size and fecundity and seasonal availability of earthworms at different altitudes in northern Kyushu, to identify factors involved in the body size variation in *C. japonicus*.

MATERIALS AND METHODS

Study sites

We selected six sites in northern Kyushu, where *C. japonicus* populations with different body sizes occurred: site 1, Mt. Sefuri

(33°26'04" N, 130°22'12" E, altitude 980 m above sea level (a.s.l.), annual mean temperature 10.2°C); site 2, Mt. Sakurei (33°21'37" N, 130°4'19" E, 820 m a.s.l., 10.8°C); site 3, Mt. Abura (33°30'43" N,

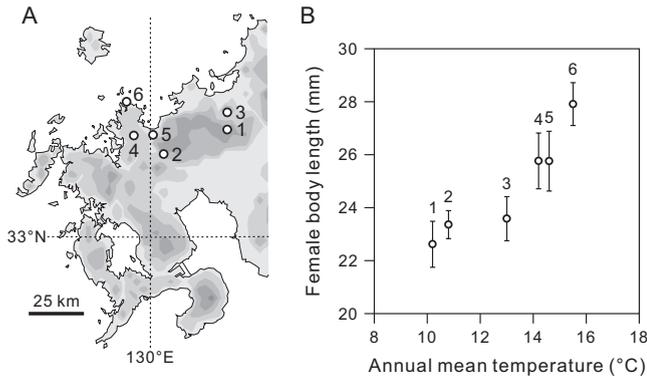


Fig. 1. (A) Locations of study sites (sites 1–6). (B) Relationship between female body length of *C. japonicus* and annual mean temperature. Circles and error bars represent means and standard deviations of body length of females collected in this study, respectively. Numbers indicate study sites.

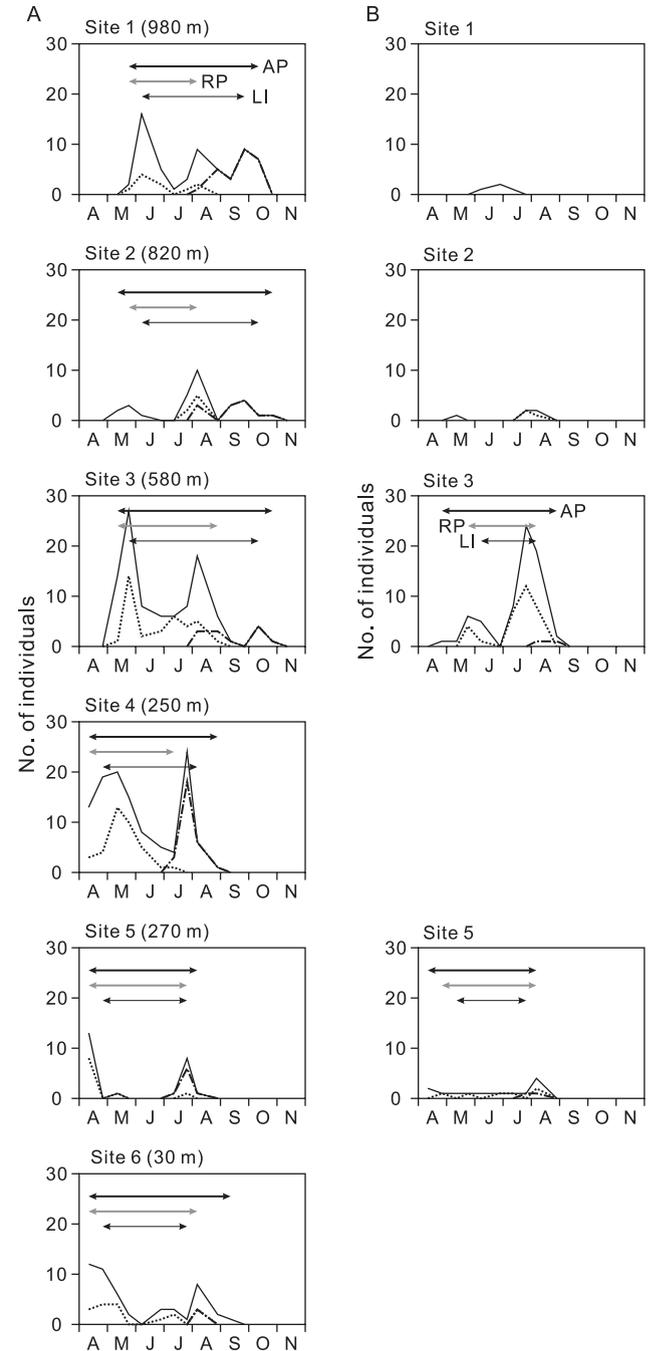


Fig. 2. Number of individuals collected per sampling time of *C. japonicus* (A) and *C. dehaanii* (B). Solid, dotted, and dash-dotted lines represent all individuals, females with mature eggs, and new adults with soft body, respectively. Black-bold, gray-bold and black-thin double-headed arrows represent activity period of adults (AP), reproductive period (RP) and period of larval incidence (LI), respectively, at each site. Altitudes of sites are indicated in parentheses. For *C. japonicus*, starts of RP and LI at site 2 were assumed to be the same as at site 1 with similar habitat temperature, because females with mature eggs were first collected in July. For *C. dehaanii*, the AP, RP, and LI could not be estimated at sites 1 and 2, due to small number of individuals collected.

130°21'52" E, 580 m a.s.l., 13.0°C); site 4, Mt. Ishidaka (33°25'28" N, 129°54'51" E, 250 m a.s.l., 14.2°C); site 5, Mt. Kagami (33°25'46" N, 130°1'26" E, 270 m a.s.l., 14.6°C); and site 6, Kabe Is. (33°32'55" N, 129°52'56" E, 30 m a.s.l., 15.5°C) (Fig. 1). Both *C. japonicus* and *C. dehaanii* occurred in sympatry at sites 1, 2, 3, and 5, whereas only *C. japonicus* occurred at sites 4 and 6. The annual mean temperatures of these sites were obtained from 1-km mesh climatic data from 1971 to 2000 (Mesh Climatic Data 2000; Japan Meteorological Business Support Center).

Sampling

Seasonal occurrences of *C. japonicus*, *C. dehaanii*, and earthworms were examined at the six sites from April to November 2010. *Ohomopterus* beetles were collected every two weeks by using pitfall traps (plastic cups of 8-cm diameter, 12-cm deep) containing 50 ml of 50% ethanol to prevent the trapped beetles from escaping or being eaten within the trap. In each sampling, 20 pitfall traps were set at each site at 3-m intervals for three days. By using the same method, we confirmed that no *Ohomopterus* beetles occurred in March 2011.

The collected beetles were transported to the laboratory at Kyoto University within one day and sorted based on species and sex. The firmness of the elytra was inspected manually to determine whether the individual was newly emerged; new adults have soft bodies within approximately one week after emergence. Female body length (from the front margin of the labrum to the apical part of the elytra following Sota et al., 2000a and Okuzaki et al., 2015) was measured using digital calipers DT-150 (Niigata Seiki Co., Ltd., Niigata, Japan) with an accuracy of 0.01 mm, and ovaries were dissected to count the number of mature eggs and ovarioles as indices of reproductive activity and fecundity (reproductive capacity). We defined the activity period as the period during which adult beetles were captured and the reproductive period as the period during which females with mature eggs were captured.

Because larvae were seldom collected by pitfall traps due to their low mobility, we estimated the period of larval incidence as the period between the two weeks after the first overwintered females with mature eggs were collected and two weeks before the last new (teneral) adults were collected. We chose two-week lags because (1) eggs of *C. japonicus* hatch 11.1 days after oviposition at 20°C, and the first instar larvae start to feed on earthworms about three days after hatching, and (2) fully-fed third (last) instar larvae burrow into the soil, pupate, emerge as adults, and appear above the ground by 21.2 days after burrowing (data from Okuzaki et al., 2015).

Earthworms (Oligochaeta: Haplotaxida) were collected by hand from the litter layer on the day of pitfall setting. Sampling was performed from 10 quadrats (50 cm × 50 cm) set arbitrarily at each site. Within 6 h after sampling, the weight of earthworms was measured

using an electric balance FZ-120i (A&D company, Ltd., Tokyo, Japan) with an accuracy of 1.0 mg. We assumed the earthworms that existed during the period of *Ohomopterus* larval incidence were potential prey of *Ohomopterus* larvae.

Statistical analysis

The effect of female body length on the number of ovarioles in *C. japonicus* and *C. dehaanii* was examined using generalized linear mixed models (GLMM) with normal distribution and identity link function. The effect of female body length on the number of mature eggs in the two species collected during the reproductive period was examined using a GLMM with Poisson distribution and log-link function. In these models, the study site was added as a random variable.

Body weights of earthworms collected during the period of larval incidence of *C. japonicus* were log-transformed and compared among the study sites by using analysis of variance (ANOVA) and Tukey's HSD test. These analyses were conducted using the statistical package JMP version 12 (SAS Institute Inc., Cary, NC).

RESULTS

Seasonal life history of *Ohomopterus* beetles

Adults of *C. japonicus* appeared from May to October and reproduced from May to August at upper sites 1–3, whereas they occurred from April to August or September and reproduced from April to July at lower sites 4–6 (Fig. 2A). The duration of the larval incidence period tended to be short at the lower sites.

The number of *C. dehaanii* captured was small, except for site 3, and differences in seasonal life cycle among the sites were unclear (Fig. 2B). However, the active and reproductive periods overlapped with those of *C. japonicus* at

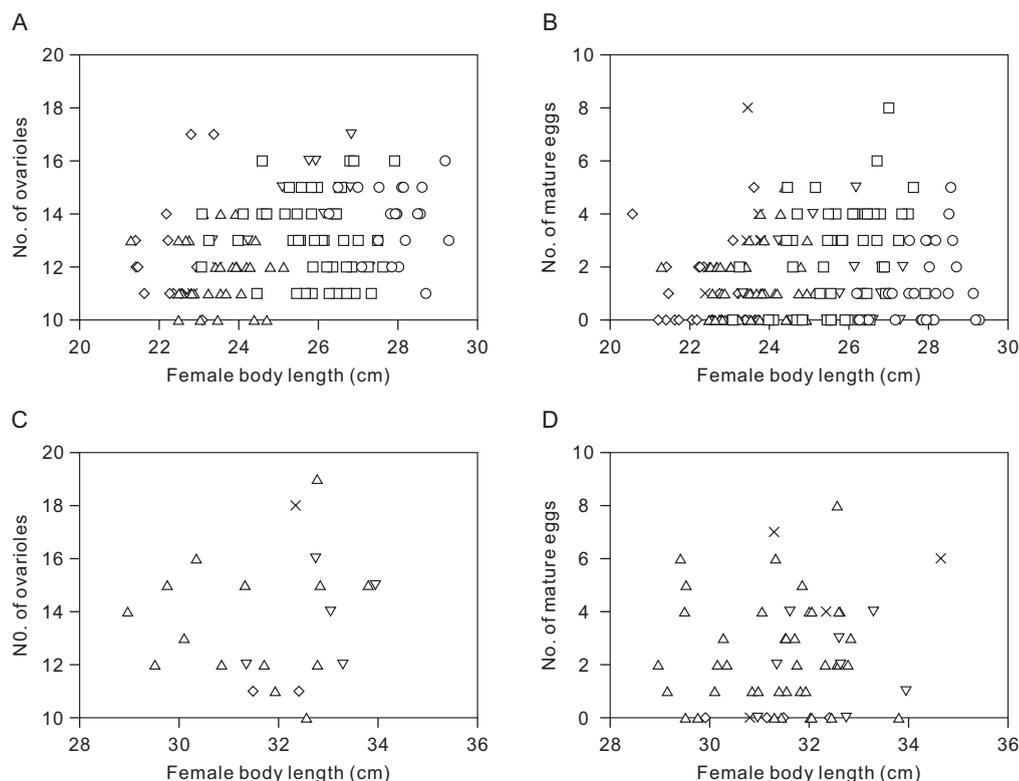


Fig. 3. Relationships between female body length, the numbers of ovarioles and mature eggs in *C. japonicus* (A, B) and *C. dehaanii* (C, D). Diamonds, crosses, triangles, squares, converse triangles, and circles represent individuals collected in sites 1–6, respectively.

sites 3 and 5 (Fig. 2).

Fecundity of *Ohomopterus* beetles

Both the numbers of ovarioles and mature eggs increased with female body length in *C. japonicus* (Fig. 3A, B; $n = 130$, $\chi^2 = 17.7$, $P < 0.0001$ for ovarioles; $n = 190$,

$\chi^2 = 15.3$, $P < 0.0001$ for mature eggs), suggesting that large body size has potentially large fecundity. For *C. dehaanii*, female body length was not related to the numbers of ovarioles and mature eggs (Fig. 3C, D; $n = 22$, $\chi^2 = 0.3$, $P = 0.5549$ for ovarioles; $n = 48$, $\chi^2 = 0.0$, $P = 0.9256$ for mature eggs), probably because of the relatively small range of variation in their body length.

Seasonal dynamics of earthworms

Earthworms, mostly Megascolecidae, appeared from

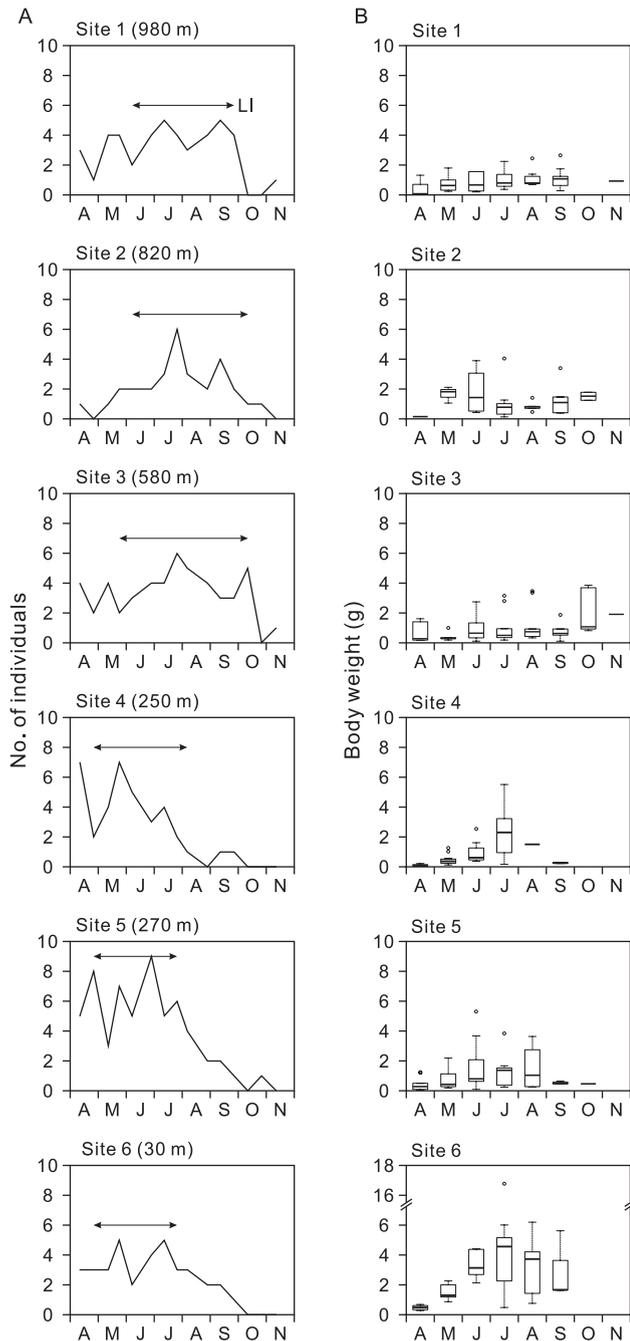


Fig. 4. (A) Number of individuals collected per sampling time of earthworms in litter layer. Double-headed arrows represent period of larval incidence (LI) of *C. japonicus* (Fig. 2A). Altitudes of sites are indicated in parentheses. (B) Seasonal change in body weight of the collected earthworms. Bold bar, box, thin bars, and circles represent median, interquartile range, highest and lowest non-outlier values, and outliers of the body weight, respectively. Data were compiled by month.

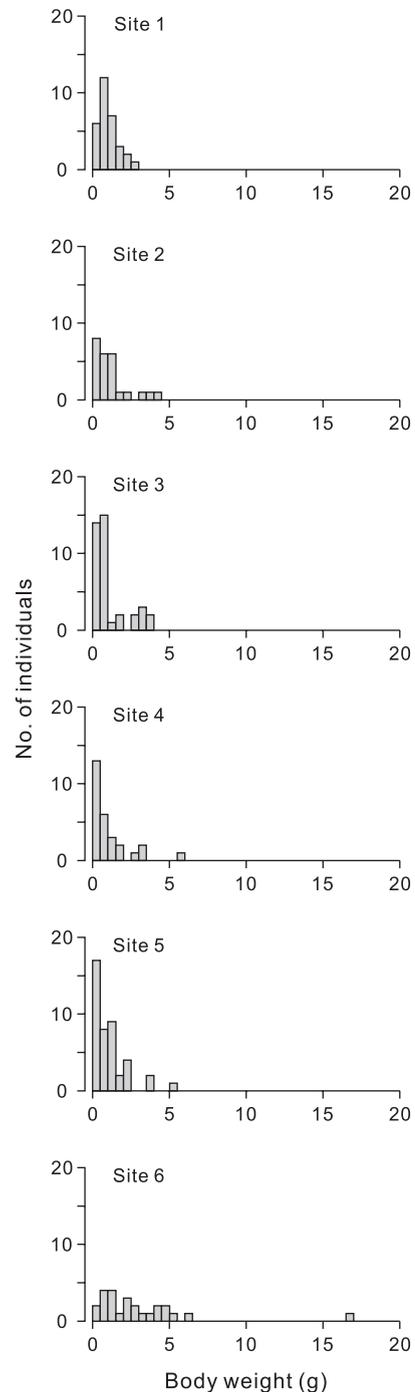


Fig. 5. Body weight distribution of earthworms during the period of larval incidence of *C. japonicus* (Fig. 2A).

April to September in all study sites (Fig. 4A). At the lower sites 4–6, the number of individuals collected decreased rapidly after July, but this trend was absent or moderate at the upper sites 1–3 (Fig. 4A). The median body weight of earthworms increased from April to July, especially at site 6, where large *C. japonicus* occurred (Fig. 4B). Body weights of earthworms collected in the period of larval incidence of *C. japonicus* differed among sites (Fig. 5: ANOVA, $F_{5,185} = 6.0$, $P < 0.0001$); earthworms at site 6 were larger than those at the other sites (Tukey's HSD, $P < 0.05$).

DISCUSSION

Factors affecting altitudinal life history variation

The numbers of ovarioles and mature eggs increased with female body size in *C. japonicus* (Fig. 3A, B), suggesting that fecundity increases with body size. Because an increase in adult body size is associated with an increase in development time in *C. japonicus* and other *Ohomopterus* species (Sota, 1985b; Tsuchiya et al., 2012; Okuzaki et al., 2015), there would be a trade-off between fecundity (correlated with adult body size) and juvenile survival rate via development time. However, the increase in juvenile development time with adult body size is not large in *C. japonicus* under the same temperature condition (e.g., 0.60 and 0.33 days per 1-mm increase for males and females, respectively, at 20°C; data from Okuzaki et al., 2015). This is because larger *C. japonicus* females produce larger larvae (i.e. large eggs), which show faster growth rates (Okuzaki et al., 2015). Therefore, attaining as large a body size as possible within the season available for larval development would be optimal for *Ohomopterus* beetles because of the advantages of a large female body size in fecundity.

However, *C. japonicus* populations occurring at low altitudes (sites 4–6) began reproduction and completed their larval development earlier than those occurring at high altitudes (sites 1–3) (Fig. 2A). Thus, the low-altitude populations did not utilize the entire warm season for reproduction and larval development. As the development of ectotherms is possible at temperatures above a certain threshold (Trudgill et al., 2005), the reproductive and larval development periods of the high-altitude populations may be restricted by the short period with suitable temperature conditions. On the other hand, the reproductive and larval developmental periods of the low-altitude populations were unlikely to be restricted by temperature conditions. These periods might instead be terminated due to the decrease in abundance of earthworms in the litter layer after July (Fig. 4A). Female *C. japonicus* with mature eggs were collected in July at sites 4 and 5 and in August at site 6, but the larvae that hatched from these eggs would find it difficult to develop into adults. Similar seasonal trends in megascolecid earthworm abundance have been reported from other warm habitats of *Ohomopterus* (Sota, 1985a). Thus, at warm habitats of low altitudes, the available time for the development of *Ohomopterus* larvae may be restricted by the availability of prey after summer. The seasonal decline of earthworm abundance may be related to physical factors (e.g., high temperature and dryness in summer) and/or species-specific life history. To address this question, it is necessary to study the life history and the effects of physical factors on seasonal population trend in earthworm species at different

altitudes.

Implication for the body size variation under sympatric and allopatric conditions

Our data provide insight into the factors causing both body size differentiation between sympatry and allopatry in *C. japonicus*, namely character release. In sympatry, *C. japonicus* and *C. dehaanii* have small and large body sizes, respectively, each of which are correlated with the habitat annual mean temperature, and the body size difference between the two species is constant (female body length, 21.4–26.8 mm and 28.6–36.9 mm in *C. japonicus* and *C. dehaanii*, respectively; cf. Okuzaki et al., 2015). In *Ohomopterus*, interspecific mating due to incomplete mate recognition is known to occur (Sota et al., 2000b; Takami and Suzuki, 2005; Usami et al., 2006; Takami et al., 2007; Kubota et al., 2013), and it can result in loss of time and gametes as well as injury to the genitalia (Sota and Kubota, 1998). Body size differences between sympatric species reduce fitness loss due to reproductive interference through interspecific mating (Okuzaki et al., 2010b). Overlapping reproductive periods between *C. japonicus* and *C. dehaanii* suggested that *C. japonicus* was potentially exposed to reproductive interference by *C. dehaanii* (Fig. 2), and the small body size of *C. japonicus* is thought to be adaptive in sympatry. The effectiveness of size differences between the two species in preventing interspecific mating must be investigated in future studies.

In allopatry (i.e., under character release), *C. japonicus* showed body size variation at the same habitat temperature, presenting unexpectedly large body sizes in some islands (female body length 23.1–29.1 mm; cf. Okuzaki et al., 2015). Thus, the body size variation could not be explained solely based on habitat temperature and the presence/absence of closely related species. In the present study, we found a large variation in the body size of earthworms among the study sites (Fig. 4). At site 6, one of islands where exceptionally large *C. japonicus* occurred, the earthworms were also large during the period of larval incidence of *C. japonicus* (Fig. 5). Predation success decreases with increasing prey size in the first-instar larvae of small *Ohomopterus* species (Okuzaki et al., 2010b). Large females of *C. japonicus* produce large eggs (Okuzaki et al., 2015) without sacrificing fecundity (present study). Therefore, natural selection in habitats where large earthworms occur during the summer may favour a large body size, and the body size variation in allopatric populations of *C. japonicus* may be associated with the variation in the size of earthworms. It is necessary to assess both geographic variations in the body size of earthworms in Kyushu and the relationship between body size of *C. japonicus* larva and predation success on different-sized earthworms.

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COMPETING INTERESTS

The authors have no competing interests to declare.

AUTHOR CONTRIBUTIONS

YO and TS designed research. YO performed research. YO and TS wrote the paper.

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