Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Parental and offspring origins independently influence larval growth in body-size-divergent populations of a burying beetle

Mamoru Takata

takata.mamoru.7z@kyoto-u.ac.jp

Kyoto University

Yuki Matsuo

Tokyo University of Agriculture and Technology

Research Article

Keywords: Geographic variation, Body size, Parental investment, Parental care, Parent–offspring interactions, Burying beetles

Posted Date: November 12th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11450653/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Abstract

Phenotypic divergence among populations can arise not only from intrinsic differences among individuals but also from variation in the social environments shaping development. In species with parental care, offspring growth depends on both parental and offspring traits, yet how these contributions are associated with geographic body-size divergence remains unclear. In natural populations of the burying beetle *Nicrophorus quadripunctatus*, adults from Tokyo are larger than those from Hokkaido. To investigate the basis of this geographic body-size divergence, we examined descendants of both populations reared under common laboratory conditions. Without manipulating brood size, Tokyo-origin adults had approximately 12% greater pronotum width than Hokkaido-origin adults. Among size-selected mothers, Tokyo females produced eggs approximately 5% larger in volume at a given maternal body size, whereas egg number and hatchling head width showed no population differences after adjusting for their respective predictors. The number of larvae retained for rearing was also similar. We then reciprocally cross-fostered one larva from each population together with an unrelated mother of either origin. Among broods in which both larvae survived, Tokyo-origin larvae were approximately 10% heavier than Hokkaido-origin larvae raised by the same foster mother. Larvae also attained greater mass with Tokyo foster mothers, independently of their own origin, with no significant interaction. The relationship between larval mass at 120 h and adult pronotum width also differed between populations. These findings support an inherited component to geographic body-size divergence and reveal population differences in maternal investment, caregiver-associated effects, offspring-associated growth, and subsequent developmental relationships.

Significance statement

Why do animals from different populations grow to different sizes? An animal’s size depends not only on its own characteristics but also on the developmental environment created by its parents. We studied burying beetles from Hokkaido and Tokyo and found that their difference in adult body size persisted under common laboratory conditions. Females also differed in egg volume relative to their body size. To investigate growth after hatching, we placed one larva from each population together with an unrelated foster mother from either population. Tokyo-origin larvae were heavier than Hokkaido-origin larvae raised by the same mother, while Tokyo foster mothers raised heavier larvae regardless of larval origin. We also found that the relationship between larval mass and adult size differed between populations. These findings show how geographic body-size variation can involve parental and offspring-associated traits across development.

Introduction

Geographic variation in body size is widespread among animal populations and provides an opportunity to investigate the evolutionary processes underlying phenotypic divergence (Blanckenhorn and Demont 2004; Chown and Gaston 2010). However, an individual's phenotype is shaped not only by its own genetic and physiological characteristics but also by interactions with other individuals (Moore et al.

1997). This is particularly relevant in species with parental care, where parents create a developmental environment that influences offspring growth (Rauter and Moore 2002). Evolutionary theory predicts that such socially mediated effects can modify the response of phenotypic traits to selection because trait expression depends on characteristics of both the focal individual and its social partners (Moore et al. 1997; Wolf et al. 1998). Consequently, geographic divergence in offspring body size may involve differentiation in both offspring traits and parental traits that influence offspring development. Understanding the mechanisms underlying body-size divergence therefore requires identifying the contributions associated with each component and their interactions.

Parental investment encompasses both pre-hatching and post-hatching components that influence offspring development through different mechanisms (Clutton-Brock 1991). Pre-hatching investment includes the allocation of resources to egg production, reflected in egg number and size (Smith and Fretwell 1974), whereas post-hatching investment includes food provisioning, protection, and regulation of the number of offspring reared (Clutton-Brock 1991; Royle et al. 2012). Offspring growth also depends on offspring-associated characteristics, including physiological requirements and responses to parental care. These parental and offspring traits may influence development independently or through their interactions. Quantitative genetic theory predicts that genetic associations between parental and offspring traits can modify evolutionary responses to selection (Kirkpatrick and Lande 1989; Moore et al. 1997). Furthermore, selection acting on parent–offspring interactions may promote coordinated evolutionary changes in parental and offspring traits (Lock et al. 2004). Thus, geographic differentiation in body size may reflect divergence in multiple reproductive and developmental traits, including the interactions between caregivers and offspring.

Burying beetles (*Nicrophorus* spp.) provide a useful model for investigating these processes because they exhibit elaborate parental investment spanning both pre-hatching and post-hatching stages. Adults reproduce on small vertebrate carcasses, which they prepare as both food resources and developmental environments for their offspring (Scott 1998; Grubmüller et al. 2026). Females lay eggs in the surrounding soil, and after hatching, parents provision larvae through regurgitation (Scott 1998; Eggert et al. 1998). Larvae can also feed directly on the carcass, allowing offspring growth to be influenced by both parental activities and offspring-associated characteristics (Eggert et al. 2026). Parents can further regulate the number of larvae reared through filial cannibalism, thereby influencing competition for the limited resources available on a carcass (Steiger et al. 2007). Previous studies have demonstrated that post-hatching parental care can mask the effects of egg size on offspring growth (Monteith et al. 2012), while direct and indirect genetic effects contribute to offspring development (Rauter and Moore 2002). Genetic correlations between parental and offspring behaviors have also been demonstrated in burying beetles (Lock et al. 2004). Furthermore, experimental selection has shown that parental care can alter the evolutionary response of adult body size (Jarrett et al. 2017). These findings highlight the potential importance of parental investment and parent–offspring interactions in the evolution of body size.

Population comparisons provide a complementary approach to investigating how these reproductive and developmental traits diverge. Previous research on *Nicrophorus* has revealed population differences

in reproductive traits, brood-size regulation, and offspring body size, with both environmental and genetic factors contributing to the observed variation (Steiger et al. 2007; Sun et al. 2020). The burying beetle *N. quadripunctatus* is particularly suitable for examining these processes because parental care during early larval development is essential for successful offspring growth (Satou et al. 2001), and parental provisioning involves chemical communication that stimulates larval begging (Takata et al. 2019). Recent comparisons between Hokkaido and Tokyo populations revealed geographic differences in adult body size and newly hatched larval size, with Tokyo individuals being larger (Takata and Matsuo 2025). Laboratory-reared females from these populations also differed in their exclusion of heterospecific larvae, indicating population differentiation in parental behavioral traits (Takata and Matsuo 2025). The two populations occur in distinct burying beetle communities, particularly regarding the presence of similarly sized competitors and the potential intensity of interspecific competition (Ohkawara et al. 1998; Nagano and Suzuki 2003; Takata and Matsuo 2025). Although these findings demonstrate geographic differentiation in morphological and parental behavioral traits, whether the adult body-size difference persists under common environmental conditions and which reproductive and developmental traits are associated with this divergence remain unresolved.

Here, we investigated geographic body-size divergence between Hokkaido and Tokyo populations of *N. quadripunctatus* using individuals maintained under common laboratory conditions. We first tested whether the previously documented difference in adult body size persisted when offspring were produced under standardized rearing conditions. We then examined population differences in size-dependent reproductive investment by analyzing the relationships between maternal body size and egg number or egg volume, as well as the relationship between egg volume and hatchling head width. We also compared the number of offspring retained for rearing under standardized conditions. To distinguish parent-associated and offspring-associated contributions to post-hatching growth, we conducted a reciprocal cross-fostering experiment in which larvae from both populations were reared together by foster mothers from either population. Finally, we examined whether the relationship between larval body mass at 120 h and adult body size differed between populations. By integrating these comparisons across developmental stages, we aimed to identify reproductive and developmental traits associated with geographic body-size divergence and clarify the respective roles of parental and offspring-associated factors in larval growth.

Materials and methods

Study populations and laboratory rearing

We used two populations of the burying beetle *N. quadripunctatus* originating from Hokkaido and Tokyo, Japan. Wild adults were collected from forest habitats in both regions between June and September during 2014–2018. Beetles were collected using plastic cup traps (122 mm in diameter and 111 mm deep) baited with approximately 40 g of chicken meat. At each sampling site, ten traps were placed approximately 300 m apart on tree trunks at a height of 1.5 m and checked after 24 h. Laboratory populations were established from the field-collected individuals, and beetles from the first to fourth

laboratory generations (F1–F4) were used in the present experiments. To minimize inbreeding, mating pairs were formed between individuals from different family lineages, with ancestry traced to the original field-collected beetles. Adult beetles were maintained individually in transparent plastic containers (4 cm high, 6 cm in diameter) containing approximately 2 cm of moist peat, at 20 ± 1°C under a 14:10 h light:dark cycle. They were fed approximately 200 mg of freshly killed mealworms (*Zophobas atratus*) three times per week.

For breeding, unrelated males and females from the same population were paired in plastic containers (8 cm high, 11.5 cm in diameter) containing approximately 2 cm of moist peat and a whole mouse carcass (4.0 ± 0.5 g). The containers were maintained in a dark incubator at 20 ± 1°C. Unless otherwise specified, females and carcasses were transferred to new containers 93 h after the beginning of breeding, and males were removed at this time. Eggs remained in the original containers, allowing newly hatched larvae to be collected separately from the females and carcasses. Hatching was monitored at 2-h intervals.

In experiments involving foster mothers, larvae were introduced only after the foster mothers' own eggs had begun to hatch. This procedure was used to avoid premature killing of introduced larvae, as female burying beetles switch from infanticidal to parental behavior around the expected hatching time of their own offspring (Müller and Eggert 1990).

For analyses of relationships between reproductive and developmental traits, individuals were deliberately selected to span broad ranges of the relevant explanatory variables rather than to represent their natural distributions within each population. To generate variation in adult body size, the number of larvae reared per carcass was experimentally manipulated during laboratory breeding. These selected samples were used to examine relationships between traits rather than to estimate population-specific trait distributions. Neither brood-size manipulation nor size-based selection was applied to the adults used for the common-environment comparison of body size.

Adult pronotum width was measured using an electronic vernier caliper (Mitutoyo Corp., Japan) to the nearest 0.1 mm.

Comparison of adult body size

To determine whether the geographic difference in adult body size persisted under common laboratory conditions, we compared 289 laboratory-reared adults from 31 broods, including both males and females. These adults were produced by parents maintained and bred under standardized conditions.

Egg production and egg volume

To examine the relationships between maternal body size and egg production or egg volume, females spanning a broad range of pronotum widths were selected from each population. To collect eggs before

hatching, females and carcasses were transferred to new containers 72 h after the beginning of breeding, and eggs remaining in the original containers were collected. At 93 h, females and carcasses were transferred again, and the additional eggs were collected. This two-stage procedure reduced the likelihood that the earliest-laid eggs would hatch before collection.

The total number of eggs collected at 72 and 93 h was recorded for each female. Egg production was measured for 119 broods (57 from Hokkaido and 62 from Tokyo).

The major and minor axes of each egg were measured using light microscopy and digital image-analysis software (cellSens Standard, version 1.6; Olympus, Japan). Egg volume was calculated by assuming an ellipsoidal shape. All eggs suitable for measurement were included in the egg-volume dataset, comprising 1,207 eggs from 85 broods (472 eggs from 41 Hokkaido broods and 735 eggs from 44 Tokyo broods). Maternal pronotum width was recorded for the analyses of both egg production and egg volume.

Relationship between egg volume and hatchling size

To examine the relationship between egg volume and hatchling size, eggs were individually isolated on moist filter paper after measurement and maintained under laboratory conditions until hatching. Newly hatched first-instar larvae were collected within 1 h of emergence, and their head widths were measured using light microscopy and digital image-analysis software. Individual tracking allowed each larva's head width to be matched with the volume of the egg from which it hatched. The analysis included 200 larvae from 41 broods (100 larvae from 21 Hokkaido broods and 100 larvae from 20 Tokyo broods).

Number of offspring reared

To compare the number of offspring retained for rearing by females from the two populations, each female was initially provided with ten newly hatched larvae under standardized laboratory conditions. Larvae collected from multiple broods within each population were pooled to obtain sufficient larvae of approximately the same age.

Each female received ten larvae originating from her own population, none of which were her biological offspring. The number of larvae remaining alive with the foster mother was recorded at 24 and 120 h after introduction. All surviving larvae remained on the carcass at these observation times, allowing the number of larvae being reared to be assessed directly. The experiment included 49 foster mothers (23 from Hokkaido and 26 from Tokyo). The pronotum width of each foster mother was also recorded.

Reciprocal cross-fostering experiment

To distinguish the contributions associated with foster-parent and offspring origin to post-hatching larval growth, we conducted a reciprocal cross-fostering experiment using larvae and foster mothers from the

Hokkaido and Tokyo populations.

Each foster mother received two newly hatched larvae, one originating from Hokkaido and the other from Tokyo. Foster mothers originated from either population. Neither larva was the biological offspring of the foster mother, even when the larva and foster mother originated from the same population.

To distinguish the two larvae within each foster brood, newly hatched larvae were briefly anesthetized on ice, and the tarsus of either the right or left hind leg was removed. One larva was marked on the right hind leg and the other on the left, with the assignment randomized independently of population origin. The two larvae were introduced simultaneously to the carcass and subsequently reared together by the same foster mother.

Larval survival and body mass were assessed 120 h after introduction, when larval body mass normally approaches its maximum in *N. quadripunctatus* (Takata et al. 2013, 2014). The pronotum width of each foster mother was also recorded.

The experiment included 72 foster broods (42 with Tokyo foster mothers and 30 with Hokkaido foster mothers), each initially containing one larva from each population. Larval survival was evaluated using all 72 broods, including those in which one or both larvae died. To compare larval body mass under equivalent brood-size conditions, the analysis was restricted to broods in which both larvae survived and were weighed at 120 h. This resulted in 48 foster broods (26 with Tokyo foster mothers and 22 with Hokkaido foster mothers), comprising 96 larvae.

Relationship between larval mass and adult body size

To examine the relationship between larval mass and adult body size, larvae spanning a broad range of body masses were deliberately selected from both populations and followed to adult emergence. Larval body mass was measured at 120 h after the beginning of larval rearing. Following the larval feeding period, individuals were transferred separately to transparent containers containing moist peat and maintained until adult eclosion. After emergence, the sex and pronotum width of each adult were recorded. The analysis included 174 individuals (45 from Hokkaido and 129 from Tokyo). Each individual originated from a different parental pair, ensuring independence among observations.

Statistical analyses

All statistical analyses were performed in R version 4.4.0 (R Core Team 2024). We used generalized linear models (GLMs), negative-binomial GLMs, and linear mixed-effects models (LMMs), depending on the response variable and experimental design. Mixed-effects models were fitted using the lme4 and lmerTest packages. Continuous explanatory variables were mean-centered where appropriate.

Adult pronotum width was analyzed using an LMM with population, sex, and their interaction as fixed effects and brood identity as a random intercept.

Egg number was analyzed using a negative-binomial GLM fitted with the glm.nb function in the MASS package. A negative-binomial distribution was used because a preliminary Poisson model indicated overdispersion. Maternal pronotum width, population, and their interaction were included as explanatory variables.

Egg volume was analyzed using an LMM with maternal pronotum width, population, and their interaction as fixed effects and brood identity as a random intercept. The relationship between egg volume and hatchling head width was examined using a separate LMM, with hatchling head width as the response variable, egg volume, population, and their interaction as fixed effects, and brood identity as a random intercept.

For the standardized experiment involving ten larvae per foster mother, the numbers of larvae reared at 24 and 120 h were analyzed separately using quasibinomial GLMs with a logit link. The response was expressed as the number of larvae remaining alive versus the number no longer present among the initial ten larvae. Maternal pronotum width, population, and their interaction were included as explanatory variables. The quasibinomial distribution was used to account for extra-binomial variation among broods. The number of larvae remaining at each observation time was evaluated relative to the original ten larvae rather than treating the number remaining at 120 h as conditional on the number at 24 h.

For the reciprocal cross-fostering experiment, larval body mass at 120 h was analyzed using an LMM with foster-parent origin, offspring origin, and their interaction as fixed effects. Foster-brood identity was included as a random intercept to account for the non-independence of the two larvae reared together.

To examine differences between larvae reared by the same foster mother, we calculated the within-brood difference in body mass by subtracting the mass of the Hokkaido-origin larva from that of the Tokyo-origin larva. Positive values therefore indicated that the Tokyo-origin larva was heavier. One-sample t-tests were used to determine whether these paired differences differed from zero within each foster-parent population. A Gaussian GLM was used to test whether the within-brood difference depended on foster-parent origin. Larval survival in the cross-fostering experiment was summarized descriptively.

The relationship between larval body mass at 120 h and adult pronotum width was examined using a Gaussian GLM. Larval mass, population, their interaction, and adult sex were included as explanatory variables. No brood-level random effect was included because observations were independent at the parental-pair level.

For each analysis, we first fitted a model containing the relevant interaction term and compared it with the corresponding additive model. When the interaction was not statistically significant, we adopted the additive model and evaluated the contribution of each main effect by removing that term individually and

comparing the resulting reduced model with the additive model. When the interaction was significant, the interaction model was retained, and effects were interpreted in the context of the interaction.

Nested LMMs were compared using likelihood-ratio tests based on maximum-likelihood estimation. Selected LMMs were subsequently refitted using restricted maximum likelihood to obtain coefficient estimates. Negative-binomial models were compared using likelihood-ratio tests, whereas Gaussian and quasibinomial GLMs were compared using F-tests. Where appropriate, estimated marginal means and population-specific regression slopes were calculated using the emmeans package. Statistical significance was assessed at $p < 0.05$. To minimize observer bias, data collection and statistical analyses were conducted blind to population origin and, where applicable, foster-parent and offspring origin.

Results

Adult body size under common laboratory conditions

Adult body size differed significantly between the two populations under common laboratory conditions (Fig. 1). Tokyo-origin beetles had approximately 12% greater pronotum width than Hokkaido-origin beetles (LMM: $\chi^2_1 = 15.42$, $p < 0.001$). Mean pronotum widths were 5.02 mm in Tokyo females and 5.14 mm in Tokyo males, compared with 4.58 mm in Hokkaido females and 4.52 mm in Hokkaido males. Neither sex ($\chi^2_1 = 0.03$, $p = 0.870$) nor the interaction between population and sex ($\chi^2_1 = 1.85$, $p = 0.173$) had a significant effect on adult body size.

Egg production, egg volume, and hatchling size

Egg number increased significantly with maternal pronotum width (negative-binomial GLM: $\chi^2_1 = 5.23$, $p = 0.022$; Fig. 2a). However, after accounting for maternal body size, egg number did not differ significantly between populations ($\chi^2_1 = 2.25$, $p = 0.134$). The interaction between maternal body size and population was also not significant ($\chi^2_1 = 0.47$, $p = 0.492$).

Egg volume increased significantly with maternal pronotum width (LMM: $\chi^2_1 = 72.59$, $p < 0.001$; Fig. 2b). At a given maternal body size, Tokyo females produced eggs with approximately 5% greater volume than Hokkaido females ($\chi^2_1 = 7.99$, $p = 0.005$). The interaction between maternal body size and population was not significant ($\chi^2_1 = 0.01$, $p = 0.905$).

Hatchling head width increased significantly with egg volume (LMM: $\chi^2_1 = 11.02$, $p < 0.001$; Fig. 2c). However, after accounting for egg volume, hatchling head width did not differ significantly between populations ($\chi^2_1 = 0.16$, $p = 0.691$). The interaction between egg volume and population was also not significant ($\chi^2_1 = 2.58$, $p = 0.108$).

Number of offspring reared

When provided with ten unrelated larvae from their own population, Hokkaido females reared an average of 6.78 larvae and Tokyo females reared 7.38 larvae at 120 h (Fig. 2d). The number of larvae reared did not differ significantly between populations (quasibinomial GLM: $F_{1,46} = 1.02$, $p = 0.319$) and was not significantly associated with maternal pronotum width ($F_{1,46} = 0.54$, $p = 0.464$). The interaction between maternal body size and population was also not significant ($F_{1,45} = 0.01$, $p = 0.908$).

Similar results were obtained at 24 h, when Hokkaido and Tokyo females reared averages of 7.09 and 7.50 larvae, respectively. Neither population ($F_{1,46} = 0.43$, $p = 0.513$) nor maternal pronotum width ($F_{1,46} = 0.34$, $p = 0.564$) significantly affected the number of larvae reared at this time, and their interaction was not significant ($F_{1,45} = 0.21$, $p = 0.652$).

Effects of foster-parent and offspring origin on larval growth

In the reciprocal cross-fostering experiment, 109 of 144 larvae (75.7%) survived to 120 h across 72 foster broods. Among larvae reared by Tokyo foster mothers, survival was 71.4% (30/42) for both Hokkaido- and Tokyo-origin larvae. Among those reared by Hokkaido foster mothers, survival was 83.3% (25/30) for Hokkaido-origin larvae and 80.0% (24/30) for Tokyo-origin larvae.

Analyses of larval body mass were restricted to the 48 foster broods in which both larvae survived to 120 h. Larval mass was significantly affected by both foster-parent origin (LMM: $\chi^2_1 = 4.97$, $p = 0.026$) and offspring origin ($\chi^2_1 = 20.85$, $p < 0.001$; Fig. 3). After accounting for offspring origin, larvae raised by Tokyo foster mothers were estimated to be 21.2 mg heavier than those raised by Hokkaido foster mothers. Similarly, after accounting for foster-parent origin, Tokyo-origin larvae were estimated to be 25.5 mg heavier than Hokkaido-origin larvae. The interaction between foster-parent and offspring origin was not significant ($\chi^2_1 = 0.68$, $p = 0.410$).

Within-brood comparisons also showed that Tokyo-origin larvae were significantly heavier than Hokkaido-origin larvae reared by the same foster mother. The mean within-brood difference was 30.0 mg in broods reared by Hokkaido foster mothers ($t_{21} = 4.27$, $p < 0.001$) and 21.7 mg in broods reared by Tokyo foster mothers ($t_{25} = 3.01$, $p = 0.006$). The magnitude of this difference did not vary significantly with foster-parent origin (Gaussian GLM: $F_{1,46} = 0.66$, $p = 0.422$).

Relationship between larval mass and adult body size

Adult pronotum width was positively associated with larval body mass at 120 h in both populations (Fig. 4). However, the relationship differed significantly between populations (Gaussian GLM: $F_{1,169} = 6.15$, $p = 0.014$), with a steeper slope in Tokyo (0.00633 mm/mg) than in Hokkaido (0.00371 mm/mg). Adult sex was not significantly associated with pronotum width ($p = 0.911$).

Discussion

The persistence of geographic variation in adult body size under common laboratory conditions provides evidence that the divergence between Hokkaido and Tokyo populations of *N. quadripunctatus* is not simply a consequence of differences in their natural environments. The larger body size of Tokyo beetles previously documented in the field (Takata and Matsuo 2025) was maintained in laboratory-reared offspring produced under standardized conditions without brood-size manipulation. This suggests that the geographic divergence has an inherited component, potentially reflecting genetic differentiation between populations. Although transgenerational environmental effects cannot be entirely excluded, the common-environment results provide a basis for investigating the reproductive and developmental processes underlying this divergence. Our subsequent experiments indicate that population differentiation involves multiple traits associated with maternal investment and offspring development, highlighting the importance of considering the entire developmental process when examining the evolution of body-size variation.

Maternal investment before hatching represents one potential source of variation in offspring development. The population difference in egg volume relative to maternal body size suggests that females from the two populations differ in size-dependent reproductive allocation. Importantly, this pattern concerns the relationship between maternal size and egg investment rather than population differences in mean egg size, because females were deliberately selected to span a broad range of body sizes. Although larger eggs produced larger hatchlings as measured by head width, the relationship between egg volume and hatchling head width was similar between populations. Egg volume may nevertheless influence other aspects of offspring development, including initial nutritional reserves and subsequent growth (Fox and Czesak 2000). In *N. vespilloides*, maternal body size influences both egg size and the quality of post-hatching care, but post-hatching maternal effects can outweigh prenatal effects on offspring growth (Steiger 2013). Similarly, the effects of egg size on offspring performance can be masked by post-hatching parental care (Monteith et al. 2012). These findings suggest that the developmental consequences of maternal investment cannot be fully understood from hatchling size alone and may depend on the subsequent caregiving environment.

The allocation of resources among offspring also depends on the number of larvae retained for rearing. Because burying beetles reproduce on discrete, limited food resources, brood size can have substantial consequences for individual offspring growth (Rauter and Moore 2002). In *N. quadripunctatus*, parents can reduce brood size through filial cannibalism, preferentially eliminating later-hatching larvae (Takata et al. 2013). Moreover, experiments in *N. vespilloides* have shown that sibling competition intensifies with increasing brood size, particularly when parents provide food to their offspring (Smiseth et al. 2007). In the present study, however, we found no evidence that females from the two populations differed in the number of larvae retained for rearing when initially provided with ten larvae on standardized carcasses. Thus, differential brood-size regulation does not appear to explain the geographic difference in offspring growth under these conditions. This does not exclude the possibility that populations differ in their responses to variation in carcass size or initial brood size, but it directs attention toward other components of parental care and offspring growth.

The reciprocal cross-fostering experiment provides particularly important insight into these components. By rearing larvae from both populations together under the care of the same foster mother, we were able to separate differences associated with offspring origin from those associated with the shared post-hatching caregiving environment. The persistence of an offspring-origin effect within foster broods indicates that geographic differences in larval growth cannot be attributed solely to the identity of the caregiving parent. Such differences may arise from inherited variation in growth potential, feeding behavior, or resource utilization, although prenatal maternal effects could also contribute. Meanwhile, the independent effect of foster-parent origin indicates that the caregiving environment itself differs between populations in ways that influence larval growth. Potential mechanisms include differences in food provisioning, carcass preparation, and other parental behaviors affecting offspring access to resources (Scott 1998; Rauter and Moore 2002). Because these behaviors were not directly quantified, our results establish the contribution of parent-associated and offspring-associated factors without identifying their specific mechanisms.

These findings have implications for the evolution of parent–offspring interactions. Offspring growth in species with elaborate parental care depends not only on offspring characteristics but also on traits expressed by caregivers. Quantitative genetic studies in burying beetles have demonstrated that direct genetic effects and indirect genetic effects arising through parental care can jointly influence offspring development (Rauter and Moore 2002). Furthermore, genetic correlations between parental provisioning and offspring behaviors have been demonstrated in *N. vespilloides*, suggesting that these traits can evolve in a coordinated manner (Lock et al. 2004). Our results are consistent with geographic differentiation in traits expressed by both parents and offspring, although population-origin effects should not be equated directly with genetic effects. Interestingly, we found no significant interaction between foster-parent and offspring origin, providing no evidence that offspring performed particularly well with foster mothers from their own population. Thus, while the results indicate independent contributions associated with parents and offspring, they do not demonstrate population-specific coadaptation between caregiving and offspring traits. Whether the underlying traits have evolved through correlated genetic responses or independent evolutionary processes remains an important question.

Geographic differentiation may also involve developmental processes operating after the period of intensive parental care. Previous research in *N. vespilloides* has demonstrated a positive relationship between larval mass at dispersal and adult body size (Schrader et al. 2022). The population-specific relationship between larval mass and adult body size suggests that variation in early growth does not necessarily translate into adult morphology in the same way in both populations. Several processes could contribute to this difference, including variation in subsequent growth, the timing of developmental transitions, or allocation of resources during metamorphosis. Because individuals spanning a broad range of larval masses were deliberately selected, the observed relationships characterize developmental associations across an experimentally expanded size range rather than the natural distribution of larval body sizes. Moreover, the relationship between larval mass and adult size was examined independently of the cross-fostering experiment, so the effects associated with caregiver

origin cannot be directly linked to adult size in the present data. Nevertheless, this finding identifies an additional developmental relationship that may contribute to geographic body-size divergence and warrants further investigation.

The ecological factors underlying the observed differentiation remain unresolved. The Hokkaido and Tokyo populations occur in markedly different burying beetle communities. In Hokkaido, *N. quadripunctatus* coexists with several species of similar body size, whereas comparable competitors are less prominent in Tokyo (Takata and Matsuo 2025). Previous work also demonstrated population differences in the exclusion of heterospecific larvae, suggesting divergence in parental traits associated with these contrasting ecological environments (Takata and Matsuo 2025). Competition for breeding resources could therefore be one selective factor contributing to differentiation in body size and reproductive strategies (Scott 1998). However, ecological conditions differ between these regions in multiple respects, and comparisons involving only two populations cannot establish the selective forces responsible for the observed divergence. Investigating additional populations across environmental and competitive gradients would help distinguish the effects of local ecological conditions from other sources of geographic differentiation.

Taken together, our findings suggest that geographic body-size divergence in *N. quadripunctatus* cannot be understood solely in terms of variation in larval growth or maternal investment considered independently. Instead, multiple reproductive and developmental processes, including caregiver-associated and offspring-associated influences on larval growth, may contribute to the expression of adult body size. Identifying the genetic and physiological mechanisms underlying these population differences will be an important next step toward understanding how parental investment and offspring development jointly shape the evolution of body size.

Declarations

Acknowledgements

We appreciate Kazuya Kobayashi for facilitating the use of the Hokkaido Forest Research Station of the Field Science Education and Research Center of Kyoto University. We thank Satoshi Koyama, Adam L. Cronin, and Toshiyuki Satoh for helpful discussion.

Funding

This work was supported by JSPS KAKENHI Grant Number JP18J01880.

Ethics approval

Not applicable. This study involved insects and did not require approval from an institutional animal ethics committee under the applicable regulations.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Data availability

The datasets supporting the findings of this study have been deposited in Figshare (reserved DOI: https://doi.org/10.6084/m9.figshare.34723230). The data are available to editors and reviewers through a private review link and will be made publicly available upon publication.

Code availability

The R Markdown code used for statistical analyses and figure generation has been deposited in the same Figshare repository and is available to editors and reviewers through the private review link. The code will be made publicly available upon publication.

Authors' contributions

M.T.: investigation, data curation, methodology, formal analysis, resources, validation, funding acquisition, visualization, conceptualization, project administration, supervision, writing—original draft, writing—review and editing. Y.M.: investigation, data curation, visualization, writing—original draft. All authors approved the final manuscript and agree to be accountable for all aspects of the work.

Declaration of AI use

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist with English-language drafting and editing, refinement of R scripts for statistical analyses, and preparation of figures from the original study data. The authors retained responsibility for all scientific decisions, statistical analyses, interpretations, and conclusions. All AI-assisted text, code, and figures were reviewed and validated by the authors, who take full responsibility for the accuracy and integrity of the final manuscript.

References

1. Blanckenhorn WU, Demont M (2004) Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? Integr Comp Biol 44:413–424. https://doi.org/10.1093/icb/44.6.413

2. Chown SL, Gaston KJ (2010) Body size variation in insects: a macroecological perspective. Biol Rev 85:139–169. https://doi.org/10.1111/j.1469-185X.2009.00097.x
3. Clutton-Brock T (1991) The Evolution of Parental Care. Princeton University Press, Princeton
4. Eggert A-K, Prang MA, Capodeanu-Nägler A et al (2026) Parental care liberates juvenile growth: a common-garden test of the evolutionary benefits of care. Evolution 80:269–281. https://doi.org/10.1093/evolut/qpaf223
5. Eggert A-K, Reinking M, Müller JK (1998) Parental care improves offspring survival and growth in burying beetles. Anim Behav 55:97–107. https://doi.org/10.1006/anbe.1997.0588
6. Fox CW, Czesak ME (2000) Evolutionary ecology of progeny size in arthropods. Annu Rev Entomol 45:341–369. https://doi.org/10.1146/annurev.ento.45.1.341
7. Grubmüller E, Meier DV, Kreil M et al (2026) Parental niche construction buffers microbial and competitive challenges and drives offspring dependence in burying beetles. Proceedings of the National Academy of Sciences 123: e2617749123. https://doi.org/10.1073/pnas.2617749123
8. Jarrett BJM, Schrader M, Rebar D et al (2017) Cooperative interactions within the family enhance the capacity for evolutionary change in body size. Nat Ecol Evol 1:0178. https://doi.org/10.1038/s41559-017-0178
9. Kirkpatrick M, Lande R (1989) The evolution of maternal characters. Evolution 43:485–503. https://doi.org/10.1111/j.1558-5646.1989.tb04247.x
10. Lock JE, Smiseth PT, Moore AJ (2004) Selection, inheritance, and the evolution of parent-offspring interactions. Am Nat 164:13–24. https://doi.org/10.1086/421444
11. Monteith KM, Andrews C, Smiseth PT (2012) Post-hatching parental care masks the effects of egg size on offspring fitness: A removal experiment on burying beetles. J Evol Biol 25:1815–1822. https://doi.org/10.1111/j.1420-9101.2012.02567.x
12. Moore AJ, Brodie ED, Wolf JB (1997) Interacting phenotypes and the evolutionary process: I. Direct and indirect genetic effects of social interactions. Evolution 51:1352–1362. https://doi.org/10.1111/j.1558-5646.1997.tb01458.x
13. Müller JK, Eggert A-K (1990) Time-dependent shifts between infanticidal and parental behavior in female burying beetles: a mechanism of indirect mother-offspring recognition. Behav Ecol Sociobiol 27:11–16. https://doi.org/10.1007/BF00183307
14. Nagano M, Suzuki S (2003) Phenology and habitat use among Nicrophorine beetles of the genus *Nicrophorus* and *Ptomascopus* (Coleoptera: Silphidae). Edaphologia 1–9. https://doi.org/10.20695/edaphologia.73.0_1
15. Ohkawara K, Suzuki S, Katakura H (1998) Competitive interaction and niche differentiation among burying beetles (Silphidae, *Nicrophorus*) in northern Japan. Entomol Sci 1:551–559. https://doi.org/10.5555/19990502470
16. R Core Team (2024) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

17. Rauter CM, Moore AJ (2002) Evolutionary importance of parental care performance, food resources, and direct and indirect genetic effects in a burying beetle. J Evol Biol 15:407–417. https://doi.org/10.1046/j.1420-9101.2002.00412.x
18. Royle NJ, Smiseth PT, Kölliker M (2012) The evolution of parental care. Oxford University Press, Oxford
19. Satou A, Nisimura T, Numata H (2001) Cost and necessity of parental care in the burying beetle *Nicrophorus quadripunctatus*. Zoolog Sci 18:975–979. https://doi.org/10.2108/zsj.18.975
20. Schrader M, Jarrett BJM, Kilner RM (2022) Larval environmental conditions influence plasticity in resource use by adults in the burying beetle, *Nicrophorus vespilloides*. Evolution 76:667–674. https://doi.org/10.1111/evo.14339
21. Scott MP (1998) The ecology and behavior of burying beetles. Annu Rev Entomol 43:595–618. https://doi.org/10.1146/annurev.ento.43.1.595
22. Smiseth P, Lennox L, Moore A (2007) Interaction between parental care and sibling competition: parents enhance offspring growth and exacerbate sibling competition. Evolution 61:2331–2339. https://doi.org/10.1111/j.1558-5646.2007.00192.x
23. Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. Am Nat 108:499–506. https://doi.org/10.1086/282929
24. Steiger S (2013) Bigger mothers are better mothers: disentangling size-related prenatal and postnatal maternal effects. Proceedings Biological sciences / The Royal Society 280:20131225. https://doi.org/10.1098/rspb.2013.1225
25. Steiger S, Richter K, Müller JK, Eggert A-K (2007) Maternal nutritional condition and genetic differentiation affect brood size and offspring body size in *Nicrophorus*. Zool (Jena) 110:360–368. https://doi.org/10.1016/j.zool.2007.06.001
26. Sun S-J, Catherall AM, Pascoal S et al (2020) Rapid local adaptation linked with phenotypic plasticity. Evol Lett 4:345–359. https://doi.org/10.1002/evl3.176
27. Takata M, Hayashi S, Thomas CE et al (2014) Asynchronous hatching in the burying beetle, *Nicrophorus quadripunctatus*, maxmizes parental fitness. J Evol Biol 27:1830–1838. https://doi.org/10.1111/jeb.12433
28. Takata M, Koyama S, Satoh T, Fugo H (2013) Asynchronous hatching and brood reduction by filial cannibalism in the burying beetle *Nicrophorus quadripunctatus*. J Ethol 31:249–254. https://doi.org/10.1007/s10164-013-0373-1
29. Takata M, Matsuo Y (2025) Genetic variation among populations in parental exclusion of heterospecific larvae in burying beetles. Popul Ecol 67:183–190. https://doi.org/10.1002/1438-390X.12208
30. Takata M, Mitaka Y, Steiger S, Mori N (2019) A parental volatile pheromone triggers offspring begging in a burying beetle. iScience 19:1260–1278. https://doi.org/10.1016/j.isci.2019.06.041
31. Wolf JB, Brodie ED III, Cheverud JM et al (1998) Evolutionary consequences of indirect genetic effects. Trends Ecol Evol 13:64–69. https://doi.org/10.1016/S0169-5347(97)01233-0

Figures

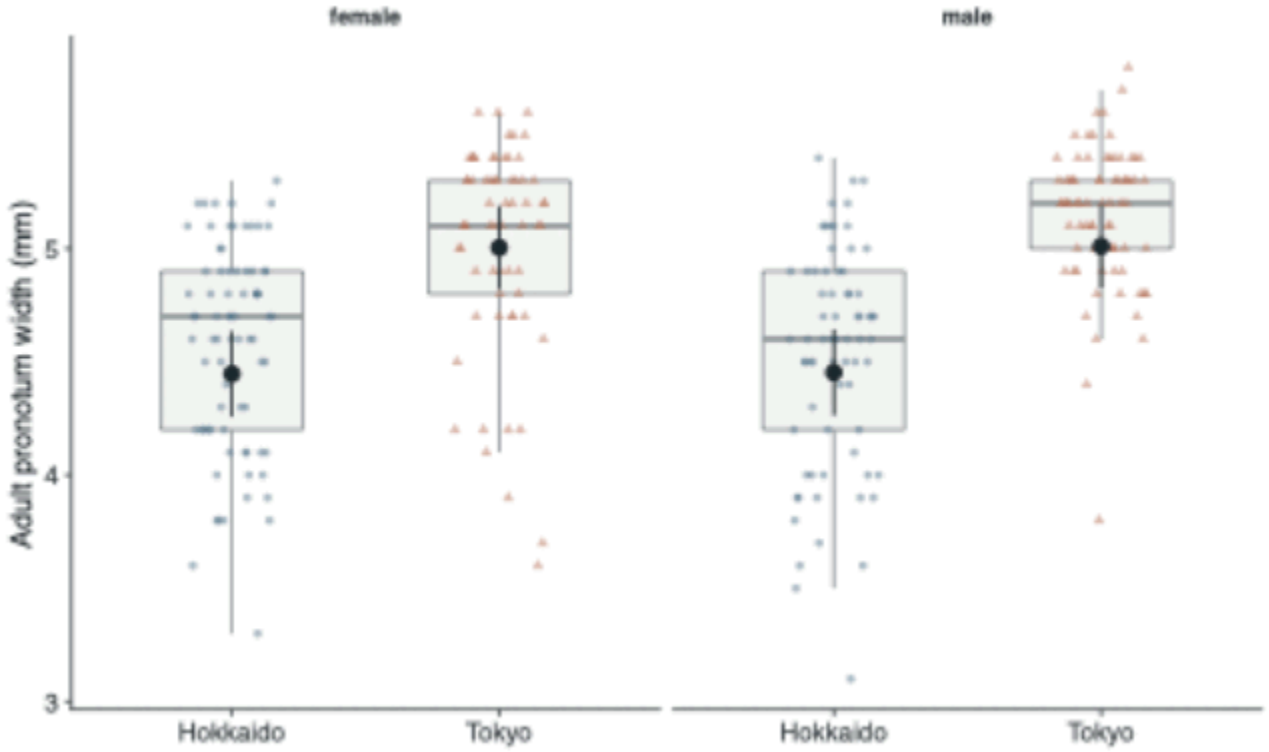


Figure 1

Adult body size under common laboratory conditions. Pronotum width of laboratory-reared adults from the Hokkaido and Tokyo populations of *Nicrophorus quadripunctatus*, shown separately for females and males. Adults were reared under standardized conditions without experimental manipulation of brood size or selection for body size. Boxes indicate interquartile ranges, horizontal lines indicate medians, and whiskers extend to 1.5 times the interquartile range. Individual observations are shown as points. Black circles and error bars represent model-estimated means and 95% confidence intervals, respectively. The analysis included 289 adults from 31 broods.

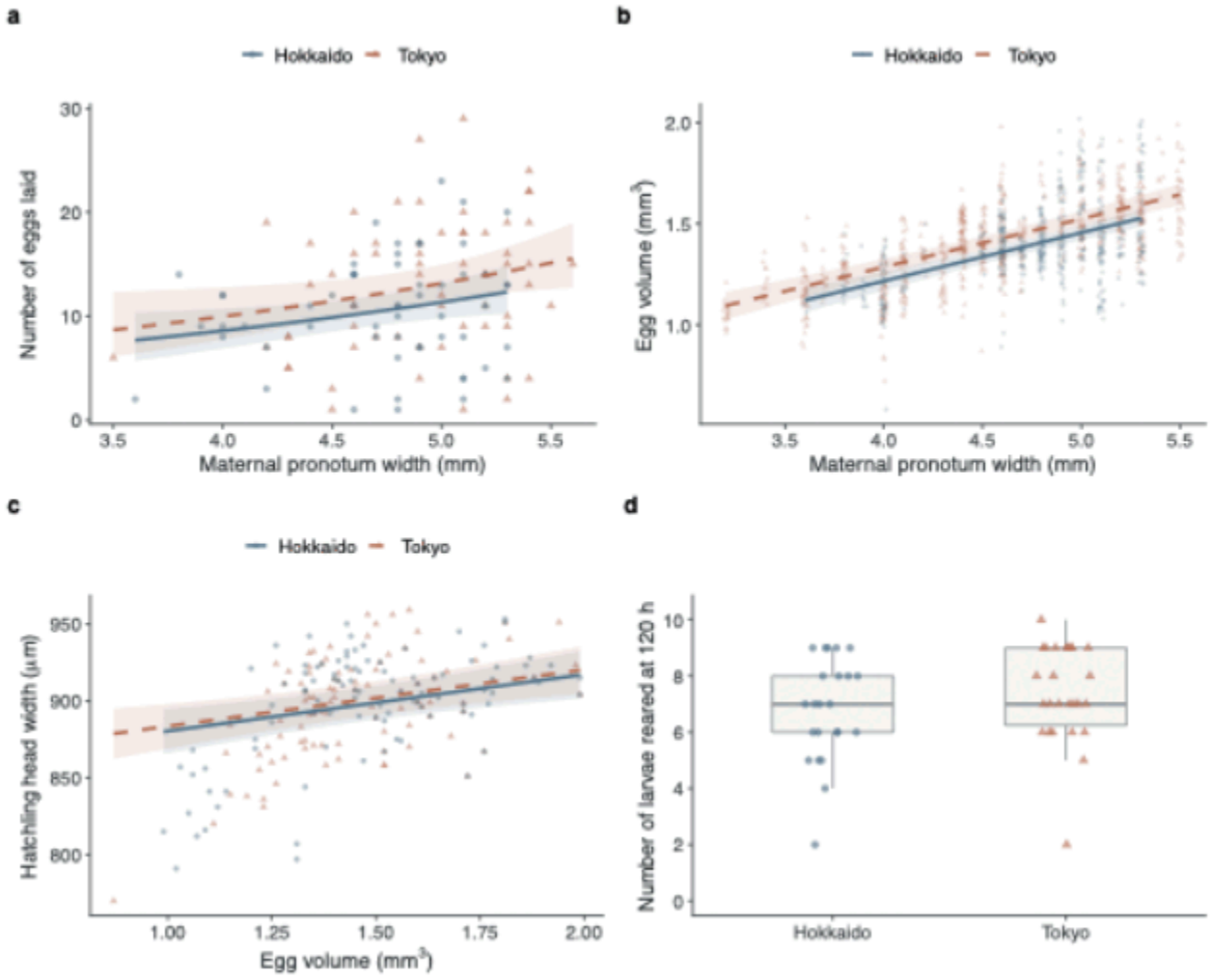


Figure 2

Reproductive investment and the number of offspring reared in the Hokkaido and Tokyo populations. (a) Relationship between maternal pronotum width and the number of eggs laid (119 broods). (b) Relationship between maternal pronotum width and egg volume (1,207 eggs from 85 broods). (c) Relationship between egg volume and hatchling head width (200 larvae from 41 broods). Points represent individual observations, lines indicate model-predicted relationships, and shaded areas represent 95% confidence intervals. (d) Number of larvae reared at 120 h after each foster mother was provided with ten newly hatched, unrelated larvae originating from her own population (49 foster mothers). Boxes indicate interquartile ranges, horizontal lines indicate medians, whiskers extend to 1.5 times the interquartile range, and points represent individual foster broods. Blue circles and solid lines indicate Hokkaido, whereas orange triangles and dashed lines indicate Tokyo.

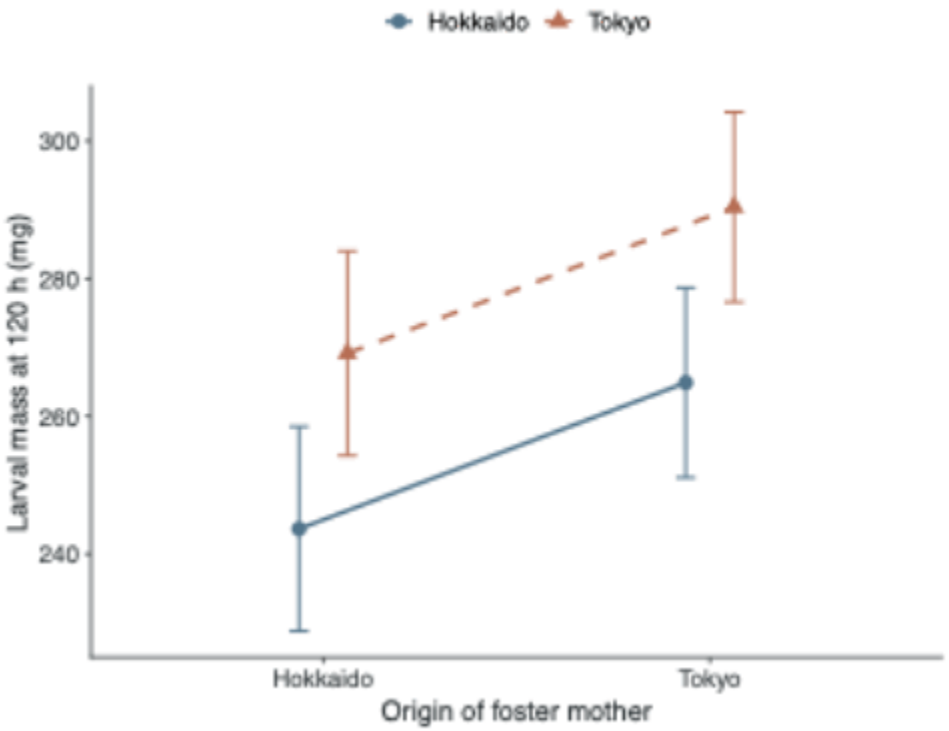


Figure 3

Effects of foster-parent and offspring origin on larval growth. Larval body mass at 120 h in a reciprocal cross-fostering experiment in which one Hokkaido-origin larva and one Tokyo-origin larva were reared together by an unrelated foster mother from either population. Points and error bars represent model-estimated means and 95% confidence intervals, respectively. Lines connect estimated means for larvae of the same origin. Blue circles and solid lines indicate Hokkaido-origin larvae, whereas orange triangles and dashed lines indicate Tokyo-origin larvae. The analysis included 96 larvae from 48 foster broods in which both larvae survived to 120 h.

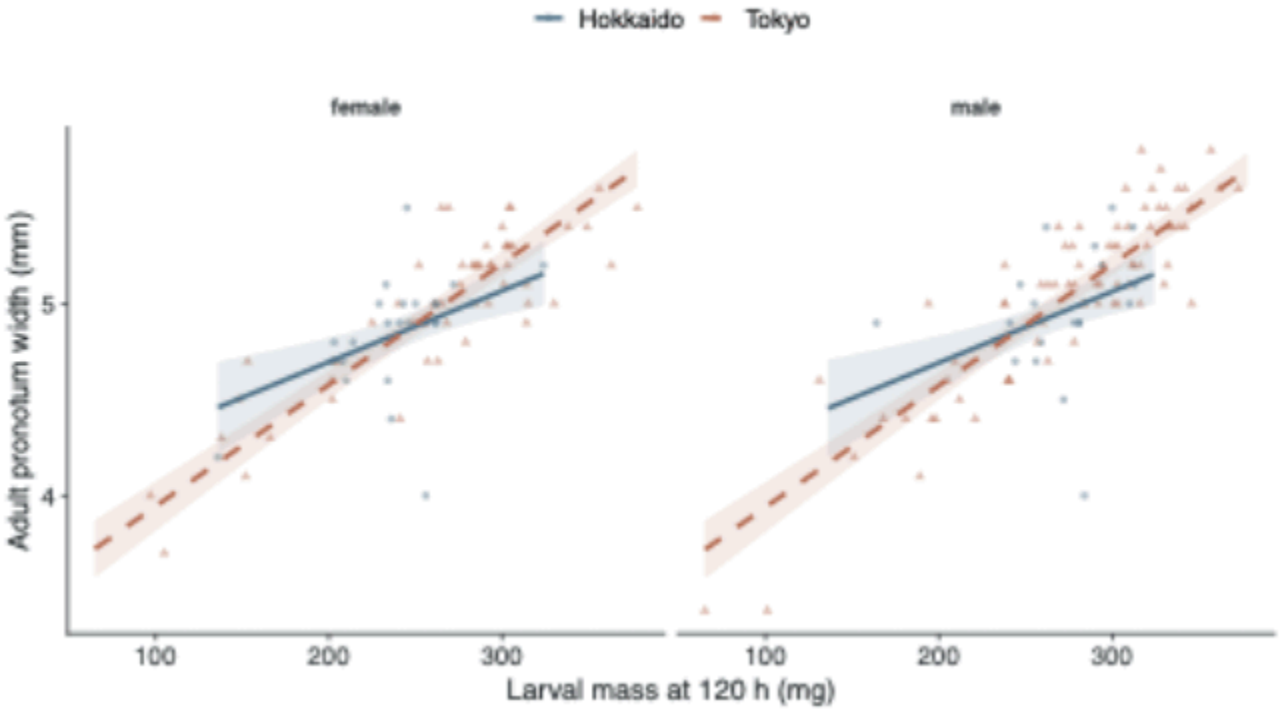


Figure 4

Relationship between larval mass at 120 h and adult body size. Relationship between larval body mass measured at 120 h and adult pronotum width in the Hokkaido and Tokyo populations, shown separately for females and males. Points represent individual observations, lines indicate population-specific relationships predicted by the fitted model, and shaded areas represent 95% confidence intervals. Blue circles and solid lines indicate Hokkaido, whereas orange triangles and dashed lines indicate Tokyo. The analysis included 174 individuals (45 from Hokkaido and 129 from Tokyo), each originating from a different parental pair.