Non-linear latitudinal cline of egg size and its consequence for larval survival in the rhinoceros beetle

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The egg size of insects often exhibits latitudinal trends, which are attributed to local adaptations to temperature or temperature-related ecological factors. Although increased egg size improves offspring fitness, the relationship between size and fitness is influenced largely by environmental variables. Additionally, there is a trade-off between the size and number of eggs if the quantity of reproductive resources is constant. Despite the key associations among offspring size, number and performance, relatively few studies have jointly considered these traits when studying the genetic differentiation of egg size. Here, we examined the effects of latitude on egg size using 14 populations of the univoltine rhinoceros beetle *Trypoxylus dichotomus* (Coleoptera, Scarabaeidae, Dynastinae) along a 1200-km latitudinal gradient under common garden laboratory conditions. We found that egg size decreased with increasing latitude in a non-linear manner. Geographical variation in egg size was independent of maternal body size, and there was no difference in female lifetime fecundity between the low-latitude (large eggs) and high-latitude (small eggs) populations. Therefore, low-latitude females invest more resources in reproduction compared with high-latitude females. We further found that the larvae of the high-latitude population showed higher mortality than those of the low-latitude population under both nutrient-poor and nutrient-rich conditions. Although the ecological factors that shape the non-linear cline of egg size were not identified in our study, geographical variation in local environments or in female reproductive strategies could have caused the latitudinal trend.

 $\label{eq:additional} ADDITIONAL\,KEYWORDS:\ fecundity-hatchling\,size-horned\,beetle-life\,history-progeny\,size-reproductive\,effort.$

INTRODUCTION

Egg size determines the initial available resources and size of offspring, strongly influencing their survival and performance, especially during early growth stages (Stearns, 1992; Fox & Czesak, 2000). While increased egg size improves offspring fitness, there is a tradeoff from a maternal perspective between the size and number of eggs produced because resources available for reproduction are limited (Smith & Fretwell, 1974; Berrigan, 1991; Stearns, 1992; Roff, 2002). Classical life-history theory suggests that females maximize their fitness by balancing the level of investment per egg with fecundity, such that the product of fecundity and survival of offspring is maximized (Smith & Fretwell, 1974; Fox & Czesak, 2000; Roff,

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2002). Progeny hatching from larger eggs generally have stronger resistance to environmental stresses, including intraspecific competition, starvation, desiccation, extreme temperature and nutritional stress (Blanckenhorn, 2000; Fox & Czesak, 2000; Fischer *et al.*, 2003). These factors affect the regimes of size–number trade-offs, resulting in genetic divergence in egg sizes under different environmental conditions (Roff, 2002). In particular, adverse conditions favour females that allocate more energy to fewer offspring. Although the effects of initial size on offspring fitness often vary between growth conditions, the effects of these variations among populations with different egg sizes have not been thoroughly examined (Potter & Woods, 2012).

The egg size of arthropods often shows latitudinal trends within a species (Fleming & Gross, 1990). Egg size increases with latitude in many species, such as

the moth Choristoneura fumiferana (Harvey, 1983), the fruit fly Drosophila melanogaster (Azevedo et al., 1996), the mosquito Wyeomyia smithii (Armbruster et al., 2001) and the damselfly Lestes sponsa (Śniegula et al., 2016b). Egg size variation is usually independent of maternal body size, which suggests adaptive differentiation (Fox & Czesak, 2000). Larger eggs are more resistant to low temperatures at higher latitudes (Harvey, 1983; Blanckenhorn, 2000; Parry et al., 2001; Fischer et al., 2003) and also have a shorter development time (Gillooly et al., 2002), particularly at high latitudes where developmental time is constrained. In D. melanogaster, evidence of a causal link between temperature and egg size has been obtained from laboratory experiments with thermal selection (Azevedo et al., 1996). In contrast, a negative relationship between latitude and egg size (the water strider Aquarius remiges; Blanckenhorn & Fairbairn, **1995**) or a flat or no cline (the dung fly Sepsis fulgens; Roy et al., 2018) has been reported in some species. The variation in latitudinal trends suggests that numerous factors other than temperature and season length (e.g. quality and abundance of resources or density of predators) that are correlated with latitudes may be responsible for latitudinal clines. However, the selective agents that shape latitudinal clines are largely unknown (Fox & Czesak, 2000); thus, the direction and form of latitudinal clines of egg size are unpredictable. In addition, although offspring size, number and performance are key life-history traits, relatively few studies have jointly considered these life-history traits when studying the genetic differentiation of egg size (Blanckenhorn & Fairbairn, 1995).

In this study, we examined the effects of latitude on egg size using 14 populations along a 1200-km latitudinal gradient (from 23.5 to 40.7°N) of the rhinoceros beetle Trypoxylus dichotomus (Coleoptera, Scarabaeidae, Dynastinae) under common garden laboratory conditions. We also compared the lifetime fecundity between small- and large-neonate populations to test the size-number trade-off. We further compared larval mortality between the smalland large-neonate populations. To determine if the advantages of a larger initial size were more evident in harsh environments, we reared neonates with high- or low-quality food. This species is univoltine (one generation per year) over its entire distribution range, which enables a simpler interpretation of the results than for species whose voltinism changes along the latitudinal gradient. The egg size of this species is positively related to maternal body size and negatively related to maternal age (Kojima, 2015). However, egg size variations among populations have not yet been explored. Gene flow was sufficiently restricted such that the larval growth rate of this species exhibited

a linear latitudinal cline in a common garden study (Kojima *et al.*, 2020). Thus, it is reasonable to expect other life-history traits, such as egg size and number, to show genetic variation among populations.

MATERIALS AND METHODS

INSECTS AND REARING CONDITIONS

Trypoxylus dichotomus is distributed throughout East Asia, including Korea, south-east China, Japan and Taiwan. It is one of the largest insects in this area, with a length of 5 cm and a mass of 10 g. A recent phylogenetic analysis of this species has indicated the presence of three distinct genetic groups (south, north, and west clusters) (Yang et al., 2021). The life cycle of T. dichotomus has been previously described by Johns et al. (2014) and Kojima et al. (2020). Briefly, adult females lay eggs in the soil in July and August, which then hatch in 13–16 days (Kojima, 2015). The egg is approximately 5 mm in diameter and 40 mg in mass. The larvae inhabit the soil humus layer, decaying wood or tree hollows and feed on decaying organic matter. After overwintering in the soil, they become pupae late the following spring. Under common garden conditions (constant 25 °C), the larval and pupal durations are approximately 200-250 and 20 days, respectively, and do not greatly vary with latitude (Kojima et al., 2020).

All experiments and insect rearing were conducted at 24.5–25 °C and 60–70% relative humidity. The temperature was close to the ambient temperature that this species usually experiences in the field during its early larval developmental stages (e.g. July and August). Ambient temperatures in July and August were not significantly different across the broad latitudinal range studied (Kojima *et al.*, 2020).

EXPERIMENT 1: GEOGRAPHICAL VARIATION OF NEONATE MASS

We examined whether neonate size changed with latitude using 11 and three populations from the North and South clusters, respectively. Detailed information on the locations of the studied populations is presented in Table 1 and Figure 1. The altitudes of the locations were 20-300 m. The adults used in this study were F1–F3 (generally F2) individuals from laboratory colonies established from six to ten wild females for each population (Kojima *et al.*, 2020). Egg collection for this experiment was performed using four to 20 females (generally four to six individuals) from each population. The number of females and the generation of females used for each population are shown in Table 1.

To obtain adults, larvae were reared individually under laboratory conditions in 430-mL (females) or

Population	Population identity	Genetic cluster	Latitude (°N)	Longitude (°E)	Locality	No. of females	Female generation(s)	No. of neonates
Aomori	1	North	40.66	141.36	Misawa City, Aomori, Japan	4	F1	51
Akita	2	North	39.65	140.58	Senboku City, Akita, Japan	6	F2	100
Kanagawa	3	North	35.61	139.47	Kawasaki City, Kanagawa, Japan	4	F2	76
Yamaguchi	4	North	34.15	131.47	Yamaguchi City, Yamaguchi, Japan	12	F1–3	203
Tsushima	5	North	34.11	129.19	Tsushima, Nagasaki, Japan	5	F2	81
Saga	6	North	33.30	130.36	Kanzaki City, Saga, Japan	6	F2	94
Kumamoto	7	North	32.85	130.87	Kikuyo Town, Kumamoto, Japan	6	F1, 2	94
Nagasaki	8	North	32.75	128.76	Fukue-jima, Nagasaki, Japan	17	F1–3	209
Kamikoshiki	9	North	31.84	129.92	Kamikoshiki-jima, Kagoshima, Japan	6	F2	74
Kanoya	10	North	31.29	130.92	Kanoya City, Kagoshima, Japan	4	F2	80
Yakushima	11	North	30.30	130.65	Yakushima, Kagoshima, Japan	20	F1–3	286
Okinawa	12	South	26.77	128.26	Okinawa-Island, Okinawa, Japan	4	F1, 2	66
Taipei	13	South	24.96	121.53	New Taipei City, Taiwan	4	F2	45
Chiayi	14	South	23.47	120.48	Chiayi City, Taiwan	6	F2	97

Table 1. Information on localities and genetic clusters (Yang *et al* 2021) of the studied populations of *Trypoxylusdichotomus*. The number of females, generation of the females and neonates used in the analysis of geographical variationin neonate size (Experiment 1) are also shown. The population identity numbers correspond to the labels used in Figure 1

860-mL (males) plastic cups filled with fermented sawdust that was commercially sold for rearing rhinoceros beetles (Dorcus Owner's shop, Osaka, Japan). During the last stage of the 3rd-instar larvae, we checked the larvae daily for pupal cells. The pupal duration was approximately 20 days, and the adults emerged from the soil and started to feed approximately 10 days after eclosion. On the day when adult activity (e.g. walking and flying) was observed, they were individually transferred to 860mL plastic cups containing wet tissue paper and insect jellies sold commercially for rearing sap-feeding beetles. Pronotal width, as an index of body size, was measured using a digital caliper. Five to 7 days later, a female and male were jointly transferred to a rectangular plastic container $(15 \times 24 \times 17 \text{ cm})$ and kept for 1 day for mating. Subsequently, the females were individually maintained in a plastic container with fermented sawdust as the oviposition substrate and insect jellies. Each male was used to

inseminate one or two females. Egg size is positively correlated with maternal body size (Kojima, 2015), which might confound the results of this experiment; therefore, we used females with prothorax widths of 18.5–22.0 mm, which corresponds to the size range of females obtained under standard rearing conditions (fig. 1 in Kojima, 2019). A previous study showed that pupal body mass was not affected by latitude, suggesting that neither Bergmann's rule nor converse Bergmann's rule applies to this species (Kojima et al., 2020). However, for the Yakushima population, smaller adults were obtained when reared under the same conditions, suggesting that the genetically determined body size of this population is smaller than that of other populations (W. Kojima, unpubl. data). Thus, we included smaller adults (e.g. 18.35, 18.36 and 18.41 mm) in this population.

Ten days later, the eggs were collected and isolated from the fermented sawdust, maintained on wet



Figure 1. Map of East Asia showing the geographical origins of *Trypoxylus dichotomus* used in the analysis of geographical variation of egg size (Experiment 1). See Table 1 for additional locality information. The North and South genetic clusters are indicated by black and grey dots, respectively. Each number corresponds to a population identity number defined in Table 1.

tissue paper, and checked daily. The egg size of this species increases as the embryos grow. As the initial egg mass is strongly and positively correlated with the neonate mass (Kojima, 2015), we used neonate mass as a substitute for egg mass in this study. Neonatal mass was measured using an electronic digital balance to the nearest 0.1 mg. The females of T. dichotomus continue to lay eggs for more than 2 months after they become active; however, the egg mass gradually decreases with age (Kojima, 2015). To minimize the effects of maternal age on egg mass, sampling was conducted using the following two rules. First, eggs laid by young females (< 15 days after they became active) were collected. Second, when females laid more than 20 eggs, the first 20 eggs were used for analysis. The order of eggs was determined by the order of the larval hatch date, assuming that the egg duration did not differ among eggs (Kojima, 2015). The sample sizes of neonates for each population are shown in Table 1.

EXPERIMENT 2: SURVIVORSHIP OF YOUNG LARVAE

We tested whether (1) larger neonates had higher survivorship within populations and (2) the survivorship of neonates was higher in populations with larger eggs. To assess the interaction between population and nutritional condition, neonates were reared under either poor or rich nutritional conditions. The fermented sawdust was used as a high-quality diet. A mixture of fermented sawdust and commercially available bark (less fermented) for stag beetle rearing (Dorcus Owner's Shop) (1:2 volume ratio) was used as the low-quality diet (Kojima, 2019). A previous study demonstrated that larvae reared using a low-quality diet developed into much smaller adults than those reared using fermented sawdust (Kojima, 2019). Based on the results of Experiment 1 (see Results), we used the Yakushima population as a 'large-neonate population', and Yamaguchi, Saga and Kumamoto populations as 'small-neonate populations'. Although these latter three sites are located to the north of Yakushima, they are not very distant (< 400 km) (Fig. 1). Thus, given that the larvae from these sites experience similar climate-mediated selective pressure to the Yakushima larvae, variation in life-history traits between the populations in common garden experiments will not be the result of adaptation to different climate conditions. The neonates obtained in Experiment 1

were randomly selected and individually transferred to 430-mL plastic cups filled with a high- or low-quality diet. The sample sizes for rich food/large neonate, rich food/small neonate, poor food/large neonate, and poor food/small neonate treated larvae were 52, 54, 100 and 129, respectively. Although the larval duration of *T. dichotomus* is 200–250 days, the majority (> 90%) of deaths occur during the1st and 2nd instars (W. Kojima, unpubl. data). Thus, we determined larval mortality at 40 days (early stage of the 3rd instar).

EXPERIMENT 3: ADULT FECUNDITY

To test the trade-off between egg number and size, we examined whether females of large-neonate populations laid fewer eggs than those of small-neonate populations. We also tested the presence of a negative relationship between egg mass and egg number. Twelve females from the large-neonate (Yakushima) population, and ten females from the small-neonate (Yamaguchi, Saga or Kumamoto) populations were randomly chosen and individually maintained in containers $(15 \times 24 \times 17 \text{ cm})$ with fermented sawdust as the oviposition substrate until death. They were provided insect jellies ad libitum. Eggs were collected every 10-12 days, and lifetime fecundity was compared between the large- and small-neonate populations. The mean values of neonate mass from the initial 16–20 eggs (the criterion for egg selection is shown in the previous section, Experiment 1) were used as indicators of egg size. We could not collect neonate mass data for six (four from Yakushima and two from small-neonate populations) out of 22 females.

STATISTICS

All analyses were performed using the R software, version 3.6.1 (R Development Core Team, 2019, Vienna, Austria). To assess the latitudinal pattern of neonate size, we fitted data on the masses of individual neonates (N = 1584) using a generalized additive mixed model (GAMM) with normal distribution and automatic smoothing, in which latitude was a smoothing parameter and maternal identity and population identity were random effects. The analysis was conducted using the gam function of the mgcv R package (Wood, 2017). We included maternal body size (pronotal width) as a covariate, for which we assumed a linear relationship with neonatal mass (Kojima, 2015). We also compared the fits of the non-linear model and linear mixed model (LMM), where maternal body size and latitude were included as explanatory variables and maternal identity and population identity as random factors, using an F test.

To test the relationship between neonate mass and survivorship, a binomial generalized linear mixed model (GLMM) was used, where population (small- or large-neonate), nutritional treatment (high- or lowquality diet), and the interaction between population and nutritional treatment were entered as explanatory variables (Model 1). Maternal identity was included as a random variable. We constructed another GLMM (Model 2), in which neonate mass was added as an explanatory variable to Model 1 to test whether the survival advantage of Yakushima larvae was explained by their initial larger size.

Lifetime fecundity was compared between the small- and large-neonate populations using a linear model. Maternal body size and population (small or large neonates) were used as explanatory variables. The relationship between the mean neonate mass and egg number was also analysed by adding the mean neonate mass to the explanatory variables in the same model.

RESULTS

EXPERIMENT 1: GEOGRAPHICAL VARIATION OF NEONATE MASS

In the comparison of the linear model (LMM) and non-linear model (GAMM), the GAMM described the data significantly better than the LMM (F = 9.77, P < 0.001). The GAMM results suggested that neonate mass changed significantly with latitude (effective d.f. = 4.46, residual d.f. = 1484.7, F = 25.2, P < 0.001), and the relationship between neonate mass and latitude was non-linear (Fig. 2). Neonate mass was not significantly related to female body size (prothorax width) (estimate = 0.558, t_{1572} = 1.648, P = 0.0995). There was no significant difference among the neonate masses of the three populations from the southern cluster (Okinawa, Taipei and Chiayi, $\chi_{2,202}^{2} = 2.260$, P = 0.323, mixed ANOVA). The neonate mass of the southernmost population from the North cluster (Yakushima) was also not significantly different from that of the South cluster populations $(\chi_{1.486}^2 = 0.055,$ d.f. = 1, P = 0.814, mixed ANOVA). However, the neonate mass decreased linearly from approximately 30 to 35°N, whereas from 35 to 40°N (northernmost area of distribution of the North cluster), the neonate mass was invariable (Fig. 2).

EXPERIMENT 2: SURVIVORSHIP OF YOUNG LARVAE

The results of Model 1 showed that the mortality of larvae of small-neonate populations was significantly higher than that of large-neonate populations (Table 2; Fig. 3A). Food treatment and the interaction between food treatment and population (large or small neonate) did not significantly affect mortality (Table 2).



Figure 2. Relationship between latitude and mean $(\pm$ SE) neonate mass of *Trypoxylus dichotomus*. The curve and 95% confidence intervals estimated by GAMM are shown. Black and grey points represent the North and South genetic clusters, respectively.

Table 2. Results of binomial GLMMs for survival of*Trypoxylus dichotomus* larvae

	Explanatory variable	$\begin{array}{c} \text{Logistic} \\ \text{Regression} \\ \chi^2 \end{array}$	d.f., residual d.f.	Р
Model 1	Food quality	2.45	1, 330	0.118
	Population	7.49	1,330	< 0.01
	Food quality × Population	0.165	1, 330	0.685
Model 2	Food quality	2.14	1,329	0.144
	Population	1.30	1,329	0.254
	Food quality × Population	0.191	1, 329	0.662
	Initial mass	12.5	1,329	< 0.001

In Model 2, the initial neonate mass was significantly positively related to larval survival (Table 2; Fig. 3B). Population (small- or large-neonate populations), food treatment, and the interaction between food treatment and population did not significantly affect larval survival, in contrast to the results of Model 1 (Table 2).

EXPERIMENT 3: ADULT FECUNDITY

We compared lifetime fecundity between the small- and large-neonate populations. Lifetime fecundity was not significantly different between the large- and small-neonate populations (Fig. 4, estimate = 30.63, t_{19} = 1.375, P = 0.185). Lifetime fecundity was positively related to female body size, but the relationship was not statistically significant (estimate = 22.46, t_{19} = 1.966, P = 0.064).



Figure 3. Results of the experiment for survivorship of larvae of *Trypoxylus dichotomus* for 40 days after hatching (Experiment 2). A, mortality of larvae of low-latitude (large-neonate) and northern (small-neonate) populations of the North cluster when provided with a high- or low-quality diet. B, the relationship between neonate mass and proportion that survived. Black and grey curves indicate the fitted values and their 95% confidence intervals, respectively. Based on statistical results (Table 2), all data are pooled.

To test the trade-off between egg mass and number, we added mean neonate mass as an explanatory variable to the model shown above. Lifetime fecundity was not significantly related to mean neonate mass (estimate = 3.90, t_{12} = 1.386, P = 0.191). Furthermore, lifetime fecundity was not significantly related to maternal body size (estimate = 9.53, t_{12} = 0.603, P = 0.557) or population size (estimate = -23.47, t_{12} = -0.554, P = 0.590).

DISCUSSION

Positive relationships between egg size and latitude have been reported for many arthropod species (Harvey, 1983;



Figure 4. Relationship between maternal body size (pronotum width) and egg number of high-latitude (small-neonate; black points) and low-latitude (large-neonate; grey points) populations of *Trypoxylus dichotomus*.

Ayres & Scriber, 1994; Azevedo et al., 1996; Armbruster et al., 2001; Bauerfeind et al., 2018). We showed that neonate mass (an indicator of egg size) was larger in the low-latitude populations of *T. dichotomus* than in the high-latitude populations, in contrast to the general trends seen in arthropods. A previous study suggested that the female body size of this species was positively correlated with egg size (Kojima, 2015); however, the latitudinal trend in egg size was significant when the effects of maternal body size were experimentally and statistically controlled. Therefore, the larger eggs in the low-latitude populations were not attributable to larger maternal body size. Although the genetically determined body size of this species does not exhibit a latitudinal cline (Kojima et al., 2020), that of Yakushima females (the southernmost population in the North cluster) was the smallest among all populations (W. Kojima, unpubl. data). Nevertheless, Yakushima females produced larger eggs than any other population in the same genetic cluster.

We also found greater survival rates of larvae in the Yakushima population than those from highlatitude (small-neonate) populations irrespective of diet quality, suggesting that producing larger eggs is beneficial to females in terms of offspring survival. Possible alternative explanations for the survival advantages of Yakushima larvae include variations in the relative egg composition (Bauerfeind *et al.*, 2018) and immune functions (Meister *et al.*, 2017). However, these explanations fail to satisfactorily account for the findings that (1) neonate survival during the early developmental stage was positively related to initial body mass, and (2) the effect of population on neonate survival became non-significant after statistically controlling for the effect of initial size (Model 2 in Table 2). Given that a previous study demonstrated that neonates from larger eggs developed into larger adults in *T. dichotomus* (Kojima, 2015), the initial size of this species probably affects multiple fitness components across its entire life history.

Egg size is not only genetically determined but also influenced by environmental factors that females experience during the developmental stages of insects (Azevedo *et al.*, 1996; Blanckenhorn, 2000; Armbruster *et al.*, 2001; Fischer *et al.*, 2003; Bauerfeind *et al.*, 2018). However, as the females used in our study were F1–F3 individuals reared under common garden conditions, geographical variation in egg size is probably based on genetic effects. Quantitative genetic analysis with a full-sib/half-sib design is required to further understand the genetic effects on egg size. For example, the egg volume of some populations of the damselfly *Lestes sponsa* expressed significant values of additive genetic variance (Śniegula *et al.*, 2016a).

We showed that egg size decreased rapidly between 30 and 35°N and was relatively invariable at both lower (25–30°N) and higher latitudes (35–40°N). Such a non-linear cline of egg size has not been reported in insects, except for the damselfly L. sponsa (Śniegula et al., 2016b), partly because non-linearity is difficult to detect by comparison with smaller numbers (e.g. fewer than five) of populations. We acknowledge that population sampling between 35 and 40°N was insufficient (Figs 1, 2), making it difficult to determine whether the cline is flat in this latitudinal range. The ecological factors that shaped the latitudinal cline of egg size in T. dichotomus were unclear in our study. The non-linear cline of life-history traits is frequently associated with variations in voltinism along latitudes (Kivelä et al., 2011; Välimäki et al., 2013). However, T. dichotomus was univoltine throughout its distribution. Moreover, as low temperatures and strong time constraints at high latitudes generally favour larger eggs over smaller ones (Fox & Czesak, 2000), these factors are unlikely to explain our results. Gene flow may be another potential explanation for the geographical patterns of egg size. Given that gene flow generally prevents genetic differentiation and local adaptation along environmental gradients (Kawecki & Ebert, 2004; Bachmann et al., 2020), a flat (no) cline would occur in the latitudinal range. This hypothesis appears unlikely in T. dichotomus considering that a previous study found a strong latitudinal gradient of genetically based larval growth rates from 23.5 to 40.7°N (Kojima et al., 2020).

If female reproductive investment is constant among populations, we would expect a negative relationship between egg size and number, resulting in geographical differentiation of egg size. However, a negative relationship between egg size and number was absent, and furthermore, female lifetime fecundity of the highlatitude population was no greater than that of the low-latitude population (Yakushima). We attribute this apparent absence of the size-number trade-off to the difference in the quantity of resources allocated to reproduction (Armbruster et al., 2001; Bauerfeind et al., 2018). Females of low-latitude populations of T. dichotomus probably invest more resources in reproduction than high-latitude populations, and consequently produce larger eggs. However, our results should be interpreted with caution because females were provided with a diet *ad libitum* during the experiment. The trade-off between size and number may be obscured by additional resources. Lifetime fecundity may also be influenced by longevity, which was not examined in the present study. Moreover, the females we used did not experience winter diapause during the larval stage, which may have altered resource allocation.

In summary, we found a non-linear latitudinal cline in the egg size of *T. dichotomus*. Taken together with previous studies on geographical variation in egg size (e.g. Harvey, 1983; Ayres & Scriber, 1994; Azevedo *et al.*, 1996; Armbruster *et al.*, 2001; Bauerfeind *et al.*, 2018), there might not be a universal intraspecific latitudinal trend across insects; therefore, latitudinal patterns cannot be explained by a single common environmental factor, such as temperature. In addition, understanding the causes of egg size clines requires consideration of not only selective pressures directly acting on egg size but also resource allocation strategies of females because of the complex association among multiple life-history traits, including egg size, egg number and offspring performance.

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DATA AVAILABILITY

All data generated for this study are provided in the Supporting Information (Tables S1 and S2).

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Egg size.Table S2. Larval size.