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ORIGINAL ARTICLE

Latitudinal cline in reproductive traits in the red flour beetle *Tribolium castaneum*

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Abstract

Several previous studies have reported geographic variation and/or latitudinal clines of morphological sexual characteristics, but there are few studies that consider reproductive traits that are not morphological. Here, we measured the proportion of females fertilized by males, frequency of reproductive failure in males, and number of female copulations of the red flour beetle *Tribolium castaneum* collected from fields in Japan to investigate the relationship between reproductive traits and latitude. Our results show substantial differences in the reproductive traits of both sexes among field populations. We identified latitudinal clines for reproductive traits in males, but not females. Moreover, female, but not male, reproductive traits were correlated with body size. Our study suggests that selection for male reproductive traits varies with latitude in *T. castaneum*.

Significance statement

It has been established that latitudinal gradients are frequently observed in the traits of numerous animal species, of which body size is a prime example. Analogously, latitudinal gradients have been documented in the reproductive traits of several animal species, indicating that sexual selection may be influenced by latitude, although the specific understanding thereof remains elusive. In this study, we quantified the male and female reproductive traits and analyzed their relationship with latitude in the red flour beetle *Tribolium castaneum*. Our findings indicate that male mating efficacy was superior at lower than at higher latitudes, but no discernible latitudinal gradient was observed in female mating frequency. These results imply that selective pressure on males of this species is stronger at lower than at higher latitudes.

Keywords Sexual selection · Sexual conflict · Geographic variation · *Tribolium castaneum*

Introduction

The evolution of sexual traits may be influenced by food availability, population density, and predation risk (Svensson et al. 2004). Therefore, it is predicted that sexual selection differs according to environmental conditions.

Environmental conditions differ based on geography (e.g., Bergmann 1848; Blanckenhorn and Demont 2004; Blanckenhorn et al. 2006; Hut et al. 2013; Leocadio-Miguel et al. 2017; Matsumura and Miyatake 2023), and these differences predict geographic variation in traits via differences in food conditions and population density. Previous studies have reported geographic variation in traits of several animal species (Bergmann 1848; Blanckenhorn et al. 2006), such as in the field cricket *Teleogryllus emma*, where individuals located at higher latitudes demonstrated a shorter developmental period than those from lower latitudes (Masaki 1967). Moreover, body size shows geographic variation in various animal taxa (Cushman et al. 1993; Sota et al. 2000; Timofeev 2001; Ashton 2002; Belk and Houston 2002; Ashton and Feldman 2003; Adams and Church 2008; Estlander et al. 2017). This body size difference often depends on the latitude; a positive relationship between body size and latitude has been reported in various species (Blanckenhorn and Demont 2004; Blanckenhorn et al. 2006). Therefore, life history traits and body size may differ based on the geographic location due to habitat environmental factors.

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Since sexual selection is dependent on environmental factors (Svensson et al. 2004), these traits may demonstrate geographic variation and/or latitudinal cline.

Geographic variation in sexually selected traits has been reported. For example, in Japanese rice fish *Oryzias latipes*, males at lower latitudes display increased mating activity than males at higher latitudes (Fujimoto et al. 2015). In the false blister beetle *Oedemera sexualis*, males possess large hind legs that function as a female-grasping apparatus, and males from lower latitudinal areas have more developed legs than those from higher latitudinal areas (Satomi et al. 2019). In the New Zealand giraffe weevil *Lasiorhynchus barbicornis*, the overall mean body and weapon size increased at higher latitudes which is consistent with Bergmann's rule; however, there was a decline in the allometric slope (Painting et al. 2014). The latitudinal clines of the reproductive traits observed in these studies may be influenced by temperature which affects copulatory behavior (reviewed in Colinet et al. 2015). For example, in the adzuki bean beetle *Callosobruchus chinensis*, male mating activity increased at higher than at lower temperature (Katsuki and Miyatake 2009). Thus, mating activity is expected to increase in males at lower than at higher latitudes. Moreover, in lower latitudinal areas where there is a longer reproductive season, the operational sex ratio is expected to be male-biased, suggesting increased sexual selection pressure (Fujimoto et al. 2015). Furthermore, in many species, sexual selection intensity depends on the population density (Conner 1989; French and Cade 1989; Arnqvist 1992; Bertin and Cézilly 2005). For example, because the population density of *O. sexualis* is higher at lower than at higher latitudes, the previous result suggests that the increased sexual selection for male hind legs is positively correlated with population density (Satomi et al. 2019). Although previous studies have reported on the geographic variation and/or latitudinal cline of male sexual traits by considering the morphological traits (e.g., Fujimoto et al. 2015; Satomi et al. 2019), there are few studies that consider the non-morphological reproductive traits.

Geographic variation in reproductive traits is observed in females such as a latitudinal cline in fecundity was demonstrated in *T. emma* where females showed higher fecundity at lower than at higher latitudes (Masaki 1967). Selection pressure for male reproductive traits also influences female reproductive traits (Lande and Arnold 1983; Andersson and Iwasa 1996; Mead and Arnold 2004). Furthermore, a previous study conducted experimental evolution under different operational sex ratios (OSRs, female:male, 9:1 or 1:6) in *T. castaneum* and examined the reproductive fitness consequences (Michalczyk et al. 2011). After 20 generations of selection, females from female-biased OSRs had decreased reproductive fitness after multiple mating events. In contrast, females from the male-biased OSRs showed no change in reproductive fitness after multiple mating events. These results suggest that higher sexual selection intensity for males may be influenced by the evolution of female traits via counter-adaptation for sexual conflict (i.e., sexually antagonistic co-evolution). Therefore, if male mating activity is higher in lower latitudinal areas, females from lower latitudes are expected to have an increased number of copulations than those from higher latitudinal areas. It is possible that changes in female reproductive traits depend on environmental factors which may influence the evolution of male reproductive traits. For example, a previous study suggested that female body size differs among field populations in different environments, and these differences in female body size can alter male mating traits and tactics of the ground beetle *Carabus japonicus* (Okuzaki 2021). Thus, it is important to examine the male and female traits simultaneously when examining the geographic variation in reproductive traits. However, few studies have investigated geographic variation and/or latitudinal cline in the intensity of female polyandry (Hill 1994; Endler 1995).

In addition, latitude-dependent temperatures may affect how often females mate and empirical studies have shown that high temperature conditions lead to sterility in insects (Sutter et al. 2019; Vasudeva et al. 2021). Therefore, in high-temperature environments, females risk reproductive failure through copulation with sterile males. Under these conditions, it has been suggested that polyandry is adaptive as a bet-hedging strategy to avoid reproductive failure (Yasui 2001; Yasui and Garcia-Gonzalez 2016; Matsumura et al. 2021). Studies on the oriole have shown that in environments where males are exposed to high temperatures, female polyandrous mating increased their reproductive success (Vasudeva et al. 2021). Therefore, in females living in low latitudes where temperatures are high, polyandry is expected to evolve because males are more likely to be sterile.

Okuzaki (2021) suggested that geographic variation in sexual traits may have been indirectly caused by geographic variation in body size. Therefore, it is necessary to examine the body size in comparison with the reproductive traits between populations.

Here, we investigate whether the proportion of females fertilized by the male, frequency of reproductive failure by the male, and number of female copulations differed between the field populations and displayed latitudinal cline in *T. castaneum.* Abe et al. (2021) reported the latitudinal cline of the circadian rhythm of *T. castaneum*, suggesting that gene flow has not occurred between the field populations in Japan. *T. castaneum* is a suitable insect for conducting this study because it is widely distributed in Japan (Abe et al. 2021). In *T. castaneum*, there are differences in the male and female reproductive traits within a population (Fedina and Lewis 2008). We hypothesized that if the sexual selection intensity for this beetle was higher in the lower latitudinal area, the proportion of females fertilized by the male and number of female copulations would decrease with latitude.

Materials and methods

Insects

T. castaneum adults were collected from 19 locations in Japan (Fig. 1; Table S1) and Table S1 shows the latitude and longitude of each sampling location. The northernmost site was the Akita Prefecture (40°18′N, 140°05′E), and the southernmost site was the Okinawa Prefecture (26°25′N, 127°69′E) (Table S1). Collections were performed in 2016 and 2017. A previous study (Ridley et al. 2011) reported that the *T. castaneum* beetle is capable of flying approximately 1 km. Although Fig. 1E and F appears to be adjacent on the map, the length between E and F is approximately 60 km; thus, it is expected that movement between these collection sites is difficult for this beetle. In this study, rice bran storage areas adjacent to a rice-polishing machine served as collection sites for *T. castaneum*. This is where rice bran that is discharged from the rice-polishing machine is stored, and such places exist in various regions of Japan. Although there are some differences among the locations, all rice bran storage sites had sufficient bran to serve as food for *T. castaneum* because humans frequently use the rice milling machines. Therefore, large food condition differences are not expected among the rice bran storage sites. Each beetle was reared in an incubator (Sanyo, Tokyo, Japan) and maintained at 25 °C with a 16-h/8-h light/dark cycle (light on at 07:00; off at 23:00). The provided food was a mixture of whole wheat grain (Nisshin Seifun, Tokyo, Japan) with brewer's yeast (Asahi Beer, Tokyo) (whole wheat grain:brewer's yeast ratio, 16:1). This rearing environment was determined following a previously described study method (e.g., Miyatake et al. 2004; Miyatake et al. 2008; Matsumura and Miyatake 2015). In this study, 2–3 generational offspring of the field-caught generation were used. After pupation, the sexes were separated based on their morphological characteristics (Park 1934).

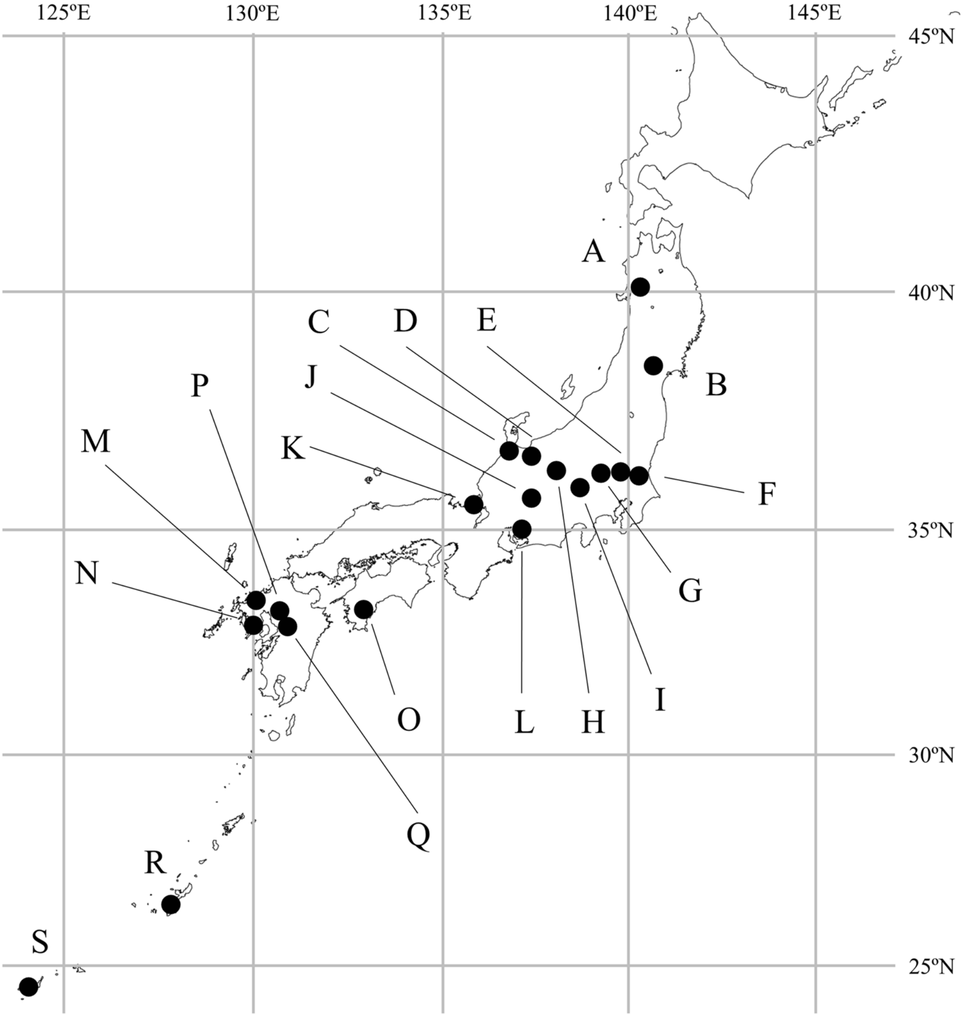

Figure 1 Collection sites from field population of *T. castaneum* in Japan

The body size was measured by photographing adult *T. castaneum* using a microscope (SZ61, Olympus, Tokyo) and camera (AUSB3-4203K, ARMSSYSTEM, Tokyo, Japan). The dorsal side of the adult beetle was photographed from directly above. The body length (head to abdomen) and prothorax width of each beetle (sample size: 10–20 beetles from 17 populations; total = 303 beetles) were measured using ImageJ software (Rasband 2011).

Sample sizes in each experiment ranged from 10 to 30 individuals in each population, although the body size of several populations could not be measured (see Table S1). Thus, some populations were excluded from the results because collections did not provide sufficient samples (Table S1).

Reproductive traits

The proportion of females fertilized by males and the frequency of male reproductive failure of *T. castaneum* from the field population was measured. Males (24–35 days old, virgin) were randomly collected from each population and marked with white paint on the middle of their elytra to distinguish the sex (sample size: 15–30 males from each population; total = 328 males). This method has been used in previous studies with *T. castaneum* (e.g., Miyatake et al. 2009; Matsumura and Miyatake 2015). Males were individually placed into a Petri dish (35 mm in diameter, 10 mm in height) with sufficient food as described above that would allow them to survive for an extended period (adult lifespan approximately 1 year). Five females (24–35 days old, virgin) from the same population as the respective male were also placed into the Petri dish (i.e., male:female = 1:5). Each male was allowed to mate with the females for 24 h. Because males of this species fail to mate at a relatively high rate (Tyler and Tregenza 2013), the number of male copulations was not recorded in this experiment. Several previous studies have used a similar 24-h mating period (e.g., Bernasconi and Keller 2001; Michalczyk et al. 2011). Each female was then moved to a separate Petri dish (35 mm in diameter, 10 mm in height) with sufficient food as described above that would allow them to survive for an extended period. After 10 days, the females were removed from the Petri dish to avoid cannibalism through excess oviposition. There are individual oviposition differences in this species and a shorter period, such as 5 days, may include females that were fertilized but did not yet oviposit (personal observation). Therefore, the oviposition period was extended to 10 days. All Petri dishes were maintained in the incubator as described above for 30 days. Whether an adult beetle was present in each Petri dish determined fertilization success. To determine the proportion of females fertilized by the male, we recorded the proportion of fertilized and unfertilized females per male. Moreover, if a male did not fertilize any of the five females, it was recorded as a “reproductive failure male.”

To measure the number of female copulations females (24–35 days old, virgin) from each population (sample size: 10 females from 13 populations; total = 130 females) were individually placed into a Petri dish (50 mm in diameter, 10 mm in height). Five males (24–35 days old, virgin) from the same population as the respective female were also placed into the Petri dish (i.e., male:female ratio = 5:1). To distinguish the sex of the beetles, the male elytra were marked with white paint as described above. The mating system of *T. castaneum* is successful when the male mounts the female and inserts their genitalia by rubbing the female body with his legs (rubbing behavior). In this observation, successful mating was determined when the male mounted the female and male rubbing behavior was present. Because it was difficult to observe whether the male inserted their genitalia into the female during mating, this was not recorded. The mating behavior of the beetles was observed in a Petri dish for 30 min and the number of copulations of the female mating with the males was observed. In this observation, we did not distinguish whether the female repeatedly mated with the same male or with different males. A previous study reported that females of this species often mate with the same male many times and are not disadvantaged by this (Pai and Yan 2020). Therefore, how many times the female mated with a particular male was not measured, but rather how many times the female mated. All observations were

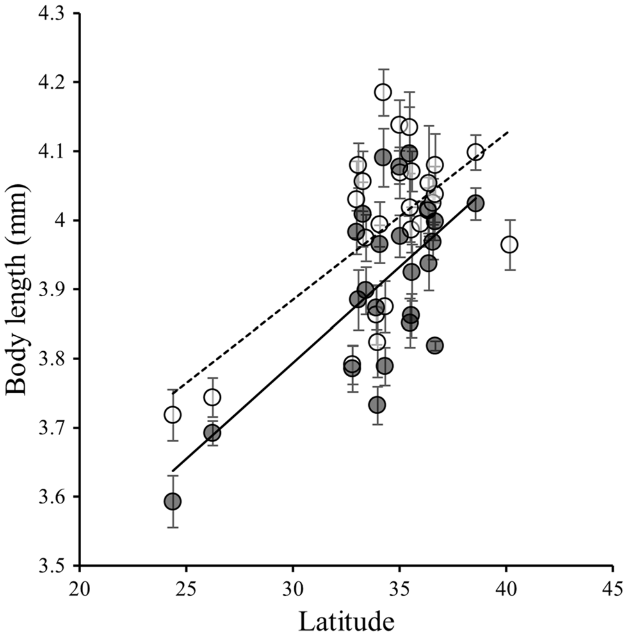

Figure 2 Relationship between body length and latitude of male (black circle, solid line) and female (white circle, dashed line) in *T. castaneum*

Table 1 Results of MANOVA for body length and prothorax width in *T. castaneum*

Factor	*d.f.*	*F*	*p*
Latitude	1	114.30	< 0.0001
Sex	1	21.96	< 0.0001
Latitude*sex	1	0.99	0.3193
Error	479		

conducted by one individual in a room maintained at 25 °C between 12:00 and 19:00.

Statistical analysis

Multivariate analysis of variance (MANOVA) was conducted for the body length and prothorax width of *T. castaneum* to determine the relationship between body size, latitude, and sex. Because there was a significantly positive correlation between body length and prothorax width ($F_{1,479}$ = 482.79, $p < 0.0001$), only the body length was used for subsequent analyses. To determine whether the proportion of females fertilized by the male varied according to population and latitude, the generalized linear model (GLM) with a binomial distribution (link = logit) was used and the proportion of females fertilized by the male (number of fertilized females/5) was used as a response variable. To determine whether the frequency of reproductive failure varied according to population and latitude, the GLM with a binomial distribution was used and the frequency of reproductive failure males (females were not fertilized) was used as a response variable. The population and latitude were analyzed separately as explanatory variables when analyzing the number of female copulations, and the GLM with gamma distribution (link = log) was used. Similarly, the population and latitude were analyzed separately as explanatory variables and added +1 to the number of female copulations to fit the gamma distribution. Spearman's rank correlation coefficient was used to test the relationship between the proportion of females fertilized by the male, frequency of reproductive failure by the male, and number of female copulations, with the body length. All statistical analyses were performed using R ver. 3.4.3 (Core 2017) and the *car* ver. 3.0.11. program (Fox and Weisberg 2018).

Results

The body length of the northernmost population was about 0.5 mm longer than that of the southernmost population (Fig. 2). MANOVA revealed that the body size significantly increased with latitude, and *T. castaneum* females were significantly larger than males (Fig. 2, Table 1). The proportion of females fertilized by the males from the 18 field populations was significantly

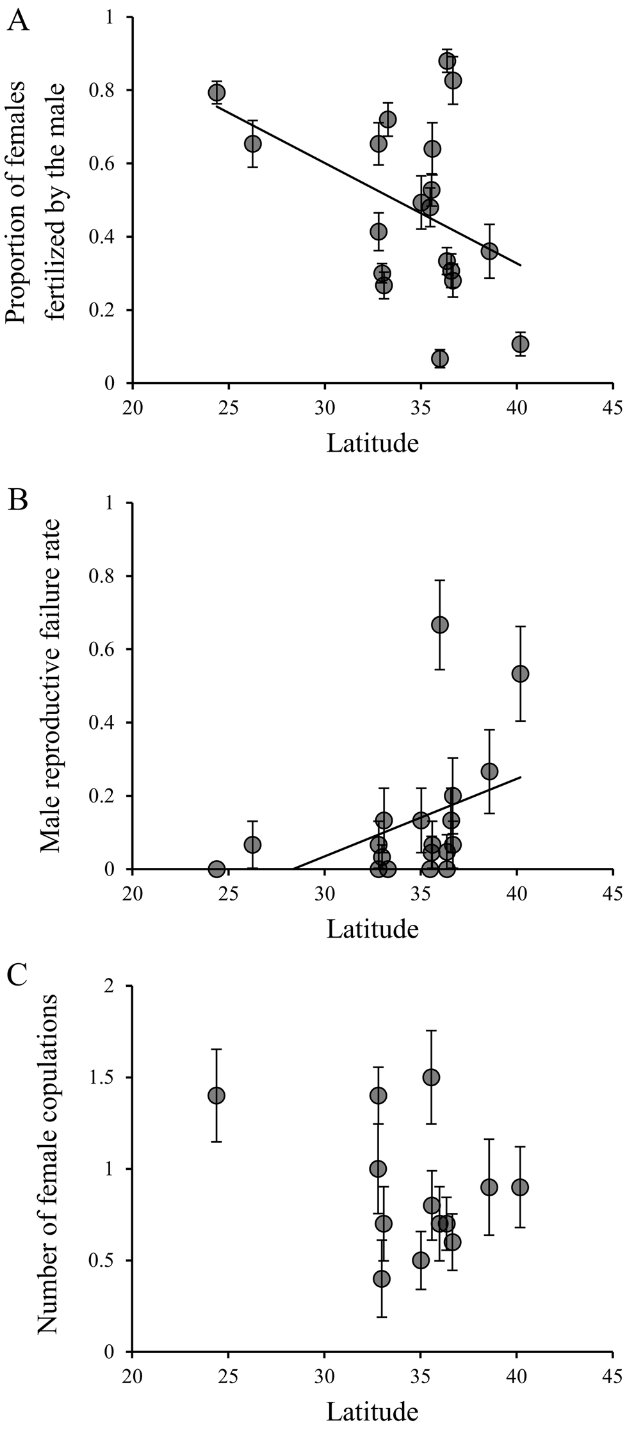

Figure 3 Relationship of latitude with proportion of females fertilized by the male (**A**), frequency of reproductive failure male (**B**), and number of female copulations (**C**) in field population. Error bars showed standard error

Table 2 Results of GLM for male and female reproductive traits

Trait	Factor	*d.f.*	χ^2	*p*
Proportion of females fertilized by the male	Population	18	381.44	< 0.0001
	Error	309		
	Latitude	1	86.34	< 0.0001
	Error	326		
Frequency of reproductive failure male	Population	18	73.32	< 0.0001
	Error	309		
	Latitude	1	26.97	< 0.0001
	Error	326		
Number of female copulations	Population	12	29.09	0.0038
	Error	117		
	Latitude	1	3.4	0.0651
	Error	128		

different between the populations (Fig. 3A, Table 2). The proportion of females fertilized by the males of the northernmost males was below 20%, and that of the southernmost males was approximately 80% (Fig. 3A). There was a significant effect of latitude on the proportion of females fertilized by the males, indicating a negative correlation between the proportion of females fertilized by the males and latitude (Fig. 3B, Table 2). The frequency of reproductive failure in males showed that more than half of the males from higher latitudes failed to fertilize the females, whereas many males from lower latitudes successfully fertilize the females (Fig. 3B). The frequency of reproductive failure in males was significantly different between field populations (Fig. 3B, Table 2). There was a significant effect of latitude on the frequency of reproductive failure in males, indicating a positive correlation between male reproductive failure and latitude (Fig. 3B, Table 2). The number of female copulations from 13 field populations showed a significant difference between populations (Fig. 3C, Table 2); however, there was no significant effect of latitude on the number of female copulations (Fig. 3C, Table 2). Statistical analysis of the correlation between the male and female reproductive traits showed a significant negative correlation between the proportion of females fertilized by males and frequency of reproductive failure in males, and a significant negative correlation between the number of female copulations and female body length (Fig. 4, Table 3). Populations with a proportion of females that were fertilized by males showed a lower frequency of reproductive failure males. Populations with larger body lengths showed a smaller number of female copulations. No significant correlations were found in the other combination (Table 3).

Discussion

In this study, there were significant differences between the male and female reproductive traits among *T. castaneum* field populations. Previous studies have reported geographic variation in the reproductive traits of various species (Outomuro and Johansson 2011; Monteiro and Lyons 2012; Hasegawa and Arai 2013; Kelly et al. 2013; Painting et al. 2014; Romiti et al. 2017). The results from this study also demonstrated geographic variation in the reproductive traits of both sexes of *T. castaneum*. Furthermore, the results indicated a latitudinal cline of the proportion of females fertilized by the male and frequency of reproductive failure males, indicating a negative and positive correlation with the latitude, respectively; i.e., the higher the latitude, the lower the proportion of females fertilized by the male and higher the male reproductive failure rate.

Previous studies that considered the morphological traits of insects have reported geographic variation and latitudinal cline in sexual traits. In the common blue-tailed damselfly (*Ischnura elegans*), Dudaniec et al. (2022) found an increase in the male body size with latitude but no effect on female body size, resulting in reduced sexual dimorphism toward the high latitudinal area. It was also reported that the sex ratios became increasingly male-biased toward the range limit, and there was evidence for an altered sexual selection regime shifting from favoring larger males in the south to favoring smaller males in the north. In the New Zealand giraffe weevil *L. barbicornis*, although the overall mean body and weapon size increased at higher latitudes, which is consistent with Bergmann's rule, there was a decline in the allometric slope, indicating that either large males decreased or small males increased their relative investment into weaponry along the cline (Painting et al. 2014). A previous study reported that as males invest more into body size

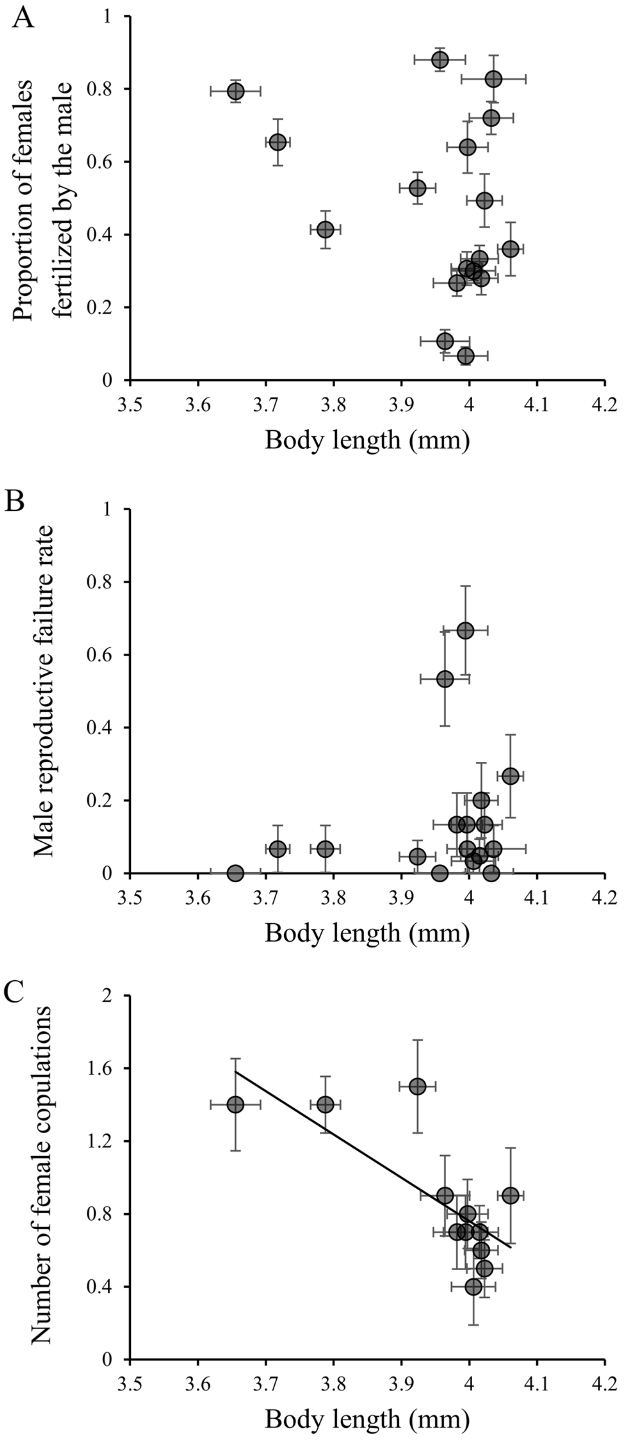

Figure 4 Relationship between body length with proportion of females fertilized by the male (**A**), frequency of reproductive failure male (**B**), and number of female copulations (**C**) in field population. Error bars showed standard error

at higher latitudes, they invest less into the relative weapon size. Therefore, several studies that have reported geographic variation and/or latitudinal cline in sexual traits considered morphological traits. However, to the best of our knowledge, this is the first study to reveal geographic variation in the proportion of females fertilized by the male and number of female copulations, and the latitudinal clines in the proportion of females fertilized by the male and frequency of reproductive failure in males.

The latitudinal cline in the male reproductive traits may be due to the influence of temperature. Temperature influences male mating behavior, where higher temperatures induce higher mating activity (Katsuki and Miyatake 2009). In lower latitudinal areas, where temperatures are higher, increased male mating activity may intensify male competition and drive selective pressure which further increased the male mating activity. Therefore, there may be differences in male reproductive traits among the populations despite observation within the same laboratory setting. Moreover, these results may be influenced by the reproductive season length. In *O. latipes*, males at lower latitudes show higher mating activity than males at higher latitudes (Fujimoto et al. 2015). Shorter reproductive season periods may lead to an equal OSR in the population, suggesting a lower sexual selection intensity pressure. Conversely, in lower latitudinal areas where there is a longer reproductive season period, the OSRs are often expected to be male-biased, suggesting a higher sexual selection intensity pressure (Fujimoto et al. 2015). Therefore, our evaluations may have occurred during the longer reproductive season period of the lower latitudinal area than of the higher latitudinal area for *T. castaneum.* Although we did not record the reproductive season and OSR in each field population, this is a critical issue that requires further investigation. Furthermore, our results may also be affected by the population density. In many species, sexual selection intensity pressure depends on the population density (Conner 1989; French and Cade 1989; Arnqvist 1992; Bertin and Cézilly 2005). For example, in *O. sexualis*, males possess large hind legs that function as a female-grasping apparatus, and the male hind femur was stouter at lower latitudes than at higher latitudes (Satomi et al. 2019). Because the population density of *O. sexualis* at lower than at higher latitudes, this suggests that the sexual selection intensity for male hind legs was positively correlated with the population density (Satomi et al. 2019). In *T. castaneum*, the population density often differs among the field populations in Japan (Matsumura 2021). Therefore, there is a possibility that our results were affected by population density. Similarly, because sexual selection strength was positively correlated with population density in *T. castaneum*, our results that male mating activity was higher at lower latitudes may have been due to the higher population density at lower latitudes. In addition, temperature is an important factor in these hypotheses. Our results suggest that the latitudinal cline of male reproductive traits was strongly influenced by temperature.

Table 3 Results of Spearman's rank correlation coefficient for each value

Variable	Vs. variable	Spearman's rank correlation coefficient (ρ)	*P*
Proportion of females fertilized by the male	Frequency of reproductive failure male	− 0.7007	0.0008
Proportion of females fertilized by the male	Number of female copulations	0.5153	0.0715
Frequency of reproductive failure male	Number of female copulations	− 0.3032	0.3139
Proportion of females fertilized by the male	Body length	− 0.0784	0.7648
Frequency of reproductive failure male	Body length	0.2317	0.3708
Number of female copulations	Body length	− 0.6679	0.0176

In the experiment on male reproduction, there was a geographic variation in the proportion of the five females who were not fertilized by the male. Moreover, the frequency of reproductive failure in males was positively correlated with latitude. In many insect species, males often fail to fertilize even if they successfully copulate. Various temporary or permanent genetic or environmental factors cause male infertility (Garcia-Gonzalez 2004; Tyler and Tregenza 2013). For example, some postemergence injuries or infectious diseases may damage the male copulatory organs, or males may temporarily exhaust their sperm stock. A previous study reported that the proportion of infertile mating across 30 insect species varied between 0 and 63%, with a median of 22% (Garcia-Gonzalez 2004). However, to the best of our knowledge, this study is the first to report a latitudinal cline in male reproductive failure. It is possible that the selection pressure toward an increased proportion of females fertilized by males may be synonymous with a decreased frequency of reproductive failure in males. A significant positive correlation was found between both traits (Table 3); however, this result should be carefully considered because it is unknown whether the failed reproduction in males was due to infertility.

The number of female copulations also showed geographic variation; however, a latitudinal cline was not found. In Fig. 3C, the relationship between the two tends to be negative, but the effect of one population at lower latitude may be greater. A previous study (Pai and Bernasconi 2008) using *T. castaneum* reported female mating up to ten times during observation for 1 h; therefore, it is unlikely that the 30-min observation period in this study was too short. Conversely, some species mated only once during the 1-h observation (Pai and Bernasconi 2008). Thus, the variability in the number of female copulations is expected to be high. Several previous studies have suggested that female polyandry is an adaptive bet-hedging strategy to avoid reproductive failure through the copulation with sterile males (Yasui 2001; Yasui and Garcia-Gonzalez 2016; Matsumura et al. 2021). In the results of this study, the proportion of infertile males was higher at higher latitudes, but the number of female copulations was not greater at higher than at lower latitudes. Since females of this species frequently copulate with multiple males (Fedina and Lewis 2008), selection pressure as a bet-hedging strategy may be relatively small.

The body size of *T. castaneum* was larger in higher than in lower latitudinal areas. This result may be caused by the "temperature-size rule" where body size increases because of the longer developmental period in lower temperature environments (Atkinson 1994). Although the *T. castaneum* used in the experiments were maintained in a laboratory with a standardized environment, the presence of a genetic basis from the evolution of the field populations may have caused this result (Partridge et al. 1994). The geographic variation of the reproductive traits may be affected by differences in body size among the field populations. For example, in *C. japonicus*, although there are geographic variations in the male reproductive traits, a latitudinal cline was not observed (Okuzaki 2021). Because reproductive traits are often associated with body size, male reproductive traits may differ geographically in response to body size. A previous study in *C. japonicus* found geographical differences in the food conditions during the developmental period (Okuzaki 2021), which affects the reproductive traits via body size. Similarly, in this study, the relationships between latitude and *T. castaneum* body size were positively correlated. Therefore, it was considered that body size affected the reproductive traits, and the number of female copulations was negatively correlated with body size. Conversely, although latitudinal clines were observed, male reproductive traits showed no significant correlation with body size. These results suggest that male reproductive traits may be directly altered geographically, whereas the female reproductive traits may be indirectly altered via geographic variation in body size. Multiple mating is considered precarious in females (Arnqvist and Rowe 2005). Females with a large body size may be able to resist excessive mating attempts from males, resulting in less copulation. Conversely, females with a smaller body size may have difficulty in resisting excessive mating attempts from males, which may increase the number of copulations. In this study, it was not possible to determine whether the females accepted copulation without resistance or were forced to mate. Therefore, more detailed investigations are required.

In this study, to remove the maternal effects from our results, each population was reared in a standardized laboratory environment for 2–3 generations. Thus, reproductive traits may have been altered during this period. However, in a previous study, field populations of *T. castaneum* were reared in the laboratory for two generations, as in the present study, and differences in behavioral traits were found among these populations (Matsumura and Miyatake 2023). Therefore, the influence of this rearing period on our results may be negligible.

In conclusion, these results reveal geographic variations and/or latitudinal clines in the male and female reproductive traits of *T. castaneum*. These study results also suggest that the female reproductive traits are affected by body size, but the effect of body size on the male reproductive traits is small. Further investigation is required to reveal other factors that play a role in the geographic variations and/or latitudinal clines in the sexual traits of *T. castaneum*.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00265-023-03359-x.

Author contributions KM and TM conceived and designed the study. KM and KW performed the experiment and data analyses. KM and TM contributed to the interpretation of dat, and drafted the manuscript. All authors approved the final version and agree to be accountable for the content therein.

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Data availability All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Declarations

Competing interests The authors declare no competing interests.

References

Abe MS, Matsumura K, Yoshii T, Miyatake T (2021) Amplitude of circadian rhythms becomes weaken in the north, but there is no cline in the period of rhythm in a beetle. PLoS ONE 16:e0245115

Adams DC, Church JO (2008) Amphibians do not follow Bergmann's rule. Evolution 62:413–420

Andersson M, Iwasa Y (1996) Sexual selection. Trends Ecol Evol 11:53–58

Arnqvist G (1992) Spatial variation in selective regimes: sexual selection in the water strider, *Gerris odontogaster*. Evolution 46:914–929

Arnqvist G, Rowe L (2005) Sexual conflict. In: Sexual Conflict. Princeton university press

Ashton KG (2002) Do amphibians follow Bergmann's rule? Can J Zool 80:708–716

Ashton KG, Feldman CR (2003) Bergmann's rule in nonavian reptiles: turtles follow it, lizards and snakes reverse it. Evolution 57:1151–1163

Atkinson D (1994) Temperature and organism size-a biological law for ectotherms? Adv Ecol Res 25:1–58

Belk MC, Houston DD (2002) Bergmann's rule in ectotherms: a test using freshwater fishes. Am Nat 160:803–808

Bergmann KGLC (1848) Uber die Verhaltnisse der Warmeokono-mie der Thiere zu ihrer Grosse. Gottinger Stud 1:595–708

Bernasconi G, Keller L (2001) Female polyandry affects their sons' reproductive success in the red flour beetle *Tribolium castaneum*. J Evol Biol 14:186–193

Bertin A, Cézilly F (2005) Density-dependent influence of male characters on mate-locating efficiency and pairing success in the waterlouse *Asellus aquaticus*: an experimental study. J Zool 265:333–338

Blanckenhorn W, Demont M (2004) Bergmann and converse Bergmann latitudinal clines in arthropods: two ends of a continuum? Integr Comp Biol 44:413–424

Blanckenhorn WU, Stillwell RC, Young KA, Fox CW, Ashton KG (2006) When rensch meets Bergmann: does sexual size dimorphism change systematically with latitude? Evolution 60:2004–2011

Colinet H, Sinclair BJ, Vernon P, Renault D (2015) Insects in fluctuating thermal environments. Annu Rev Entomol 60:123–140

Conner J (1989) Density-dependent sexual selection in the fungus beetle, *Bolitotherus cornutus*. Evolution 43:1378–1386

Core TR (2017) R Foundation for Statistical Computing; Vienna, Austria: 2018. R: A language and environment for statistical computing. In

Cushman JH, Lawton JH, Manly BFJ (1993) Latitudinal patterns in European ant assemblages: variation in species richness and body size. Oecologia 95:30–37

Dudaniec RY, Carey AR, Svensson EI, Hansson B, Yong CJ, Lancaster LT (2022) Latitudinal clines in sexual selection, sexual size dimorphism and sex-specific genetic dispersal during a poleward range expansion. J Anim Ecol 91:1104–1118

Endler JA (1995) Multiple-trait coevolution and environmental gradients in guppies. Trends Ecol Evol 10:22–29

Estlander S, Kahilainen KK, Horppila J, Olin M, Rask M, Kubečka J, Peterka J, Říha M, Huuskonen H, Nurminen L (2017) Latitudinal variation in sexual dimorphism in life-history traits of a freshwater fish. Ecol Evol 7:665–673

Fedina TY, Lewis SM (2008) An integrative view of sexual selection in *Tribolium* flour beetles. Biol Rev Camb Philos Soc 83:151–171

Fox J, Weisberg S (2018) An R companion to applied regression. Sage publications

French BW, Cade WH (1989) Sexual selection at varying population densities in male field crickets, Gryllus veletis andG. pennsylvanicus. J Insect Behav 2:105–121

Fujimoto S, Miyake T, Yamahira K (2015) Latitudinal variation in male competitiveness and female choosiness in a fish: are sexual selection pressures stronger at lower latitudes? Evol Biol 42:75–87

García-González F (2004) Infertile matings and sperm competition: the effect of "nonsperm representation" on intraspecific variation in sperm precedence patterns. Am Nat 164:457–472

Hasegawa M, Arai E (2013) Divergent tail and throat ornamentation in the barn swallow across the Japanese islands. J Ethol 31:79–83

Hill GE (1994) Geographic variation in male ornamentation and female mate preference in the house finch: a comparative test of models of sexual selection. Behav Ecol 5:64–73

Hut RA, Paolucci S, Dor R, Kyriacou CP, Daan S (2013) Latitudinal clines: an evolutionary view on biological rhythms. Proc R Soc B 280:20130433

Katsuki M, Miyatake T (2009) Effects of temperature on mating duration, sperm transfer and remating frequency in Callosobruchus chinensis. J Insect Physiol 55:113–116

Kelly CD, Folinsbee KE, Adams DC, Jennions MD (2013) Intraspecific sexual size and shape dimorphism in an Australian freshwater fish differs with respect to a biogeographic barrier and latitude. Evol Biol 40:408–419

Lande R, Arnold SJ (1983) The measurement of selection on correlated characters. Evolution 37:1210–1226

Leocadio-Miguel MA, Louzada FM, Duarte LL, Areas RP, Alam M, Freire MV, Fontenele-Araujo J, Menna-Barreto L, Pedrazzoli M (2017) Latitudinal cline of chronotype. Sci Rep 7:5437

Masaki S (1967) Geographic variation and climatic adaptation in a field cricket (Orthoptera: Gryllidae). Evolution 21:725–741

Matsumura K (2021) Relationship between death-feigning behavior and population density in a beetle. J Ethol 39:363–367

Matsumura K, Miyatake T (2015) Differences in attack avoidance and mating success between strains artificially selected for dispersal distance in *Tribolium castaneum*. PLoS ONE 10:e0127042

Matsumura K, Miyatake T (2023) Latitudinal cline of death-feigning behaviour in a beetle (*Tribolium castaneum*). Biol Lett 19:20230028

Matsumura K, Miyatake T, Yasui Y (2021) An empirical test of the bet-hedging polyandry hypothesis: Female red flour beetles avoid extinction via multiple mating. Ecol Evol 11:5295–5304

Mead LS, Arnold SJ (2004) Quantitative genetic models of sexual selection. Trends Ecol Evol 19:264–271

Michalczyk Ł, Millard AL, Martin OY, Lumley AJ, Emerson BC, Gage MJG (2011) Experimental evolution exposes female and male responses to sexual selection and conflict in *Tribolium castaneum*. Evolution 65:713–724

Miyatake T, Katayama K, Takeda Y, Nakashima A, Sugita A, Mizumoto M (2004) Is death–feigning adaptive? Heritable variation in fitness difference of death–feigning behaviour. Proc R Soc B 271:2293–2296

Miyatake T, Nakayama S, Nishi Y, Nakajima S (2009) Tonically immobilized selfish prey can survive by sacrificing others. Proc R Soc B 276:2763–2767

Miyatake T, Tabuchi K, Sasaki K, Okada K, Katayama K, Moriya S (2008) Pleiotropic antipredator strategies, fleeing and feigning death, correlated with dopamine levels in *Tribolium castaneum*. Anim Behav 75:113–121

Monteiro NM, Lyons DO (2012) Stronger sexual selection in warmer waters: the case of a sex role reversed pipefish. Public Library of Science San Francisco, USA, p e44251

Okuzaki Y (2021) Effects of body size divergence on male mating tactics in the ground beetle *Carabus japonicus*. Evolution 75:2269–2285

Outomuro D, Johansson F (2011) The effects of latitude, body size, and sexual selection on wing shape in a damselfly. Biol J Linn Soc 102:263–274

Pai A, Bernasconi G (2008) Polyandry and female control: the red flour beetle *Tribolium castaneum* as a case study. J Exp Zool B Mol Dev Evol 310:148–159

Pai A, Yan G (2020) Long-term study of female multiple mating indicates direct benefits in *Tribolium castaneum*. Entomol Exp Appl 168:398–406

Painting C, Buckley T, Holwell G (2014) Weapon allometry varies with latitude in the N ew Z ealand giraffe weevil. J Evol Biol 27:2864–2870

Park T (1934) Observations on the General Biology of the Flour Beetle, *Tribolium confusum*. Q Rev Biol 9:36–54

Partridge L, Barrie B, Fowler K, French V (1994) Evolution and development of body size and cell size in drosophila melanogaster in response to temperature. Evolution 48:1269–1276

Rasband WS (2011) ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA. https://imagej.nih.gov/ij///index.html

Ridley A, Hereward J, Daglish G, Raghu S, Collins P, Walter G (2011) The spatiotemporal dynamics of *Tribolium castaneum* (Herbst): adult flight and gene flow. Mol Ecol 20:1635–1646

Romiti F, De Zan LR, de Gasperis SR, Tini M, Scaccini D, Anaclerio M, Carpaneto GM (2017) Latitudinal cline in weapon allometry and phenology of the European stag beetle. Nat Conserv 19:57

Satomi D, Koshio C, Tatsuta H, Kudo SI, Takami Y (2019) Latitudinal variation and coevolutionary diversification of sexually dimorphic traits in the false blister beetle Oedemera sexualis. Ecol Evol 9:4949–4957

Sota T, Takami Y, Kubota K, Ishikawa R (2000) Geographic variation in body size of some Japanese Leptocarabus species (Coleoptera, Carabidae): the" toppled-domino pattern" in species along a geographic cline. Entomol Sci 3:309–320

Sutter A, Travers LM, Oku K, Delaney K, Store S, Price TA, Wedell N (2019) Flexible polyandry in female flies is an adaptive response to infertile males. Behav Ecol 30:1715–1724

Svensson EI, Kristoffersen L, Oskarsson K, Bensch S (2004) Molecular population divergence and sexual selection on morphology in the banded demoiselle (*Calopteryx splendens*). Heredity 93:423–433

Timofeev SF (2001) Biology Bulletin of the Russian Academy of Sciences, vol 28. Springer, pp 646–650

Tyler F, Tregenza T (2013) Why do so many flour beetle copulations fail? Entomol Exp Appl 146:199–206

Vasudeva R, Dickinson M, Sutter A, Powell S, Sales K, Gage MJG (2021) Facultative polyandry protects females from compromised male fertility caused by heatwave conditions. Anim Behav 178:37–48

Yasui Y (2001) Female multiple mating as a genetic bet-hedging strategy when mate choice criteria are unreliable. Ecol Res 16:605–616

Yasui Y, Garcia-Gonzalez F (2016) Bet-hedging as a mechanism for the evolution of polyandry, revisited. Evolution 70:385–397