Intraspecific variation of sex ratio and body size along latitude in the termite Reticulitermes speratus (Isoptera: Rhinotermitidae)

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Abstract

The Japanese subterranean termite *Reticulitermes speratus* exhibits a female-biased alate sex ratio and a wide north-south distribution area. The possible causes of the biased sex ratio can be tested by examining alate sex ratios and their geographical variation. Considering the need for adaptation to the winter fasting period, a body size cline of alates may be present in this species. In this study, we examined variations in the sex ratio, head width, and dry weight of *R. speratus* alates in 157 colonies from 16 populations on the Japanese archipelago. The alate sex ratio was biased toward females; however, no colonies without males were found, thus contradicting the hypothesis that parthenogenetic colonies founded by two female alates bias the sex ratios. The sex ratio bias was more pronounced at lower temperatures (higher latitudes), which supports the hypothesis that asexual queen succession causes sex ratio bias. Interestingly, a distinct split sex ratio was observed at low-temperature sampling sites, indicating the existence of another factor causing disruptive selection. The head widths and dry weights of alates were larger at lower temperatures and higher latitudes. This is the first study of an intraspecific latitudinal body size cline in termites, which is common in a temperate ant species and many ectotherms.

Introduction

Traits of termite alates are probably prone to strong natural selection. Termite colonies are typically founded by a pair of male and female alates (the king and queen) following extensive synchronous mating flights (Nutting 1969, Kahn and Ahmad 2018). Estimated mortality rates of the alates and royal pair in an incipient colony are very high because of intensive predation during nuptial flights (Nutting 1969), frequent infection with pathogenic microorganisms in incipient colonies (OK, personal observation), and intense intra-colonial competition and aggression which is expected considering the highly concentrated distribution of incipient colonies (Kitade et al. 2004). The mode of colony formation and the eusocial life of termites are remarkably similar to those of ants; however, unlike ants and other eusocial hymenopteran insects with haplodiploid sexual determination, termites are diploid in both sexes. Thus, termite colonies typically do not exhibit intra-colony relatedness asymmetry, thus natural selection allows for a 1:1 reproductive sex ratio, as in most other animal species. However, the rhinotermitid termite *Reticulitermes speratus* (Kolbe) exhibits a female-biased alate sex ratio in field colonies (Kitade et al. 2010). This termite species can reproduce parthenogenetically (Matsuura and Nishida 2001, Hayashi et al. 2003), and under experimental conditions, a female alate pair of this species can establish a colony through parthenogenesis (Matsuura and Nishida 2001). It was therefore proposed that this extraordinary colony foundation mode may be the cause of the female-biased sex ratio of alates in this species. Colonies originating from parthenogenesis in female-female pairings should consist exclusively of females, which can be tested by examining whether female-only alate-producing colonies occur in the field.

Kobayashi et al. (2013) proposed a different hypothesis to explain the female-biased alate sex ratio in *R. speratus* based on kin selection. Because the lifespan of the primary *R. speratus* queen is much shorter than that of the primary king, in most field colonies, the primary king reproduces with a parthenogenetically-produced secondary queen (referred to as asexual queen succession [AQS]; Matsuura et al. 2009); however, in a very mature colony where the primary king has died, a secondary king will succeed it to mate with the secondary queens. Such reproduction gives rise to differences in reproductive patterns of the primary king and queen, where natural selection favors the production of more female alates than males. This hypothesis was supported by field data showing that the alate sex ratios of *Reticulitermes* species with AQS were female-biased, whereas those without AQS were not (Kobayashi et al. 2013). These two hypotheses are not mutually exclusive, leaving open the possibility that the parthenogenic colony formation may be a secondary factor. Moreover, examining geographical variations in sex ratios may help test the validity of these hypotheses. This is because the optimal sex ratio of a population as predicted by a hypothesis may change to reflect differences in ecological factors such as temperature.

R. speratus is the termite species with the widest north-south distribution in Japan (Park et al. 2006, Morimoto 1968), and across its range, the difference in mean temperature in January is 14°C between the northern (southern Hokkaido: -4°C) and southern (Tokara Islands: 10°C) extents of its distribution (Morimoto 1975). In *R. flavipes* (Kollar) and *R. grassei* (Clemént), which are widely distributed in temperate zones, monogamous colonies are dominant in low-latitude populations, whereas the proportion of inbreeding colonies is high in high-latitude populations (Vargo and Husseneder 2011). In *Reticulitermes* termites, monogamy and inbreeding are general characteristics of young and mature colony developmental stages, respectively (Thorne et al. 1999). Therefore, assuming that high-latitude *R. speratus* populations include a larger proportion of mature colonies, the hypothesis of Kobayashi et al. (2013) can also be tested using geographical variation in the reproductive sex ratio. If this hypothesis is true, the higher the latitude, the more female-biased is the population sex ratio.

Life history theory considers body size one of the most important traits of an organism (Stearns 1992). In mammals, species inhabiting higher latitudes tend to have larger body sizes (Bergmann's rule: Bergmann 1847). The latitudinal body size cline, that body increases with latitude, has also been observed in many ectotherms, including insects (Stevenson 1985; Blanckenhorn and Demont 2004; Blanckenhorn et al., 2006). Adaptation to low-temperature conditions was proposed as the cause of latitudinal clines in insects, as artificial selection experiments have shown that large body size is selected for in low-temperature environments (Anderson 1966, 1973; Cavicchi et al. 1985, 1989; Partridge et al. 1994); however, it is still unclear whether temperature is the direct cause of body size discrepancies occurring in many animals, because numerous environmental and ecological variables change along with temperature over a latitude gradient (Stillwell 2010).

Among social insects, *Leptothorax acerborum* (Fabricius), a myrmicine ant distributed in the temperate zone of the Northern Hemisphere, shows a distinct tendency for individual body size to increase in higher-latitude populations (Heinze et al. 2003). Higher-latitude ant communities also tend to have larger mean body sizes of their component species (Cushman et al. 1993). Considering social insect colonies as superorganisms, the mass of the superorganisms is determined by the size distribution of individuals and the colony size. Kaspari and Vargo (1995) showed that ant colony size also tended to be larger at higher latitudes.

Because termites exhibit a very similar ecology to that of ants, a similar trend in the relationship between latitude and body/colony size would be expected in termites; however, the relationships between body size, colony size, and latitude in termites reported to date do not match those of ants. The average body size of the termite species in a community tends to be smaller at higher latitudes (Liu et al. 2022). We may have to consider that some authors have questioned the validity of analyzing the latitude-body size relationship through interspecies comparisons and have suggested limiting the analysis to intraspecific comparisons (Mayr 1956). Termite colonies are also rather small at high latitudes (Porter and Hawkins 2001). In termites, however, body size comparisons within species have not been performed among populations at different latitudes. Intraspecific comparisons in termites are essential to elucidate common and different patterns in size–latitude relationships and their selective causes and discrepancies between ants and termites.

In this study, we sampled 157 colonies of *R. speratus* containing alates or last-instar nymphs from 16 sampling sites covering most of their distribution area. To test the hypotheses regarding the female-biased alate sex ratio, we investigated geographic variations in the sex ratios of workers and alates (or last-instar nymphs). Alate body size (head width and dry weight) was also investigated to determine whether an intraspecific latitudinal body size cline occurs in temperate termite species.

Materials and methods

Sample collection

To investigate the sex ratios of alates and workers of *R. speratus*, partial colonies containing alates and workers were collected at 10 sites on the Japanese archipelago. The collection sites and numbers of colonies were: Hakodate (Site 1, Hokkaido pref., 41°74.71' N, 140°90.83' E, N = 9); Akita (Site 2, Akita pref., 39°68.35' N, 140°06.44' E, N = 15); Shizukuishi (Site 3, Iwate pref., 39°69.60' N, 141°00.17' E, N = 11); Sendai (Site 4, Miyagi pref., 38°12.32' N, 140°58.36' E, N = 12); Mito (Site 5, Ibaraki pref., 36°42.12' N, 140°37.20' E, N = 13), Iida (Site 8, Nagano pref., 35°54.65' N, 137°81.57' E, N = 9); Ako (Site 12, Okayama pref., 34°72. 34' N,131°96.45' E, N = 7); Aki (Site 15, Kochi pref., 33°51.71' N, 133°81.15' E, N = 10); Nichinan (Site 16, Miyazaki pref., 31°54.07' N, 131°37.05' E, N = 11; Table 1).

The sex ratio of the last-instar nymphs of *R. speratus* can be a reliable estimate of the alate sex ratio, as all the last-instar nymphs in a colony that appeared in autumn completely disappear from the colony just after the nuptial flight period (the following spring), indicating that almost all of them develop into alates by the nuptial flight period (OK, unpublished observation). Thus, to obtain the sex ratio data from sufficient localities, partial colonies containing last-instar nymphs and workers were collected from six sites: Tokai (Site 6, Ibaraki pref., 36°44.37' N, 140°60.29' E, N = 10); Niigata (Site 7, Niigata pref., 37°91.61' N, 139°01.55' E, N = 6); Toyama (Site 9, Toyama pref., 36°66.16' N, 137°10.36' E, N = 8); Hakui (Site 10, Ishikawa pref., 36°92.58' N, 136°76.75' E, N = 1); Ohmi Hachiman (Site 11, Shiga pref., 35°18.49' N, 136°08.95' E, N = 11); Shimonoseki (Site 14, Yamaguchi pref., 33°97.31' N, 130°95.89' E, N = 5; Table 1).

Sampling was carried out between 2009 and 2011. Each colony, including nest wood, was transported to the laboratory and kept in a plastic box (400 × 730 × 310 mm) at 8 °C to prevent caste differentiation, and sex ratios were assessed within 20 days.

Assessment of sex ratios

To investigate the sex ratios of alates and workers, we randomly selected 200 living alates or last-instar nymphs and 100 workers from the colonies described above. The individuals were sexed by examining the morphology of their abdominal sternites (Zimet and Stuart 1982) using a stereomicroscope and were stored in 70% ethanol. The sex ratio of alates (or last-instar nymphs) to workers in each colony was calculated as the ratio of females to all individuals surveyed. In colonies where the number of alates or nymphs was less than 200, and in colonies at sites 9 and 10, all collected alates or nymphs were counted, and the sex ratio was calculated.

Investigation of alate body size

The body sizes of the alates were investigated in 64 colonies of 10 alate-containing sites. We randomly selected ten alates of each sex from the preserved specimens of each colony and measured head width (maximum distance across compound eyes) using a digital microscope (VHX-600; KEYENCE). We also measured the mean dry weight of alates from each colony: 15 alates per sex from a colony were randomly selected and dried at 70 °C for 24 h. The total weight was measured using an electronic scale (AE260; Mettler Toledo).

Statistical analysis

Bias in the alate and worker sex ratios of all individuals and each population was examined using a binomial test. The effects of annual mean temperature on alate–female ratios were analyzed using a generalized linear model (GLM). We assigned alate sex to a response variable with a beta-binomial error distribution. The annual mean temperature, colonies, and their interactions were used as explanatory variables. First, the data were fitted to a full model and then subjected to backward stepwise model selection using the Akaike information criterion (AIC). Annual mean temperature data were obtained from the meteorological observatory nearest to the collection sites in the Japan Meteorological Agency web database (http://www.jma.go.jp/).

We performed a permutation test to examine the effects of annual mean temperature on the variation in the alate and worker sex ratios of each colony. First, we calculated the correlation coefficient (r) between the standard deviations (SDs) of the alate or worker sex ratios of the 88 colonies and annual mean temperature. The SD values between two randomly selected colonies were then replaced 500 times, and the correlation coefficient was calculated. By repeating this process 1,000 times, a frequency distribution of r was obtained. Statistical significance (two-sided, $\alpha = 0.05$) was judged by comparing the original correlation coefficient with the frequency distribution.

GLM analysis was performed to investigate the effect of temperature on the head widths of alates and workers. We assigned the mean head width of ten alates or workers as the response variable, with a normal error distribution, and annual mean temperature, sex, and the interaction between them were used as explanatory variables. Stepwise model selection using the AIC was performed. The effect of temperature on alate dry weight was also examined using GLM analysis. We assigned the average dry weight of alates as a response variable, with a normal error distribution, annual mean temperature, sex, and the interaction between them as explanatory variables. Stepwise model selection using the AIC was performed.

All the statistical analyses were carried out using R 3.0.1 (R Core Team 2013). The function glm.binomial.disp() in the dispmod package was used for GLM analysis with beta-binomial error distribution of the response variable. The function stepAIC() of the R package the MASS was used for the stepwise model selection.

Results

Sex ratio

When the data from all colonies were combined, the female ratio of workers was nearly 0.5 (Table 1; female ratio = 0.51; $n = 13{,}500$, binomial test; $P > 0.05$). The female ratio of each colony was not significantly different from 0.5, except for two populations: sites 4 (Sendai) and 5 (Mito). The variation in worker sex ratio, measured as the *SD* of the female ratios of colonies in a sampling site, averaged 0.016 (range, 0.39–0.61; $n = 135$; Fig. 2a).

In contrast, the compiled sex ratio data for alates were significantly female-biased (female ratio = 0.59; $n = 31{,}496$; binomial test, $P < 0.05$; Table 1). In particular, all the alates examined in a colony from Niigata were female. The sex ratios of alates (or last-instar nymphs) varied widely among colonies: the *SD* of the colony female ratios averaged 0.411 (range, 0.00–0.83; $N = 147$ colonies; Fig. 2b) and was much larger than that of workers.

GLM analysis with the reproductive sex ratio as the response variable and AIC-based model selection indicated that the best model included mean temperature as an explanatory variable (Table 2). The reproductive sex ratio was more female-biased at high-latitude sampling sites with low annual mean temperatures; however, the fact that the AIC value of the best model (207.9) was very close to that of the null model (208.0) and that the regression coefficient of the mean temperature was not significantly different from 0 (Wald test, $P = 0.07$) suggests that the effect of temperature was minimal.

Inter-colonial sex ratio variation of alates was significantly higher at lower mean annual temperatures in the habitat(Fig. 3b; $r = -0.687$; Permutation test, $P = 0.002$). The alate sex ratio showed a unimodal distribution in the low-latitude (high annual temperature) populations of the Japanese archipelago (Fig. 4c); however, the variation was greater in mid-latitude (intermediate annual temperature) populations (Fig. 4b), and in high-latitude (low annual temperature) populations the variation was even greater, resulting in a distinct bimodal distribution (Fig. 4a). There was no clear trend between the variation in worker sex ratio among colonies and habitat temperature ($r = 0.301$; Permutation test, $P = 0.288$).

Body size

The mean head widths of the female alates of *R. speratus* were larger than those of males: males: 0.831 ± 0.015 mm (mean ± *SD*); females: 0.844 ± 0.016 mm. Dry weight was also higher in females than in males: males: 0.829 ± 0.072 mg; females: 0.968 ± 0.076 mg (Fig. 5c, d).

The best model, selected by GLM analysis and model selection with alate head width as the response variable included mean temperature and sex as explanatory variables (Table 4). Head width was larger at sampling sites at lower latitudes with lower mean annual temperatures, and that of females was larger than that of males (Fig. 5a, b). Similarly, the best model, selected by GLM analysis and model selection with alate dry weight as the response variable, included mean temperature and sex as explanatory variables (Table 4). Dry weight was larger at sampling sites at higher latitudes with lower mean annual temperatures and was higher in females than in males (Fig. 5c, d); however, because the Wald test result for the regression coefficient was not significant, the effect of temperature (latitude) was probably not strong.

Discussion

All 157 colonies investigated in this study included workers of both sexes. These data indicate that colony foundation by a female-female pair does not occur in the field in this species, or if it does occur, however rarely, the colonies cannot grow to a size sufficient to produce alates. Judging from the microsatellite genotype data of reproductives in field colonies (Matsuura et al. 2009), it is unlikely that offspring produced by parthenogenesis in female-female pairs are integrated in other colonies and are involved in reproduction. Our results indicate that the hypothesis of Matsuura and Nishida (2001) cannot explain the bias in the alate sex ratio of this species.

The sex ratio of alates was significantly biased toward females in all populations, whereas that of workers was not significantly biased and differed from a 1:1 ratio, indicating that the sex ratio bias of alates observed in this species is a general phenomenon throughout the distribution range. Kitade et al. (2010) examined colonies of this species in the Ibaraki population and found that the variation in the alate sex ratio among colonies was much greater than that among workers. This study showed that this trend was observed regardless of the collection site and that the variation in the alate sex ratio is particularly large in high-latitude populations.

Ants equipped with a haplodiploid sex-determination system can oviposit male and female eggs selectively by fertilization, and this mechanism can be used to regulate alate sex ratios. Although this mechanism cannot be applied to termites with an XY sex-determination system, the above results indicate that *R. speratus* colonies can drastically alter the sex ratio of nymphs that eventually differentiate into alates. The genetic caste determination models proposed by Hayashi et al. (2007) and Matsuura et al. (2018) present the mechanisms by which *Reticulitermes* can affect the sex ratios of nymphs and workers. The former model predicts that nymphal production would not occur in colonies with an original king and queen (Hayashi et al. 2007; Kitade et al. 2011); however, subsequent studies confirmed nymphal production in these colonies (OK, unpublished data) and in genetically equivalent colonies (primary king and secondary queen colonies of *R. speratus*; Matsuura et al., 2009). Therefore, the model proposed by Hayashi et al. (2007) cannot be directly applied to field colonies of this species. The epigenetic caste determination model of Matsuura et al. (2018) is intriguing, but it probably requires further empirical confirmation to verify its validity, especially concerning the presence of sex-specific epimarks (Vargo 2019).

Although the trend was not strong, the degree of female bias in the alate sex ratio of each population was greater at the sampling sites with lower temperatures, and this trend was not observed for workers. If the tendency that the frequency of inbreeding colonies is higher at higher latitudes (Vargo and Husseneder 2011) is also applies to *R. speratus*, this trend supports the hypothesis that AQS results in the female-biased sex ratio (Kobayashi et al. 2013; but see also Hellemans et al. [2019] for AQS species with balanced alate sex ratio). Intriguingly, however, the variation in the alate sex ratio exhibited a clear trend of being greater in colder (higher latitude) populations. Although the frequency distribution of the sex ratio was unimodal in low-latitude (high-temperature) populations, the sex ratio variation became larger in intermediate populations and bimodal in high-latitude (low-temperature) populations. Because the AQS hypothesis (Kobayashi et al. 2013) predicted a unique optimal sex ratio of colonies in a population and, thus, an unimodal frequency distribution, our data suggested that another factor strongly affected the alate sex ratio in *R. speratus*. Possible causes of this split sex ratio include local mate competition (Hasegawa & Yamaguchi, 1995), avoidance of sib-mating (Noirot, 1989), and infection of sex-distorting bacteria like *Wolbachia* (Bandi et al. 1997, Lo and Evans 2007, Sinotte et al. 2020). To elucidate the cause of the sex ratio variation in this species, it should help to identify geographical differences in these possible causal factors: population reproductive structures, colony densities, and infection rates of *Wolbachia*.

This study is the first to show that termite body size tends to increase in higher-latitude habitats, based on a comparison between populations within a species. This trend was also observed in the ant *Leptothorax acerorum*, a social insect with a similar life history (Heinze et al. 2003). If this body size cline is based on adaptation, the results support the idea that common selection pressures occur in ants and termites.

Heinze et al. (2003) suggested that the most likely cause of the body size cline of *L. acerorum* is an adaptation that increases endurance to fasting forced by long periods of low temperatures at high latitudes. This explanation also applies very well to *R. speratus*, considering its life history and the results of GLM analyses, indicating that the annual mean temperature affected both the head width and body weight of alates.

Heinze et al. (2003) examined other possible adaptive hypotheses to explain the size clines of ectotherms, such as a) larger animals are more likely to disperse to higher latitudes and b) competition is less intense in northern habitats, but they concluded that these do not apply to *L. acerorum*. This was similar to what was observed in termites. Because the mobility of *R. speratus* is not high and the difference in body size is limited to a maximum of 1.06-fold of the head width, it is unlikely that only large individuals are able to migrate to colder high-latitude areas because of differences in dispersal ability. It is also unlikely that reduced competition at high latitudes is the cause because termites other than *R. speratus* are not distributed in the study area north of 35° N, but distinct size clines were also observed between these study areas.

The tendency for colony size to increase at higher latitudes, as observed in ants, has not been observed in termites (Porter & Hawkins 2001). Kaspari and Vargo (1995) proposed the hypothesis that a larger ant colony size at higher latitudes is an adaptation to longer fasting periods, based on the results of fasting experiments of ant colonies of different sizes at room temperature; however, because interactions in a colony, including nutrient exchange, are thought to cease at low temperatures, there may be room to re-examine whether an increase in colony size can truly be considered an adaptation to a longer period of fasting. Bernadou et al. (2016) revealed that the body size of *L. acervorum* increased at higher altitudes, but the colony size did not.

It should be noted that there has been no confirmation that the body size trends observed in *R. speratus* are due to adaptations (see Stillwell 2010). Even if adaptation is the cause, the observed body size data should be a combination of selected genetic and individual response components to the environment. We propose conducting experiments in which kings and queens derived from different populations at different latitudes are reared under matching temperature conditions to confirm whether the body size characteristics of different populations are indeed inherited.

Declarations

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Contributions All authers designed the sutdy and conducted field sampling and experiments; FM and OK analyzed data and wrote first draft of manuscript. All authors contributed to discussing the results and revising the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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Tables

Tables 1 to 4 are available in the Supplementary Files section.

Figures

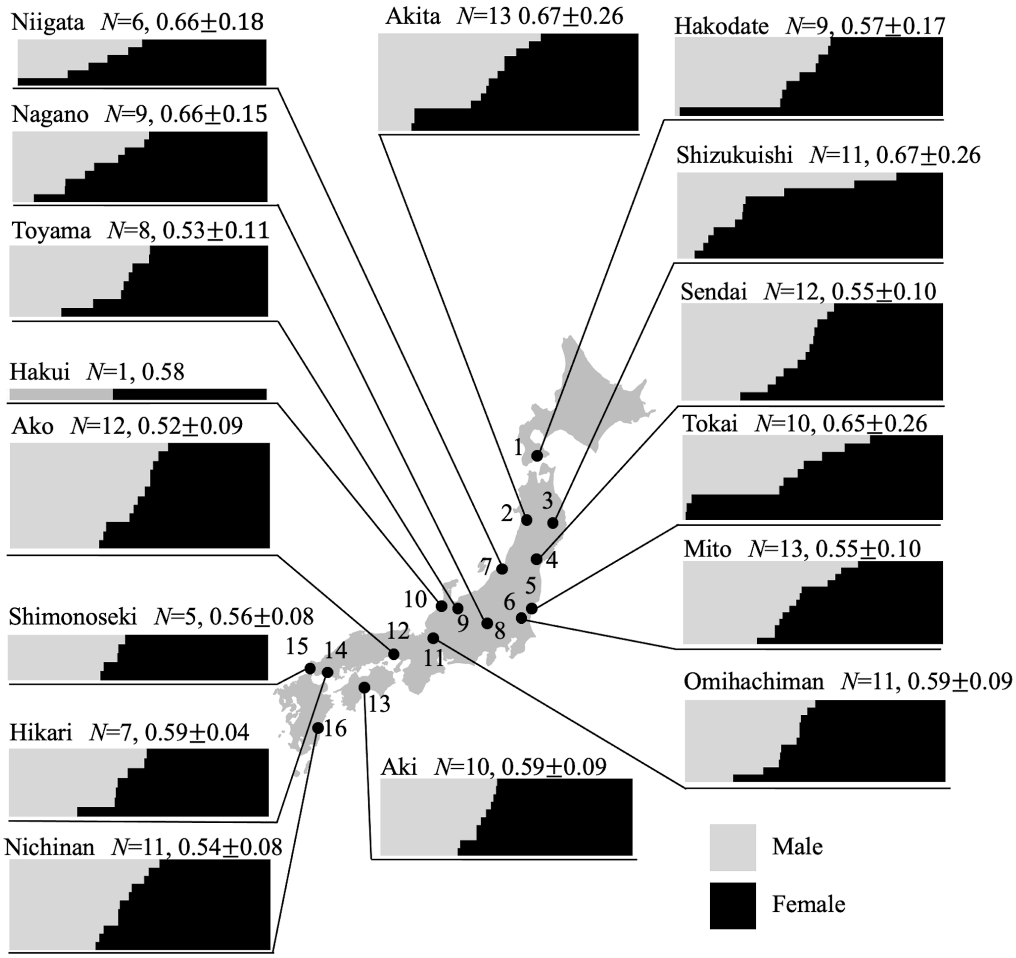

Figure 1

Collection sites of *Reticulitermes speratus* in this study and alate sex ratio of each colony. A total of 147 colonies were collected at 16 sites in Japan during 2009-2011. The numerals of the collection sites correspond to those of materials and methods and Tables 1 and 2.

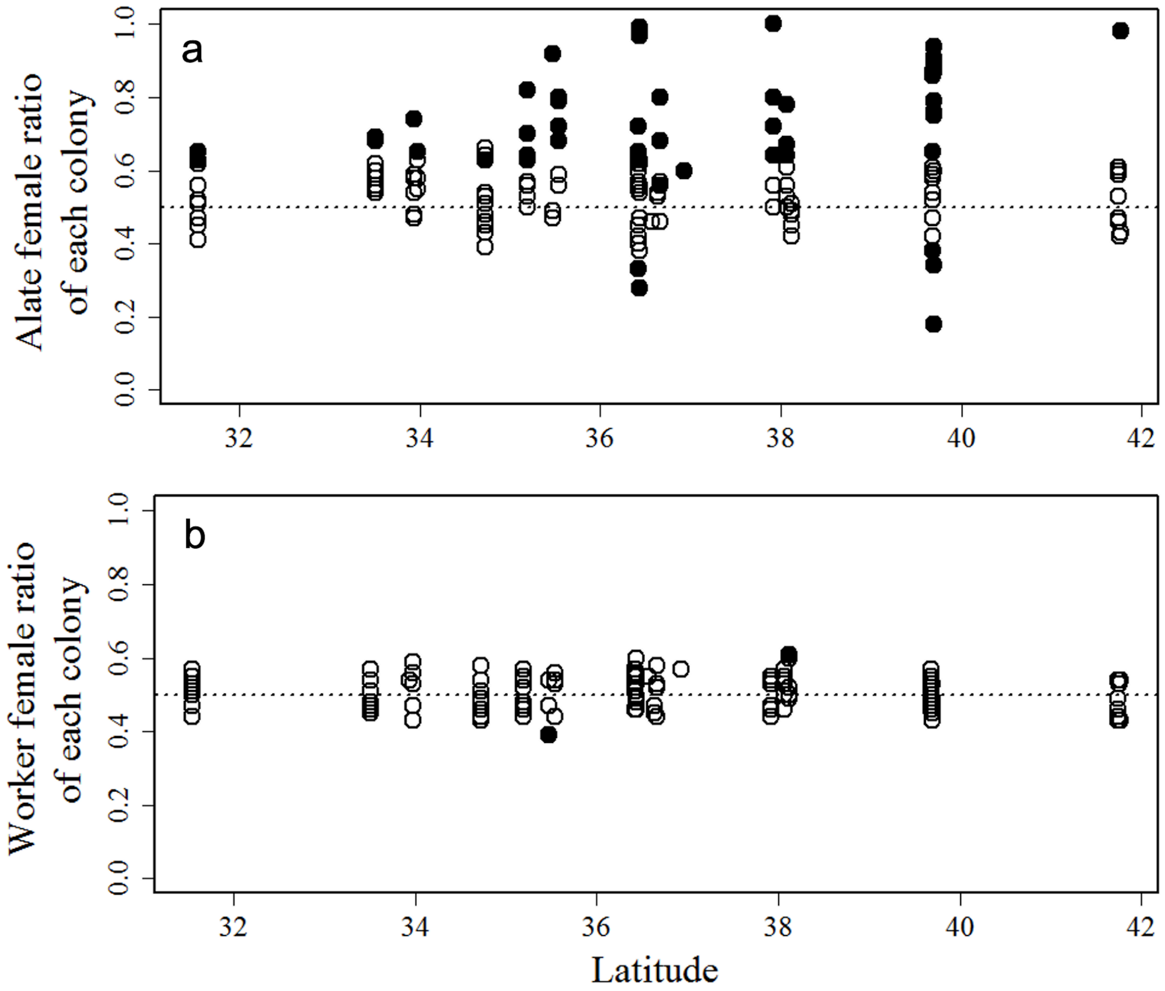

Figure 2

Female ratios of alates or last instar nymphs in 6 sites (a) and workers (b) in each colony. Filled circles indicate colonies significantly biased from 0.5 (binomial test and Holm's correction, $P < 0.05$). Open circles indicate colonies with an unbiased sex ratio (binomial test and Holm's correction, $P > 0.05$).

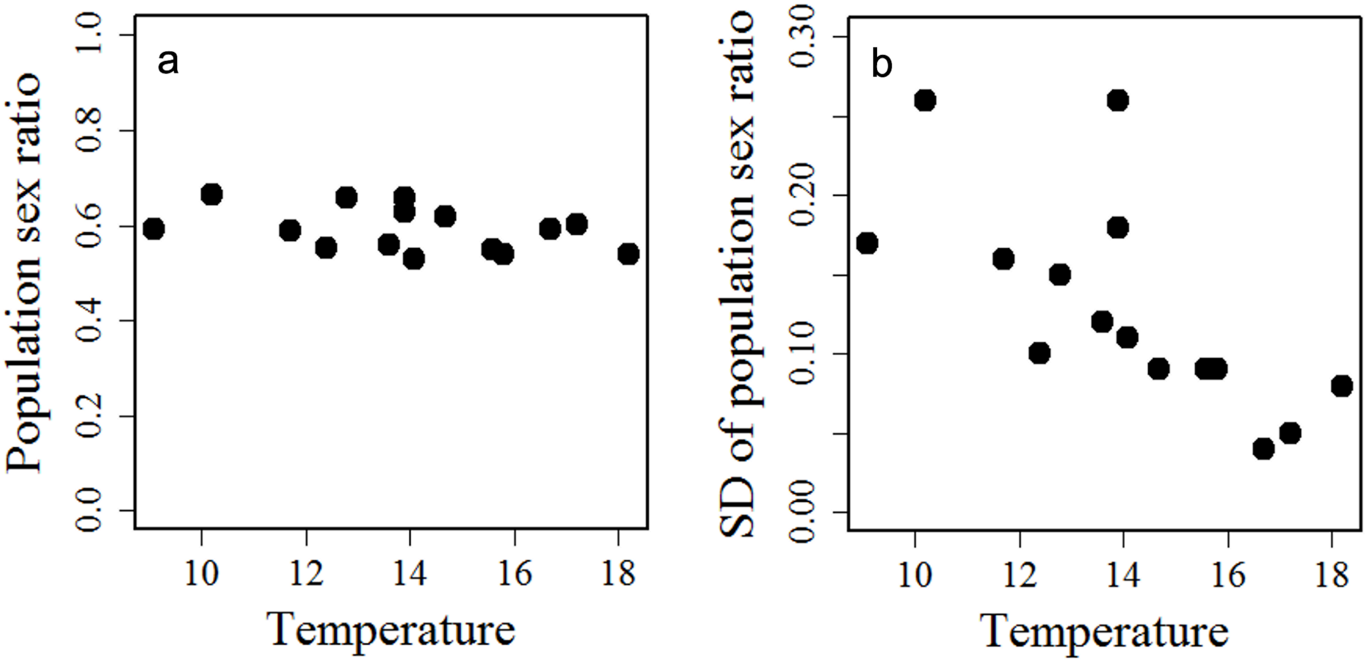

Figure 3

Relationships between annual mean temperature and population sex ratios (a), and those between annual mean temperature and variability of colony sex ratios (b).

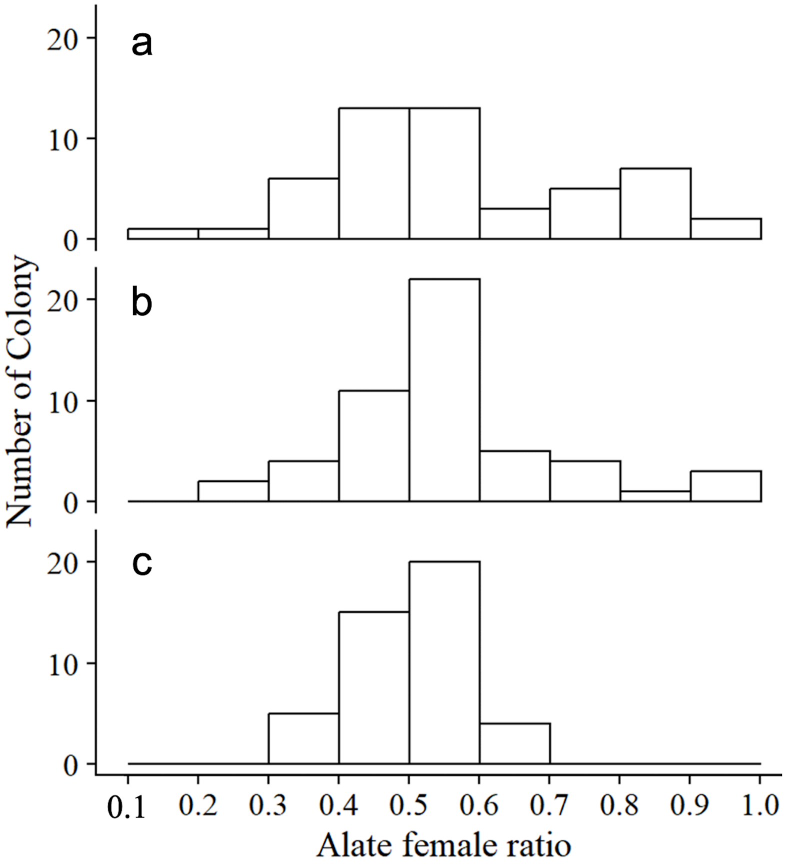

Figure 4

Frequency distribution of colonies' alate sex ratio. a, Northern populations (41°75'N to 37°91'N, *n*=53); b, Mid populations (36°93'N to 35°18'N, *n*=54); c, Southern populations (34°73'N to 31°54'N, *n*=53).

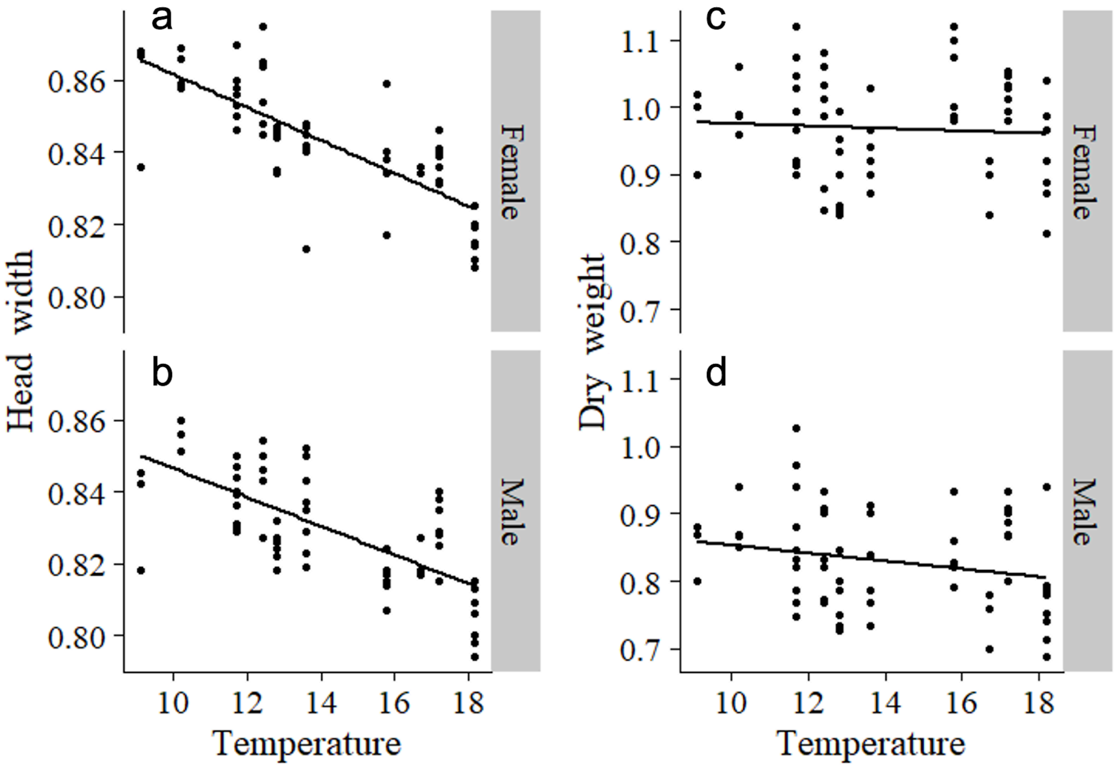

Figure 5

Relationships between temperature and head widths of male alates (a), and female alates (b), temperature and dry weights of female alates (c), and male alates (d). Bold lines indicate regression equations estimated by GLM.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Table2.docx
- Table3.docx
- Table4.docx