

1 Identification of the critical period for socioenvironmental 2 influences on caste determination in a termite

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14 Abstract

15 Caste determination in social insects is influenced by both innate and socioenvironmental factors. In
16 termites with bifurcated caste pathways—where individuals develop along either the nymphal (alate-
17 reproductive) or apterous (worker-soldier) line—such as *Reticulitermes speratus*, heritable factors present
18 at the egg stage can bias caste differentiation, while subsequent socioenvironmental influences can further
19 modify caste fate. As hemimetabolous insects, they retain the pluripotency to molt into other castes until
20 they differentiate into their terminal caste. However, whether an individual is able to perceive a particular
21 external stimulus or not is expected to depend on its developmental stage. Here, we demonstrate that in
22 *Reticulitermes speratus*, the critical period during which individuals are influenced by foster parents occurs
23 during the early first instar stage. A fostering experiment was conducted using parthenogenetically
24 produced larvae whose caste fate (nymph or worker) changes depending on the foster parents, specifically
25 whether they are reared by workers or colony founders. Foster parents altered the caste fate of larvae within
26 10 days post-hatching but had no effect beyond this period. Even under the same foster parent conditions,
27 larvae that hatched earlier were significantly more likely to differentiate into workers, suggesting that
28 parthenogenetic eggs laid during the early foundation stage differ in caste predisposition from those laid
29 later. This study identified the critical period for socioenvironmental influences on the caste fate of offspring,
30 contributing to a deeper understanding of the complex interplay between innate factors and environmental
31 influences in termite caste determination.

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33 **Key words:** Caste determination, Socioenvironmental factor, Parthenogenesis, Critical period, Totipotency,

34 Asexual queen succession

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36 Declarations

37 **Conflict of interest statement**

38 The authors declare that they have no conflicts of interest with the contents of this article.

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Introduction

In social insect colonies, individuals follow distinct developmental pathways to differentiate into castes with specialized morphologies (Wilson 1971). Extensive research has explored the socioenvironmental mechanisms underlying caste differentiation, which occurs among colony members sharing a common genetic background. These studies have demonstrated that factors such as pheromones, nutrition, season, temperature, and humidity play critical roles in regulating caste development (Haverty and Howard 1981; Howard and Haverty 1981; Wheeler 1986). In addition to these external influences, innate factors are also implicated in caste differentiation. For example, in ants, maternal factors deposited into eggs can influence caste fate (Passera and Suzzoni, 1979; Schwander et al., 2008; Libbrecht et al., 2013). In termites, caste determination mechanisms are highly diverse across taxa, ranging from species in which individuals retain developmental pluripotency with strong socioenvironmental influence, to species in which caste fate is largely fixed at the egg stage by maternal factors (see Roisin and Korb, 2011; Lanzrein, 1985). In species with bifurcated caste pathways, such as *Reticulitermes speratus*, recent studies suggest that heritable factors present at the egg stage can bias caste differentiation (Matsuura et al., 2018; Nozaki et al., 2018; Matsuura, 2020; Takata et al., 2023). Whether caste differentiation is driven primarily by a single factor or by the interaction of multiple factors varies considerably among species (Schwander et al., 2010). Theoretical frameworks based on juvenile hormone regulation (Nijhout and Wheeler, 1982; Korb and Belles, 2017) and early experimental work (Lüscher, 1960) have proposed the existence of temporally limited windows during which external or hormonal factors can influence caste fate. In the latter case, identifying these critical developmental periods is essential for understanding the mechanisms of caste determination in social insects.

Innate factors influencing caste determination in termites are exemplified by the reproductive system known as asexual queen succession (AQS), where workers and alates are produced sexually, while neotenic queens are generated through parthenogenesis (Matsuura et al. 2009; Matsuura 2017). Colonies of *R. speratus* are typically established by a monogamous pair of primary reproductives (a king and a queen) originating from alates (winged adults). The primary queen produces neotenic secondary queens via parthenogenesis, which eventually replace her. Subsequently, these secondary queens are replaced by later generations of asexually produced secondary queens. This system allows colonies to enhance their reproductive output while avoiding inbreeding, as numerous secondary queens are produced parthenogenetically. To date, AQS has been identified in seven termite species, spanning both non-termitid families (Heterotermitidae) and termitid groups (Termitinae: *Termes* group and *Syntermitinae*) (Matsuura et al. 2009; Vargo et al. 2012; Luchetti et al. 2013; Fougeyrollas et al. 2015; Fougeyrollas et al. 2017; Hellemans et al. 2017; Hellemans et al. 2019). In *R. speratus*, recent work by Takata et al. (2023) has shown that heritable factors can influence caste fate and sex allocation, and that developmental bias may be

established before oviposition. This finding supports the idea that, at least in species with bifurcated caste pathways, innate factors play a significant role in caste determination.

As described above, daughters produced through parthenogenesis exhibit an innate predisposition to differentiate into nymphs more readily than those produced through sexual reproduction. However, even daughters produced via parthenogenesis can differentiate into workers depending on socioenvironmental conditions. In *R. speratus*, in addition to colonies typically established by monogamous pairs, colony founding by female–female or male–male pairs has also been documented (Matsuura and Nishida 2001; Matsuura et al. 2002). Under natural conditions, same-sex founding pairs (female–female or male–male) have an extremely limited capacity to develop into mature colonies; field evidence for mature colonies originating from such pairs is scarce to absent (Matsuura et al. 2018; Morooka et al. 2005). By contrast, under laboratory and semi-natural settings, same-sex pairing followed by colony fusion can enhance survival and enable later reproduction, indicating an adaptive conditional tactic in risky post-dispersal contexts. These alternative pairings are considered context-dependent adaptive strategies. In particular, male–male pairing has been shown under laboratory and semi-natural conditions to enhance survival and provide opportunities for future reproduction through colony fusion, representing a conditional tactic under conditions of high predation pressure while searching for partners on the ground after dispersal (Mizumoto et al. 2016). In colonies founded by female–female pairs, only parthenogenetically produced daughters are produced, and the first brood raised by the founders exclusively differentiates into workers. Whether and how often such tactics are realized in natural *R. speratus* populations remain to be rigorously tested, calling for targeted field verification. Interestingly, in cases where incipient colonies established by female–female pairs are attacked and usurped by large colonies, the parthenogenetic eggs taken by the host colony and raised by its workers differentiate into nymphs, creating a pathway for social parasitism (Tamaki et al. 2021). Thus, even among daughters produced through parthenogenesis, caste differentiation is influenced by who raises them.

In this study, we conducted fostering experiments in *R. speratus* to identify the critical period during which socioenvironmental factors influence caste differentiation. To this end, we manipulated the identity of the caregivers of parthenogenetically produced larvae by dividing the rearing process into two phases (see Fig. 1): Phase I, in which larvae were reared by workers, and Phase II, in which larvae were reared by colony founders. The treatment groups differed in the duration of Phase I (0, 5, 10, 15, or 20 days), allowing us to test how the timing of exposure to different social environments affects caste fate. This design was based on previous findings that parthenogenetic eggs laid by female–female pairs almost exclusively differentiate into nymphs when reared by a large number of workers (Tamaki et al. 2021), suggesting that worker-mediated cues can promote nymph differentiation. Accordingly, we hypothesized that larvae reared exclusively by founders (i.e., no Phase I) would primarily differentiate into workers, whereas longer durations of worker-only rearing (Phase I) would increase the proportion of nymph differentiation up to a plateau. This approach enables the experimental identification of the critical period during which

socioenvironmental input can modify caste fate in termites.

Materials and Methods

Preparation of parthenogenetically produced eggs

In March and April 2024, five colonies (colony A, B, C, D, and E) containing final instar nymphs (pre-alates) were collected in Kyoto Prefecture, Japan, to obtain alates (Table 1). All subsequent processes were carried out individually for each colony. These colonies, together with nest wood, were transported to the laboratory and kept at 20°C in darkness until the nymphs molted into alates. The nest woods containing the alates were then kept at 25°C under constant light to induce swarming. After swarming, the alates were separated by sex, and their wings were removed. Two females (female-female pair) were placed in petri dishes (ca. 35 × 11 mm) with a 30 × 5 mm brown-rotted pinewood medium (BPC) block (Mitaka, Akino, and Matsuura 2023) as bait and stored at 25°C in darkness to initiate egg-laying. The number of pairs was 112, 140, 140, 140, and 168 for colonies A, B, C, D, and E, respectively. Eggs laid by the female-female pairs were collected every two days and used in "Preparation of larvae". The number of eggs was 1,124, 869, 1,500, 1,789, and 925 for colonies A, B, C, D, and E, respectively.

Preparation of foster workers and founders

Alongside the preparation of female-female pairs, petri dishes containing two males (male-male pairs) and a BPC block were also prepared for use as foster founders in phase II of following fostering experiment (see Fig 1). The number of pairs was 168, 140, 168, 196, and 84 for colonies A, B, C, D, and E, respectively. They were stored at 25°C in darkness. To obtain foster workers used in "Preparation of larvae" and phase I of the fostering experiment, the nest woods after swarming were partially dissected 3 to 7 days before these phases. Approximately 2,000 to 4,000 workers were extracted from each colony, and 30 randomly selected workers were placed in petri dishes containing a BPC block and stored at 25°C in darkness. To prevent the inclusion of eggs laid by foster parents during the experiment, male-male pairs were used as foster founders. However, foster workers were randomly selected regardless of sex, as there was no risk of worker-laid eggs being mixed into the samples. For further details, refer to the "Preparation of larvae" and "Fostering experiment" sections, which provide a comprehensive explanation of the time required for ergatoid differentiation and the duration of the experiment.

Preparation of larvae

The eggs collected in "Preparation of parthenogenetically produced eggs" were divided into groups of 20 to 30 and placed into petri dishes with the foster workers within one hour. The dishes were checked daily, and newly hatched larvae were collected, with their hatching dates recorded. Since the timing of hatching

may influence the probability of nymph differentiation, two values were calculated using the recorded hatching dates to examine this effect. The first value, age index, represents how many days have passed since the first larva hatched in each colony. The first hatching date was set as day 0, the next day as day 1, and so on. The second value, birth order, ranks the larvae based on their hatching sequence within each colony. Larvae that hatched on the first recorded hatching day were all assigned rank 1. The rank of larvae hatching on subsequent days was determined by adding the total number of larvae that had already hatched, with all larvae hatching on the same day sharing the same rank. A total of 809, 515, 942, 1,173, and 528 larvae from colonies A, B, C, D, and E, respectively, were used for the fostering experiment. Worker-laid eggs were not mixed among these samples, as it takes 27.6 ± 1.2 days for female workers to differentiate into ergatoids (Miyata et al. 2004), and the egg stage lasts approximately 35 days (Matsuura and Kobayashi 2007). Since the preparation of larvae was completed within 38 days, this is well below the 62-day minimum required for eggs laid by newly differentiated ergatoids to develop into larvae. Therefore, contamination of our larval samples by offspring produced by ergatoid reproductives can be confidently ruled out.

Fostering experiment

To identify the sensitive period influenced by foster parents, we conducted a fostering experiment focused on first-instar larvae. Our experimental plan was stepwise: we first aimed to test whether the sensitive period existed during the first instar. If no such period was detected, we had planned to perform additional experiments targeting the second instar. However, the results clearly indicated that the sensitive period occurred during the early first instar, and no effects were observed beyond this stage. Therefore, experiments targeting the second instar were not conducted. The fostering experiment consisted of two phases: Phase I, in which parthenogenetically produced larvae were reared by workers, and Phase II, in which they were reared by colony founders. Five treatment groups were established, each with a different Phase I duration: 0 days (i.e., larvae transferred directly to Phase II), 5, 10, 15, and 20 days (see Fig. 1). Prior to the main experiment, we conducted a preliminary study to determine the duration of the first instar under our laboratory conditions. This experiment revealed that the first instar lasts 14.29 ± 0.18 days ($n = 24$). Based on this result, we set the Phase I durations to span 0–20 days, thereby covering the entire first instar and extending slightly beyond to assess whether sensitivity persisted into the second instar.

The larvae collected in “Preparation of larvae” were divided into groups of 20 to 30 and placed into petri dishes with the foster workers within one hour. Each dish was randomly assigned to one of the treatments and stored at 25°C in darkness during the duration of each treatment (phase I). Worker-laid eggs were not mixed among these samples, as it takes 27.6 ± 1.2 days for female workers to differentiate into ergatoids (Miyata et al. 2004), which is longer than the duration of phase I. Then, the larvae were collected from the dishes and transferred in groups of five into new petri dishes containing foster founders (male–male pairs) for Phase II. Phase II was designed to simulate the condition of an incipient colony, in which

larvae receive care only from the founding reproductives, prior to the emergence of workers. According to Shimada and Maekawa (2010), colonies founded by male–female pairs contain on average 2.6 ± 1.5 workers and 3.1 ± 1.1 larvae 50 days after foundation. Based on these data, the maximum number of larvae that a founder pair can feasibly rear in such a setting is estimated to be around five. Therefore, in our experiment, five larvae were used per dish in Phase II to match the capacity of a realistic founding colony.

We used male–male founder pairs as foster reproductives in order to prevent contamination from newly laid eggs, which would occur if female founders were present. This ensured that only the development of the original experimental larvae was observed, free from interference by new progeny. From phase I to phase II, a total of 680, 420, 720, 895, and 390 larvae were transferred from colonies A, B, C, D, and E, respectively. When the larvae reached the third instar, their caste was identified by the presence of wing buds. Sex identification was not conducted because parthenogenetically produced offspring were all female (Matsuura and Nishida 2001; Matsuura et al. 2002).

Statistical analysis

To investigate the factors influencing the caste fate of parthenogenetic daughters, generalized linear models (GLMs) were applied. In the model, the binomial objective variable was the proportion of nymphs (the number of subjects that developed into nymphs given the total number of subjects as the trial number), the explanatory variables were the length of phase I (treated as a numerical variable), the age index, and the colony ID (here after model 1). An alternative model (here after model 2) was performed, replacing the age index with the birth order as an explanatory variable. In model 1, the length of Phase I, the age index, and the colony ID were examined as factors explaining the variation in the proportion of nymphs, while in model 2, the birth order was investigated. To investigate the critical period for socioenvironmental factors after hatching, GLM was applied, and Tukey HSD post hoc tests were used to compare the proportion of nymphs across different lengths of phase I. In the model, the binomial objective variable was the proportion of nymphs, the explanatory variables were the length of phase I (treated as a factor), the age index, and the colony ID. To investigate whether the hatching date or the birth order has a greater influence on caste fate, GLMs were applied. In one model, the explanatory variable was the age index, and in the other, the explanatory variable was the birth order. In both models, the binomial objective variable was the proportion of nymphs. The Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) values were calculated for both models. Since the age index had smaller AIC and BIC values compared to the birth order, the effect of the age index on the proportion of nymphs was examined using GLMs to investigate the influence of innate factors on caste fate. To separate the effect of the length of phase I from the analysis, the data were divided by the length of phase I, and five models were performed. In these models, the binomial objective variable was the proportion of nymphs, and the explanatory variables were the age index and the colony ID. To address the issue of multiple testing, we applied the Benjamini–Hochberg false discovery rate (FDR) correction to the p-values obtained from the GLMs conducted separately for each

treatment group. All statistical analyses were performed using R v.4.4.1 software (R Core Team 2020). For GLMs, likelihood ratio tests (LRTs) were used to determine the statistical significance of each explanatory variable. A significance value of $p < 0.05$ was considered to indicate statistical significance.

Table 1 Termite colony information.

Colony	Location	Sampling date	Latitude	Longitude
A	Ichijoji Kotani, Kyoto, Kyoto	29 March 2024	35.04	135.80
B	Eikando, Kyoto, Kyoto	10 April 2024	35.01	135.80
C	Iwakuramuramatsu Kyoto, Kyoto	25 April 2024	35.09	135.78
D	Iwakuramuramatsu, Kyoto, Kyoto	25 April 2024	35.09	135.78
E	Kitashirakawa Kiyozawaguchi, Kyoto, Kyoto	04 April 2024	35.04	135.80

Results

Both innate factors prior to hatching and socioenvironmental factors after hatching influenced the caste fate of parthenogenetic daughters, with the length of phase I (model 1: GLM LRT, $\chi^2 = 83.630$, $df = 1$, $p < 0.001$), the age index (model 1: GLM LRT, $\chi^2 = 53.083$, $df = 1$, $p < 0.001$), the birth order (model 2: GLM LRT, $\chi^2 = 71.791$, $df = 1$, $p < 0.001$) and the colony (model 1: GLM LRT, $\chi^2 = 177.700$, $df = 1$, $p < 0.001$) explaining the variation in the proportion of nymphs. The length of phase I had a positive effect on the proportion of nymphs (Fig. 2a). The post hoc analysis showed that the proportion of nymphs increased with the length of phase I from 0 days to 10 days, but no significant differences were observed beyond 10 days (Tukey HSD post hoc tests, Fig 2a). The age index had greater influence on proportion of nymphs than the birth order (GLM, age index: AIC = 2,017.101, BIC = 2,025.911, birth order: AIC = 2,027.329, BIC = 2,036.140). The age index had a positive effect on the proportion of nymphs across all treatment groups with varying lengths of phase I (Fig. 2b): for 0 days (GLM, estimate = 0.135, intercept = -2.740, $\chi^2 = 13.107$, $df = 1$, $p < 0.001$), for 5 days (GLM, estimate = 0.129, intercept = -0.681, $\chi^2 = 11.219$, $df = 1$, $p < 0.01$), for 10 days (GLM, estimate = 0.124, intercept = 0.426, $\chi^2 = 15.173$, $df = 1$, $p < 0.01$), for 15 days (GLM, estimate = 0.075, intercept = -0.403, $\chi^2 = 6.713$, $df = 1$, $p < 0.01$), and for 20 days (GLM, estimate = 0.187, intercept = 0.318, $\chi^2 = 30.679$, $df = 1$, $p < 0.001$). The mortality rates for each phase and treatment group are provided in Supplementary File 1.

Discussion

This study identified, for the first time, the critical period during which socioenvironmental factors influence whether individuals differentiate along the nymphal or apterous developmental line. While earlier studies have suggested the presence of temporally sensitive phases affecting caste differentiation in termites (Lüscher 1960, Watson 1977; Lanzrein 1985), these focused primarily on later caste divergences (e.g., soldier or neotenic differentiation). In contrast, our study provides direct experimental evidence for a critical period affecting the initial bifurcation of caste fate—nymphal versus apterous—in species with bifurcated developmental pathways. Differences in foster parents influenced caste differentiation of larvae during the 0–10 days post-hatching period. This period corresponds to the early first-instar stage, which lasts approximately 14 days under our experimental conditions. In contrast, changes in foster parents beyond this period had no effect on caste fate, indicating that the critical period for socioenvironmental influence is limited to this early stage of larval development. This temporal pattern is consistent with findings in *Cryptotermes secundus*, where caste fate was shown to be most sensitive to juvenile hormone signaling during the early phase after molting (Korb et al. 2021). Although the two species differ in their developmental systems—*R. speratus* exhibiting bifurcated pathways and *C. secundus* a linear one—both studies converge on the idea that caste fate is largely determined within a narrow window early in the post-molting or post-hatching period, suggesting a general principle that developmental plasticity is highest during the early stages of each molt cycle. Additionally, innate differences in caste predispositions were discovered among parthenogenetic eggs laid during the early stages of colony foundation by female-female pairs, where later-hatching individuals tended to exhibit higher proportion of nymphs, as indicated by their “age index” and “birth order.” These findings demonstrate that while innate factors establish the direction of caste differentiation, the caste fate can also be modified by socioenvironmental influences within a specific critical period after hatching.

Previous studies have shown that parthenogenetically produced offspring of *Reticulitermes speratus* can differentiate into either workers or nymphs depending on their rearing conditions (Hayashi et al., 2007; 2013). However, those studies primarily used eggs laid by secondary reproductives and focused on broader environmental contrasts. Numerous studies have also demonstrated that socioenvironmental factors—including the presence of nestmates, temperature, humidity, nutritional conditions, and the associated changes in gene expression—play critical roles in shaping caste differentiation across termite species (Liu et al. 2005; Hayashi et al. 2007; Scharf et al. 2007; Korb et al. 2009; Miura and Scharf 2010; Tarver et al. 2012; Hayashi et al. 2013; Yaguchi et al. 2019). Our study builds on this foundation by clearly identifying the specific critical period during early larval development when social factors such as foster parent identity exert their influence. This fine-scale temporal resolution offers a novel perspective on the developmental plasticity of caste determination in termites. Unlike earlier studies focusing on maternal or epigenetic effects prior to or during oviposition, our findings emphasize the post-hatching modulation of caste fate, highlighting the dynamic and responsive nature of termite development to social environments. This perspective aligns with and extends previous findings on socioenvironmental regulation of development

(e.g., Lüscher 1960; Miura 2006; Korb et al. 2009; Hayashi et al. 2013), reinforcing the concept that termite caste differentiation is shaped by both inherited and social cues acting at distinct developmental stages.

Individuals that follow the worker pathway (apterous line) may later undergo further divergence, differentiating into soldiers or worker-derived neotenics (ergatoid reproductives). Conversely, those that follow the nymph pathway (nymphal line) experience another divergence, either differentiating into secondary reproductives, which remain in the colony to take over reproduction, or developing into alates, which leave the nest to establish new colonies (Roisin 2000; Roisin and Korb 2010). Extensive research has been conducted on socioenvironmental factors influencing these later caste divergences, with primer pheromones such as soldier and queen pheromones identified as key regulators of differentiation (Lefeuve and Bordereau 1984; Bordereau 1985; Matsuura et al. 2010; Tarver et al. 2011; Mitaka et al. 2017). However, in termites with bifurcated caste pathways, the specific agents that determine the critical division between the nymphal and apterous developmental lines have remained largely unresolved. While previous studies have proposed developmental models and suggested the existence of temporally sensitive phases affecting caste fate (Lüscher 1960; Watson 1977; Lanzrein 1985), the precise timing of when socioenvironmental factors exert their influence during early caste bifurcation has not been clearly demonstrated. Our study successfully identified a critical period during the early first instar during which foster parent identity affects caste fate. This represents a crucial step toward identifying the underlying regulatory factors—such as feeding materials, pheromones, or caregiving behaviors—that operate during this window. Importantly, these socioenvironmental cues are unlikely to act in isolation but instead interact with internal physiological mechanisms, such as endocrine signaling pathways, to influence caste fate (Korb and Belles 2017; Korb et al. 2021). Our findings thus contribute to a growing understanding of how external and internal factors jointly shape the developmental trajectories underlying termite social organization.

Daughters produced through parthenogenesis exhibit an innate propensity to differentiate into the nymph pathway and further into neotenic queens more readily than daughters produced through sexual reproduction (Nozaki et al. 2018; Matsuura et al. 2018; Tamaki et al. 2021). Interestingly, even among the parthenogenetic eggs laid by the same female-female pair, earlier-produced individuals exhibited a higher tendency to differentiate into workers, even when experiencing the same socioenvironment. In *R. speratus*, the founding queen initially produces larger eggs, transitioning to smaller eggs over time (Matsuura and Kobayashi 2010). The tendency of early-produced eggs to differentiate more readily into workers may be attributed to this shift in egg size or to changes in the concentration of cytoplasmic factors within the eggs as their size decreases. Moreover, our recent transcriptomic study revealed differences in the expression of juvenile hormone (JH)-related genes between parthenogenetic and sexually produced embryos (Kamiyama et al. 2025), suggesting that caste propensity may be influenced by endocrine programming at the egg stage. Such intrinsic variation in JH-related gene expression might also underlie the observed differences in nymph differentiation between early- and late-produced parthenogenetic eggs in the current study, reflecting physiological shifts during the early phase of colony foundation.

Tamaki et al. (2021) demonstrated that parthenogenetic eggs produced by primary reproductives can entirely differentiate into nymphs when reared by approximately 3,000 workers in the presence of both a king and queen. In contrast, in our study, the nymph differentiation rate plateaued at approximately 50%, even with prolonged exposure to workers. This discrepancy likely reflects differences in the number of caregivers, the absence of reproductives, or other contextual factors such as colony social structure. Notably, in our experiment, the maximum duration of worker-only rearing (Phase I) was 20 days, covering the entire first instar and approximately the first 5 days of the second instar. While our results indicate that no further increase in nymph differentiation occurred beyond 10 days, we cannot fully exclude the possibility that an additional sensitive period exists between 20 and 30 days post-hatching. However, given that larvae reared by workers for up to 20 days—including the early second instar—did not show further caste plasticity, despite the clear presence of a sensitive period during the early first instar, it seems unlikely that a major sensitive window exists beyond this point. Another key difference emerges when comparing our results with those of a study by Matsuura et al. (2004), which reported that fewer than 1% of individuals in colonies founded by female–female pairs followed the nymphal pathway after 400 days, whereas approximately 25% of individuals in our Phase I 0-day group—reared exclusively by founders—differentiated into nymphs. Although both experiments involved parthenogenetic larvae raised by founders, differences in the social context—including the identity of caregivers during the egg stage—may have influenced the outcomes. In our study, eggs were cared for by workers until hatching, potentially exposing them to socioenvironmental cues not present in the field colonies. Moreover, due to practical constraints, larvae in the 0-day group remained with workers for up to 24 hours after hatching, during which time they may have received caste-influencing stimuli. Additional variation may arise from the sex of the founders; previous studies have shown that kings and queens, as well as male and female ergatoids, differ in their effects on caste fate (Hayashi et al. 2013; Wu et al. 2023), suggesting that our use of male–male founder pairs may have affected outcomes differently than female–female pairs.

Supplementary Information

The complete raw data from the experiment is provided in Supplementary file 1. The complete analysis code is provided in Supplementary file 2.

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Author Contributions

Riho Kamiyama: Formal analysis; investigation; methodology; data curation; visualization; writing – original draft.

Mamoru Takata: Methodology; supervision; writing – review and editing.

Kenji Matsuura: Conceptualization; funding acquisition; investigation; methodology; supervision; writing – review and editing.

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Data availability

The data used in this study are available in the electronic supplementary materials.

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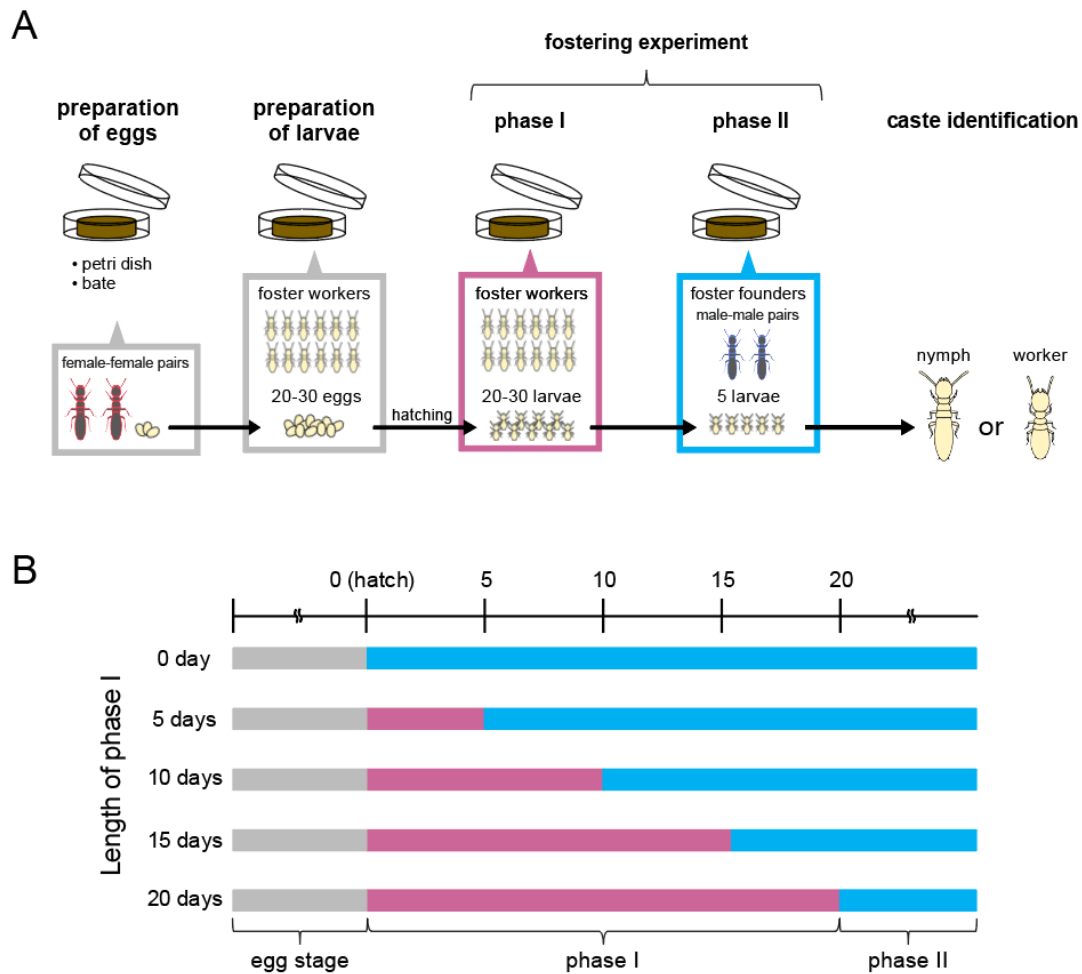


Fig. 1 Schematic diagram of the fostering experiment. **A** Experimental procedures. To obtain parthenogenetic daughters for the fostering experiment, eggs were first collected from female-female pairs during the "preparation of eggs" step. In the subsequent step, "preparation of larvae", the eggs were cared for by foster workers until hatching. The fostering experiment consisted of two phases: in phase I, larvae were cared for by foster workers, and in phase II, by foster founders. Finally, in the "caste identification" step, larvae were identified as either workers or nymphs once they developed to the third instar. The female-female pairs, foster workers, and foster founders were all sourced from the same colony. **B** Treatment groups in the experiment. There were five different period for phase I: 0 (i.e., skipping phase I and moving directly to phase II), 5, 10, 15, and 20 days.

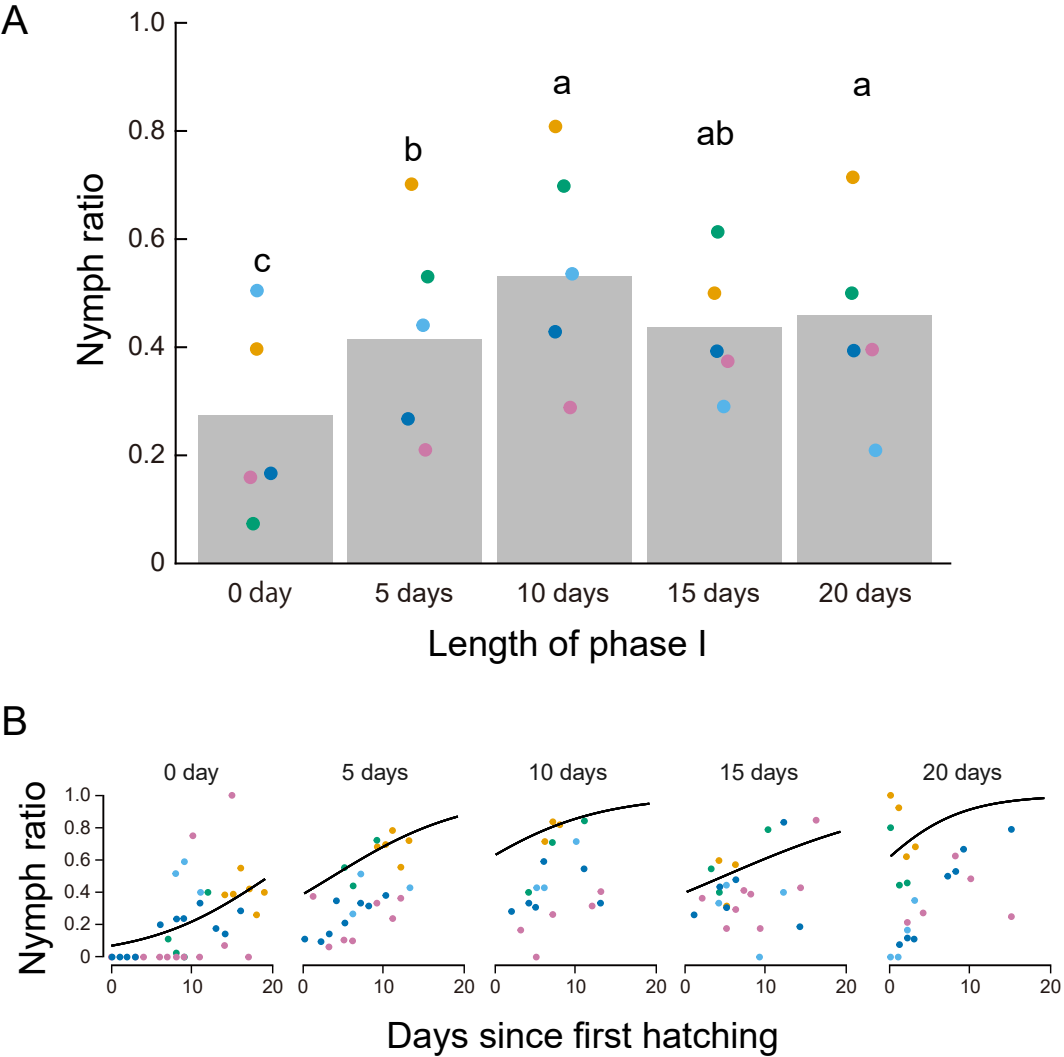


Fig. 2 Innate and socioenvironmental effects on caste fate. **A** The effect of the length of phase I on the proportion of nymphs. The bars represent the proportion of nymphs calculated from the total number of workers and nymphs across all colonies, while the plots, while the plots are color-coded to indicate different colonies. Different letters denote significant differences at the 5% level (Tukey's HSD post hoc tests, $p < 0.05$). **B** The effect of age index on the proportion of nymphs. The graph is divided by the length of phase I. The plots, color-coded, represent the proportion of nymphs for each colony.