Enhanced heterozygosity from male meiotic chromosome chains is superseded by hybrid female asexuality in termites

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Enhanced heterozygosity from male meiotic chromosome chains is superseded by hybrid female asexuality in termites

Short title: Enhanced heterozygosity in male and asexual termites

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Although males are a ubiquitous feature of animals, they have been lost repeatedly in diverse lineages. The tendency for obligate asexuality to evolve is thought to be reduced in animals whose males play a critical role beyond the contribution of gametes, for example via care of offspring or provision of nuptial gifts. To our knowledge, the evolution of obligate asexuality in such species is unknown. In some species that undergo frequent inbreeding, males are hypothesized to play a key role in maintaining genetic heterozygosity through the possession of neo-sex chromosomes, although empirical evidence for this is lacking. Because inbreeding is a key feature of the life cycle of termites, we investigated the potential role of males in promoting heterozygosity within populations, through karyotyping and genome-wide SNP analyses of the drywood termite *Glyptotermes nakajimai*. We showed that males possess up to 15 out of 17 of their chromosomes as sex-linked (sex and neo-sex) chromosomes, and that they maintain significantly higher levels of heterozygosity than do females. Furthermore, we showed that two obligately asexual lineages of this species - representing the only known all-female termite populations - arose independently via intra-specific hybridization between sexual lineages with differing diploid chromosome numbers. Importantly, these asexual females have markedly higher heterozygosity than their conspecific males, and appear to have replaced the sexual lineages in some populations. Our results indicate that asexuality has enabled females to supplant a key role of males, which represents a novel driver of the loss of males in animals.

Keywords: genetic heterozygosity, inbreeding, hybrid asexuality, neo-sex chromosomes

Significance

The evolution of asexuality is thought to be prevented when males play a critical role beyond that of gamete provision. We demonstrated enhanced high numbers of neo-sex chromosomes and heterozygosity in males of the termite *Glyptotermes nakajimai*, which appears to compensate for inbreeding within termite colonies. Furthermore, we showed that two asexual *G. nakajimai* lineages have evolved via independent intra-specific hybridizations between sexual lineages with differing diploid chromosome numbers. This has resulted in markedly higher levels of heterozygosity of females than males in the sexual lineage. Our study illustrates that asexual females may replace the role of males in maintaining heterozygosity, implying a novel route to the evolution of asexuality.

Although asexual populations should have a two-fold reproductive advantage over their sexual relatives (1), sexual reproduction is the rule in almost all animals and plants (2). This is probably because sexual reproduction enables gene pools to be constantly mixed, generates new combinations of genes, and facilitates adaptation to complex and heterogeneous environments (3). Nevertheless, obligately asexual lineages have evolved repeatedly in diverse animal taxa (2, 4, 5), which remains an important unsolved problem in evolutionary biology. Many biologists have approached this problem by considering the advantages of asexuality, and how the disadvantages of asexuality can be circumvented (6–8). In each case, it is thought that the evolution of asexuality should be prevented when males have crucial roles in the biology and life cycle of a species or population (e.g., paternal care for offspring and nuptial gifts for females) (1, 9, 10). Indeed, to our knowledge, the evolution of obligate asexual lineages from ancestors whose males play a critical role beyond that of gamete provision is unknown.

In inbred populations of some species, males potentially play a key role in maintaining heterozygosity through the possession of neo-sex chromosomes (11). Such chromosomal systems are found in some animals and plants, arising as a result of reciprocal translocations or centric fusions between sex chromosomes and autosomes (12–15). Under male heterogamety (i.e., XY = male, XX = female), it has been hypothesized that autosomes that are linked to the Y chromosome (i.e., neo-Y chromosomes) during meiosis never become homozygous by descent in the absence of crossing over, allowing maintenance of heterozygosity (11). Therefore, neo-Y chromosomes would help lineages that undergo frequent inbreeding to reduce genetic costs of inbreeding in males. However, to our knowledge, there have been no empirical tests of this hypothesis. Furthermore, the potential

role of males in maintaining heterozygosity is also expected to reduce the tendency for males to be lost through the evolution of asexuality.

Termites provide an ideal model to explore the role of males in animal species, in particular those species which undergo regular inbreeding. This is because, although almost all termite species undergo outbreeding during swarms of virgin reproductives, inbreeding as a result of sibling-sibling or parent-sibling reproduction within nests appears to be a key feature of the life cycle of many species (16, 17). Nevertheless, reduced genetic heterozygosity in termites caused by inbreeding can result not only in individual-level costs (e.g., reduced fecundity) but also in colony-level costs (e.g., reduced disease resistance) (18, 19). Such inbreeding is thought to have given rise to a striking karyological feature of many termite species: the formation of chains (or rings) of several chromosomes [sex chromosomes (i.e., X and Y chromosomes) plus autosomes (i.e., neo-X and neo-Y chromosomes)] during male meiosis, whereby the Y chromosomes and some autosomes (i.e., neo-Y chromosomes) segregate together as a single linkage group to male-determining sperm (i.e., a neo-Y chromosome system) (14, 20, 21). Heterozygote advantage in the face of inbreeding has been postulated to account for the evolution of this system (11), although extensive genetic analyses examining the effects of neo-Y chromosome systems have not yet been conducted.

We have recently investigated the biology of *Glyptotermes nakajimai* Morimoto (Isoptera: Kalotermitidae) (22), a species of drywood termite found in southern areas of the mainland of Japan, as well as islands further south (23). We examined sex and caste ratios within colonies, sperm storage of egg-laying queens, and hatching success of unfertilized eggs. We discovered the presence of up to 25 secondary (neotenic) reproductives (i.e., offspring of primary kings and queens) in most field colonies, suggesting that inbreeding occurs in this species. Despite the presumed role of males in maintaining heterozygosity in termite populations (described above), we have discovered a number of asexual (all-female) *G.*

nakajimai populations – the first case of an evolutionary transition from mixed-sex to all-female asexual societies (22). Although individuals from asexual and sexual populations are indistinguishable by external morphology and cuticular hydrocarbon profiles (23), previous molecular phylogenetic analyses have shown that asexual and sexual populations respectively form separate monophyletic groups (22). Notably, individuals of asexual populations have an uneven number of chromosomes (2n = 35), in contrast to those of sexual populations (2n = 34) (22). An uneven number of chromosomes in a diploid organism, in particular in females, can arise through hybridization between closely related lineages that differ in diploid chromosome number (e.g., refs. 24, 25). Such hybrids are expected to be sterile due to chromosome pairing incompatibilities during meiosis, providing an opportunity for the evolution of asexuality (13, 26). Importantly, hybrid asexuals in other species are known to often exhibit high and fixed heterozygosity due to the combination of two different genomes (27).

To investigate the evolution of asexuality in species that undergo inbreeding, we used *G. nakajimai* as a model species. We performed a series of analyses based on genome-wide single-nucleotide polymorphisms (SNPs) generated in representatives across the distribution of this species, and examined the karyotypes of selected populations. We sought to address the following questions: (i) what is the population genetic structure of *G. nakajimai*, and how are sexual and asexual *G. nakajimai* individuals related to each other? (ii) Do male *G. nakajimai* possess neo-sex chromosomes, and does heterozygosity vary between males, sexual females, and asexual females? (iii) Did asexual *G. nakajimai* arise via hybridization, as predicted on the basis of chromosome number?

Results and Discussion

Multiple Genetic Clusters Among Sexual and Asexual *G. nakajimai*. We compared 4,191 biallelic SNPs across 84 individuals from sexual and asexual populations of *G. nakajimai* (Fig. 1*A* and Dataset S1*A*). Principal coordinate analysis (PCoA) revealed three distinct clusters (Fig. 1*B*): (i) individuals derived from three sexual populations on small islands in southern Japan, and one on the main island of Honshu [collectively referred to hereafter as sexual lineage 1 (SL1)]; (ii) individuals derived from two asexual populations in Shikoku and three asexual populations in Kyushu [hereafter, asexual lineage 1 (AL1)]; and (iii) individuals derived from three asexual populations in Shikoku [hereafter asexual lineage 2 (AL2)]. Individuals from AL1 and AL2 respectively formed tight genetic clusters, indicative of a lack of genetic variation among members of each asexual lineage. In contrast, SL1 individuals were segregated into four sub-clusters (Fig. 1*B*), reflecting the four different geographically-separated populations (Fig. 1*A*). Significant genetic differentiation between each pair of the four populations of SL1 (i.e., pairwise F_{ST}) was detected (range = 0.376–0.548, $P < 0.001$). On the other hand, all pairwise population F_{ST} values within each of the asexual lineages were lower and non-significant (AL1: range = 0.004–0.019, $P = 0.245$–0.502; AL2: range = 0.000–0.007, $P = 0.253$–0.535) (SI Appendix, Table S1). An analysis using STRUCTURE revealed that the genetic variability observed in individuals from field colonies of *G. nakajimai* was best explained using $K = 10$ (Ln $P = -154{,}082$), and recovered the same genetic clustering as the PCoA (Fig. 1*C*). The two asexual *G. nakajimai* lineages (i.e., AL1 and AL2) are sympatrically distributed in Shikoku (Ashizuri and Muroto populations), and we found that individuals of the two lineages can coexist within a single colony (Fig. 1*B*). This is explained by the fact that incipient colonies of the asexual *G. nakajimai* are founded by multiple queens (range = 2–25) (22).

Neo-sex Chromosomes in Males and Differences in Heterozygosity Levels Between Males, Sexual Females, and Asexual Females. All sexual populations of *G. nakajimai* showed negative mean inbreeding coefficient (F_{IS}) values (-0.228–-0.031), with males displaying lower F_{IS} values than females in each of four populations (SI Appendix, Table S2). This suggests that mechanisms exist to avoid inbreeding in males of *G. nakajimai*. Indeed, similar to the case for other drywood termite species, we observed meiotic chromosome chain-formation in *G. nakajimai* (Fig. 2*A*), with 30 linked-chromosomes found in males (i.e., kings) (n = 2) collected from a population of SL1 (Okinawa Island population). Therefore, out of a total of 17 haploid chromosomes, 15 Y + neo-Y chromosomes are expected to be inherited as a unit. A significant proportion of the genome should therefore be linked, and not undergo recombination with the 15 'homologous' X + neo-X chromosomes when in the male germline (28). This is among the highest number of end-to-end linked chromosomes in male meiosis of any animal or plant (29) and expected to lead to this portion of the genome remaining heterozygous. On the other hand, X + neo-X chromosomes, when in the female germline, retain the capacity to undergo recombination with their homologous chromosomes, potentially allowing heterozygosity to become reduced in females under inbreeding. In addition, examination of meiotic chromosomes from a male (i.e., a king) (n = 1) collected from a second population of SL1 (Ogasawara Islands population) revealed a chain of 12 chromosomes plus 11 bivalents (SI Appendix, Fig. S1). Variability in neo-sex chromosome number within a species has previously been reported among different populations of drywood termite species (20, 30).

In agreement with previous hypotheses on the effects of neo-sex chromosomes (11), we found that male *G. nakajimai* possessed significantly higher mean levels of heterozygosity than females in sexual populations [10.5% vs 7.8% of alleles (SI Appendix, Table S3); P < 0.001, Tukey's honestly significant difference (HSD) test] (Fig. 2*B* and Dataset S1*B*). Mean

heterozygosity was up to 47.6% higher in males than females [range 18.9–47.6% across four sexual populations (SI Appendix, Table S3)]. However, females in both AL1 and AL2 asexual populations were found to possess significantly high heterozygosity levels (approximately four-fold) when compared with both female and male individuals from sexual populations ($P < 0.001$, Tukey's HSD test) (Fig. 2*B* and Dataset S1*B*).

The Mode of Reproduction in Asexual *G. nakajimai*. To date, all known examples of asexual reproduction in lower termites (i.e., all termites excluding the most derived family Termitidae) involve automixis with terminal fusion (31). Under this mode of reproduction (and also under automixis with gamete duplication), offspring are homozygous for a single maternal allele (if no crossing-over takes place), and are expected to contain an even number of chromosomes. The high heterozygosity displayed by asexual *G. nakajimai* (Fig. 2*B*), and the fact that they invariably possess 2n = 35 chromosomes, indicates they do not reproduce via automixis.

On the other hand, clonal reproduction via mitosis (i.e., apomixis) produces offspring that are heterozygous at loci that were also heterozygous in the mother (26, 31), and an uneven number of chromosomes can be maintained through reproduction (32, 33). To assess the mode of reproduction in *G. nakajimai*, we compared SNP genotypes between queens (i.e., mothers) and their larvae (i.e., offspring) in laboratory-founded colonies whose natal colony had been collected from an asexual population (Ashizuri population), where all individuals (two queens and one larva from each of three laboratory-founded colonies) were identified as AL1 by additional PCoA (SI Appendix, Fig. S2). The offspring inherited all or nearly all heterozygous SNP loci (99.5–100% of the loci) from the mother, whereas a small portion of homozygous SNP loci of the mothers changed to heterozygous in the offspring (0–0.6% of the loci) (Table 1 and Dataset S1*A*). Given the presence of new mutations among asexual

offspring, this result is suggestive of apomixis where almost all heterozygosity is maintained. In addition, we conducted a crossbreeding experiment with the asexual and sexual *G. nakajimai*. As expected for apomixis where meiosis is suppressed, the hatching success of hybrid eggs of the asexual and sexual *G. nakajimai* was much lower than that of unfertilized eggs of the asexual *G. nakajimai* ($P < 0.0001$, Fisher's exact probability test) (SI Appendix, Fig. S3). Only 9 of 59 hybrid eggs (15.3%) developed into larvae, possibly being triploid (infertile) individuals (Dataset S1*C*). Further work involving cytological observation of chromosomes during oogenesis and embryogenesis of both AL1 and AL2 is required to confirm clonal reproduction as the mode of reproduction in asexual *G. nakajimai*.

Hybrid Origin of Asexual *G. nakajimai*. Under the assumption that asexual *G. nakajimai* individuals arise via clonal reproduction, their high levels of heterozygosity have two potential origins. One is the accumulation of mutations in allele pairs [i.e., the Meselson effect, as seen in ancient asexual animals and plants (34, 35)]; another is through hybridization of genetically divergent parent taxa. In the former case, asexual individuals that show high levels of divergence from one another at nuclear loci are also expected to display divergence at mitochondrial loci. In the case of AL1 and AL2, which show clear divergence at nuclear loci (Fig. 1*B*), examination of a 702 base pair (bp) fragment of the mitochondrial cytochrome *c* oxidase subunit II (*COII*) gene between AL1 and AL2 revealed 100% identity (GenBank accession numbers MT387025–MT387032 and MT387033–MT387036 respectively). This suggests that high levels of heterozygosity within asexual individuals have not arisen through gradual accumulation of mutations over long periods of evolutionary time.

Instead, the origin of asexual *G. nakajimai* can be reasonably explained through intra-specific hybridizations between sexual lineages having different chromosome numbers, 2n = 34 and 2n = 36, respectively. Each parental lineage would have contributed n = 17 and n = 18

chromosomes respectively to their hybrid offspring, explaining the presence of 2n = 35 chromosomes in asexual individuals (22). Following initial hybridization, females would have then undergone clonal reproduction (see above section). The complete identity of *COII* sequences between AL1 and AL2 individuals suggests the maternal ancestors of the two lineages are genetically similar or very closely related. Therefore, the genetic differences between AL1 and AL2 (Fig. 1*B*) can be primarily attributed to differences between their paternal ancestors. Interestingly, the STRUCTURE analysis at $K = 2$ revealed that individuals of AL2 possessed mixed genetic components from AL1 and SL1 (Fig. 1*C*). Given the presence of reproductive barriers between the asexual and sexual lineages (described above), this implies that AL2 comprises hybrids with half ancestry from SL1 as the paternal ancestor [which has 2n = 34 chromosomes (22)] and another (unidentified) sexual lineage as the maternal ancestor (consequently having 2n = 36 chromosomes; SL2 in SI Appendix, Fig. S4). AL1 is predicted to have arisen from hybridization between this same maternal ancestor, and a third unidentified sexual lineage with 2n = 34 (paternal ancestor; SL3 in SI Appendix, Fig. S4).

Other termite species that exhibit facultative asexual reproduction are known to produce eggs without openings for sperm entry (micropyles) (36). We counted the number of micropyles of eggs collected from a field colony of each of two populations of the asexual *G. nakajimai*, as well as those collected from a field colony of each of two populations of the sexual *G. nakajimai*. We found that all examined eggs of the asexual *G. nakajimai* possessed a substantial number of micropyles [Tokushima population (which probably contains only individuals of AL2, as mentioned below): 48.29±4.26 SEM; range = 32–66; $n = 7$, Saiki population (which probably contains only individuals of AL1, as mentioned below): 46.50±4.10 SEM; range = 35–61; $n = 8$] (SI Appendix, Fig. S5 *A* and *B* and Dataset S1*D*). In addition, no significant difference in the number of micropyles was observed between eggs of

the asexual and sexual *G. nakajimai* (colony: $F_{2, 26} = 0.13$, $P = 0.88$; Reproductive type: $F_{1, 26} = 2.80$, $P = 0.11$; nested ANOVA with colonies nested within reproductive types) (SI Appendix, Fig. S5*B*). Thus, the evolution of asexuality in *G. nakajimai* cannot be explained by the production of eggs without micropyles.

The Evolution of Asexuality in *G. nakajimai*. To estimate when AL1 and AL2 originated, we performed pairwise comparisons of SNP genotypes between individuals within each lineage. Of the 4,191 loci, 103 and 145 were the largest number of SNP differences between individuals within AL1 and AL2, respectively (Dataset S1*A*). Given that the mode of reproduction in the asexual lineages of *G. nakajimai* appears to be apomixis (see above), the number of generations of the two lineages can be roughly calculated as the largest number of SNP differences between individuals within a lineage divided by the number of new SNP mutations per generation, divided by two. The above-mentioned comparison of SNP genotypes between the mothers and their offspring in laboratory-founded colonies showed that the number of new SNP mutations in one generation was about 11.33 (calculated from the data in Table 1). Thus, the calculated generation numbers of AL1 and AL2 are 9 and 13, respectively. In drywood termites, new queens are produced after colony maturation, which requires about 4 years (37), and the reported maximum of queen lifespan is 14 years (38). As a result, the estimated ages of AL1 and AL2 were 18–63 [i.e., (9 × 4–14) / 2] and 26–91 [i.e., (13 × 4–14) / 2] years, respectively. These results suggest that the two asexual lineages originated recently (within the last few hundred years). To further examine the maternal origin of AL1 and AL2, we sequenced a 144 bp fragment of the mitochondrial *A+T-rich* region. We detected 3 changes across this region between AL1 and AL2 (GenBank accession numbers MT387011–MT387017 and MT387018–MT387024 respectively). Based on intraspecific rates of insect mitochondrial evolution, we estimated the divergence time of the

maternal lineage of AL1 and AL2 as 104,000–333,000 years ago (39). This suggests that the maternal ancestors of AL1 and AL2 might have had different sequences in their *A+T-rich* regions.

An analysis of contemporary gene flow between populations revealed evidence for migration between asexual, but not sexual, populations (Fig. 3 and SI Appendix, Table S4). These results indicate that the Tokushima and Sata populations may be the primary source for other populations in AL1 and AL2, respectively. Notably, the Ashizuri and Muroto populations were predicted to have received migrants from both asexual lineages, in contrast to the presence of only one type of asexual lineage in each of other populations (Fig. 3). These results, in combination with the predicted origins of both AL1 and AL2 within the last few hundred years (described above), suggest that human movement of one or more sexual lineages of *G. nakajimai* may have led to novel hybridization events, and the appearance of novel asexual lineages. Based on our widespread sampling across the breadth of the distribution of *G. nakajimai*, it appears that these novel asexual lineages have replaced two of their predicted sexual ancestors (i.e., SL2 and SL3; SI Appendix, Fig. S4) on the mainland of Japan, since the only sexual lineage we detected was SL1 [which was found only at the southernmost part of Honshu (Kushimoto)]. We hypothesize that such replacement of sexual lineages by asexual lineages would have been facilitated by the high levels of heterozygosity in asexual lineages compared with sexual lineages (inferred from our comparison of heterozygosity levels in AL1 and AL2 with SL1; Fig. 2*B*), despite the presence of neo-sex chromosomes in the sexual lineages. The twofold rate of production of females by asexuals compared with sexuals is another advantage that would promote the spread of the former.

Hybridization between closely related social insect lineages has been shown to have unusual outcomes with regard to the production of different castes within colonies. In *Pogonomyrmex* spp. harvester ants, it has led to the genetic determination of the queen caste,

and worker offspring with high heterozygosity (40, 41). In *G. nakajimai*, all colony members possess relatively high heterozygosity in relation to their sexual relatives, and caste determination appears unaffected as a result of hybridization.

Conclusion

Although inbreeding is generally thought to be risky due to the negative effects of deleterious alleles on fitness when in the homozygous state (42, 43), some animals and plants (e.g., social animals and selfing plants) frequently undergo inbreeding as a part of their life-history (44–46). This can be partly explained by potential benefits of inbreeding, such as reproductive assurance, local adaptation, and inclusive fitness (44, 47). However, how such organisms persist over evolutionary time in the face of presumed genetic consequences of inbreeding is not well understood. Frequent inbreeding within a population enables purging of the genetic load, but a number of studies have shown that efficient purging of deleterious mutations may not occur even in consistently inbred lineages (48, 49). Our study indicates that the evolution of neo-sex chromosomes in *G. nakajimai* results in enhanced heterozygosity in males compared with females, potentially reducing the genetic costs of inbreeding at the colony level in this species. Nevertheless, sexual *G. nakajimai* populations appear to have been replaced on Kyushu and Shikoku by recently evolved and highly heterozygous asexual lineages (as a result of their hybrid origin). Our results indicate that asexual females can supplant a key role of males, which represents a novel driver of the loss of males in animal lineages.

Materials and Methods

Termite Collection. We collected 17 mature colonies of *G. nakajimai* from four sexual populations [Honshu (Kushimoto), Amami-Oshima Island, Okinawa Island, and Ogasawara Islands, Japan] and six asexual (all-female) populations [Shikoku (Ashizuri, Muroto, and Tokushima) and Kyushu (Sata, Toi, and Saiki), Japan] from November 2014 to May 2021. The colonies were transported back to the laboratory with colonized wood. The nest woods were dismantled and all colony members [reproductives (queens and kings), soldiers, workers, nymphs, alates, and young instars] were extracted using an aspirator and forceps. The eggs were also collected if they were present. Individuals from each colony were placed in a moist unwoven cloth in a 90-mm Petri dish and preserved at −25 °C until sexing was carried out. The sex of individuals was determined based on the configuration of the caudal sternites (22) under a stereomicroscope (SZX7; Olympus). Portions of workers and nymphs from each colony were kept in the laboratory as stock colonies in 90-mm Petri dishes that contained damp chips of sliced Oregon pine wood at 25 °C under constant darkness until subsequent experiments.

Genome-wide SNP Analyses. We conducted high-throughput genome-wide SNP genotyping of individuals from sexual and asexual populations of *G. nakajimai*. Five female and five male workers randomly chosen from each of the four field colonies of sexual populations collected in Kushimoto (colony code: IZ150430A), Amami-Oshima Island (colony code: NK150527C), Okinawa Island (colony code: HD160328C), and Ogasawara Islands (colony code: CC151014G), ten female workers were randomly chosen from each of the four field colonies of asexual populations collected in Ashizuri (colony code: AS141111K), Muroto (colony code: MR150217B), Sata (colony code: ST160304C), and Toi (colony code: TI150728A), two female workers randomly chosen from each of the two field colonies of asexual populations collected in Tokushima (colony code: TO150911B) and Saiki

(colony code: SK150715A), and two queens and one larva from each of the three laboratory-founded colonies whose natal colony had been collected in Ashizuri (colony code: AS141111C) (details of the laboratory-founded colonies are described below) were used for genotyping. The termite individuals were preserved in 99.5% (vol/vol) ethanol for genotyping. DNA was extracted from the whole body (excluding gut) of each individual using the High Pure PCR Template Preparation Kit (Roche). Genotyping was performed by Diversity Arrays Technology Pty. Ltd. using DArTseq (50–53). Four methods of complexity reduction were tested in the *Glyptotermes* termites and double digestions with PstI-SphI method were selected. Further genotyping methodology details are published elsewhere (54). Approximately 152,000 sequences per barcode per sample were identified and used in marker calling. After quality-filtering using the R package "dartR v0.93" (55), our data yielded 4,191 SNPs (average call rate 100%, average reproducibility rate 100%) (Dataset S1*A*).

To visualize genetic similarities and differences among individuals and populations, we generated a PCoA for individuals from the field colonies using the R package "dartR v0.93" (55).

To investigate patterns of population structure and admixture among populations, we performed a Bayesian clustering analysis of the SNP data using STRUCTURE v2.3.4 (56) implemented in parallel through StrAuto 1.0 (57). Markov chain Monte Carlo simulations were performed under the assumption of one to ten genetic clusters (K), with 10 replicates of 500,000 iterations for each value of K and with 10% burn-in. All analyses allowed admixture and independent allele frequencies. The Markov chains reached convergence and alpha values were stable after 200,000 iterations. Owing to the known problem of inferring population clustering from ΔK (58, 59), the optimal K value was inferred using a hierarchical approach by sequential STRUCTURE analyses of clusters identified at each step (60). The

386 results of each replicate of *K* were summarized using CLUMPAK v1.1.2 (61) and
387 STRUCTURE HARVESTER (web) v0.6.94 (62) to obtain marginal likelihoods. Bar plots
388 were generated using DISTRUCT v1.1 (63).
389 To estimate the direction and magnitude of contemporary gene flow among populations,
390 we analyzed the SNP data using a Bayesian approach (64) in BayesAss v3 (65). Each run
391 was 8×10 steps, with a burn-in of 2×10^7 steps and sampling every 8,000 steps. The mixing
392 parameter of ΔA (allele frequencies) was optimized at 0.6 to ensure appropriate acceptance
393 rates.
394 Based on the evidences of the SNP analyses that the asexual *G. nakajimai* contains two
395 lineages (i.e., AL1 and AL2), we further compared the percentage of heterozygous loci
396 within individuals between males of SL1, females of SL1, females of AL1, and females of
397 AL2 (Dataset S1*B*) using nested ANOVA followed by Tukey's HSD test (Statistica 10;
398 StatSoft). Percent data were arcsine-transformed prior to analysis. In addition, we performed
399 the following analyses. We measured pairwise population F_{ST} for SL1, AL1, and AL2 by
400 analysis of molecular variance (AMOVA) with 9,999 permutations in GENALEX 6.5 (66).
401 We calculated mean F_{IS} values for males and females in each sexual populations, for males in
402 each sexual populations, for females in each sexual populations, and for females in each
403 asexual populations using GENALEX 6.5 (66).
404
405 **Cytological Analysis.** To examine the male mitotic and meiotic karyotypes of the sexual *G.*
406 *nakajimai*, we used two primary kings from two field colonies collected from one of the
407 sexual populations, Okinawa Island (colony code: NJ210511A and NJ210511B), and a
408 neotenic king from one of the field colonies from Ogasawara Islands. The mitotic and
409 meiotic chromosomes of these individuals were successfully observed using the lactic acid
410 dissociation drying method (modified from refs. 67, 68). Demecolcine (colcemid) was used

411 to block cells in metaphase. The chromosomes of kings from Okinawa Island were stained
412 with 4′,6-diamidino-2-phenylindole (DAPI) and observed with a confocal microscope
413 (FV1000; Olympus). The chromosomes of a king from Ogasawara Islands were stained with
414 3% Giemsa and observed with an optical microscope (TBR-1; Yashima Optical).
415
416 **Mitochondrial *A+T-rich* and *COII* Sequencing.** Based on the evidences of the SNP
417 analyses that the asexual *G. nakajimai* contains two lineages, we compared mitochondrial
418 *A+T-rich* and *COII* sequences between them. The extracted DNA of 14 and 12 individuals
419 (including at least one individual of each lineage from each population when present) that
420 genotyped as described above were used for *A+T-rich* and *COII* sequencing, respectively. A
421 fragment of *A+T-rich* was amplified by PCR using the following custom primer set, modified
422 from ref. 69: Forward primer (5'-TATTTTGGTGGTGGTTGGTGCAC-3'), reverse primer
423 (5'-CCTACAAACACAATAACAFC-3'). PCR for *A+T-rich* was performed on a MyCycler
424 thermal cycler system (Bio-Rad) with initial denaturation at 95 °C for 2 min, followed by 35
425 cycles of denaturing at 94 °C for 30 s, annealing at 52 °C for 30 s, extension at 72 °C for 90 s,
426 and a final extension at 72 °C for 5 min. A fragment of *COII* was amplified by PCR using the
427 primer set, TL2-J-3037 (5'-ATGGCAGATTAGTGCAATGG-3') and TK-N-3785 (5'-
428 GTTTAAGAGACCAGTACTTG-3') (70). The PCR for *COII* consisted of initial
429 denaturation at 94 °C for 1 min, followed by 35 cycles of denaturing at 94 °C for 30 s,
430 annealing at 50 °C for 30 s, extension at 72 °C for 40 s, and a final extension at 72 °C for 3
431 min. PCR products for *A+T-rich* and *COII* were sequenced in both directions in a
432 commercial sequencing facility (Macrogen Inc.), and forward and reverse chromatograms
433 were edited using BioEdit 7.0.4.1 (71) and resulted in a 144 nucleotide sequence and a 702
434 nucleotide sequence, respectively. The *A+T-rich* and *COII* sequences obtained in this study

were deposited in the DDBJ/EMBL/GenBank nucleotide sequence databases under accession numbers MT387011–MT387036.

Divergence time was estimated based on the *A+T-rich* sequences and intraspecific rates of mitochondrial evolution (39).

Micropyle Analysis. To count the number of micropyles of eggs, we used all collected eggs of two field colonies of asexual populations [7 eggs of a colony from Tokushima (colony code: TO150911B) and 8 eggs of a colony from Saiki (colony code: SK150715A)] and those of sexual populations [5 eggs of a colony from Kushimoto (colony code: SN150430C) and 10 eggs of a colony from Amami-Oshima Island (colony code: NZ150526A)] of *G. nakajimai*. The number of micropyles of eggs was counted under scanning electron microscope (VE-8800; Keyence). To compare the numbers of micropyles of eggs between the asexual and sexual *G. nakajimai* (Dataset S1*D*), we used nested ANOVA followed by Tukey's HSD test.

Investigation of the Mode of Asexual Reproduction. To investigate the mode of reproduction in the asexual *G. nakajimai*, we genotyped the primary queens and larvae in the laboratory-founded colonies. Virgin female alates were obtained from a colony collected from one of the asexual populations, Ashizuri (colony code: AS141111C). Colonies of asexual populations are founded by more than two female alates (young queens) (22), probably due to the necessity of grooming partners that would be essential to survive in a pathogen-rich environment because termites cannot clean the whole of their body through self-grooming (67). Therefore, two virgin female alates were randomly chosen from the colony and placed in 35-mm Petri dish that contained layers of a filter paper and two damp chips of Oregon pine wood (22.5 × 22.5 × 4 mm), as described in a previous study (72). This procedure was replicated 20 times. The laboratory-founded colonies were kept at 25 °C under

constant darkness for 500 days. Although 17 of 20 laboratory-founded colonies could not survive for 500 days, two queens and one larva (all survived individuals) were obtained from each of the rest three laboratory colonies (I–III). The six queens and three larvae were genotyped as detailed above. Using the SNP data, we calculated the percentage of SNP identity between individuals. The percentage of SNP identity between an offspring (larva) and its two possible mothers (queens) was compared, and the queen with the highest genetic similarity to an individual larva was the inferred mother. The percentage of heterozygous in an individual larva for the SNPs where the inferred mother was heterozygous was calculated, and then the observed proportion of heterozygosity in the offspring were compared with the expected proportion of heterozygosity in candidate modes of asexual reproduction.

Based on the evidences of the SNP analyses that the asexual *G. nakajimai* contains two lineages (i.e., AL1 and AL2), we further conducted an additional PCoA for individuals both from the field colonies and the laboratory-founded colonies using the R package "dartR v0.93" (55) to determine whether individuals of the laboratory-founded colonies belong to AL1 or AL2.

Crossbreeding Experiment with the Asexual and Sexual *G. nakajimai*. To investigate the possibility of hybridization between the asexual and sexual *G. nakajimai*, we performed a crossbreeding experiment. Virgin alates were obtained from two stock colonies of asexual populations collected in Muroto (colony code: MR150910D) and Sata (colony code: ST160304C) and of sexual populations collected in Kushimoto (colony code: IZ150430A) and Ogasawara Islands (colony code: HH151016D), separated by sex before swarming began, and maintained in 90-mm Petri dishes containing moist unwoven clothes until they shed their wings (i.e., dealates). Then, individual dealates were randomly chosen from each colony and assigned to either pairs of a female from an asexual population and a male from a sexual

485 population (FM pairs) or pairs of females from an asexual population (FF pairs), where FM
486 pairs consisted of four different combinations ($F_{MR150910D}M_{IZ150430A}$, $F_{MR150910D}M_{HH151016D}$,
487 $F_{ST160304C}M_{IZ150430A}$, and $F_{ST160304C}M_{HH151016D}$) and FF pairs consisted of two different
488 combinations ($F_{MR150910D}F_{MR150910D}$ and $F_{ST160304C}F_{ST160304C}$). Each combination was
489 replicated ten times. Pairs were placed in a 52 × 76-mm glass cell that contained mixed
490 sawdust bait blocks, as described in a previous study (67). The glass-cell colonies were kept
491 at 25 °C under constant darkness for 100 days. We counted eggs and larvae by checking the
492 glass-cell colonies every 3 days. The hatching success, calculated as percentage of eggs
493 hatched within 100 days after colony foundation, was compared among eggs of glass-cell
494 colonies founded by FM pairs and those of glass-cell colonies founded by FF pairs using
495 Fisher's exact probability tests with sequential Bonferroni correction (Statistica 10; StatSoft).
496 Because egg protection behavior by reproductives is indispensable for egg survival, data for
497 the glass-cell colonies in which at least one reproductive died were excluded from the
498 analysis. In addition, we genotyped the reproductives (primary queens and kings) and
499 newborn larvae in glass-cell colonies founded by FM pairs at two polymorphic microsatellite
500 loci (*Gly8* and *Gly18*) as described before (22), and data for asexual offspring in the colonies
501 of FM pairs were excluded from the analysis (SI Appendix, Table S5). Moreover, because
502 there were no significant differences between the combinations and between the glass-cell
503 colonies within pair types (i.e., FM pairs and FF pairs), respectively ($P > 0.05$, Fisher's exact
504 probability test with sequential Bonferroni correction [Statistica 10; StatSoft]), we pooled the
505 data for both the combinations and the glass-cell colonies of each pair type and compared the
506 hatching success between eggs of FM pairs (i.e., hybrid eggs of the asexual and sexual *G.*
507 *nakajimai*) and those of FF pairs (i.e., unfertilized eggs of the asexual *G. nakajimai*) (Dataset
508 S1*C*) using Fisher's exact probability tests.
509

Data Availability. DNA sequences are available from GenBank under accession numbers MT387011–MT387036. All other data used in this study are available in Dataset S1.

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Author contributions: T.Y., K.M., and N.L. designed research; T.Y., N.M., and S.H. provided resources; T.Y., Y.-K.T., T.N., N.M., and S.H. performed experiments; T.Y., Y.-K.T., C.V.D.W., and N.L. analyzed data; T.Y. and N.L. wrote the first draft of the paper and all authors contributed substantially to revisions.

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691 **Table 1. Genotypes of offspring produced by laboratory-founded colonies of the asexual *Glyptotermes nakajimai***

Individual	H_O1 (no. of hetero-/homozygous SNP loci)	H_O2 (no. of hetero-/homozygous SNP loci)
Colony I		
Offspring-1	1.000 (1698/0)	0.000 (0/2493)
Colony II		
Offspring-1	0.995 (1690/8)	0.006 (14/2479)
Colony III		
Offspring-1	0.996 (1702/7)	0.002 (5/2477)

692 H_O1, observed proportion of heterozygosity in the SNP loci of the offspring for the locus that were heterozygous in the mother;
693 H_O2, observed proportion of heterozygosity in the SNP loci of the offspring for the locus that were homozygous in the mother.
694

695 **Figure legends**

696 **Fig. 1.** Population genetic structure in *Glyptotermes nakajimai.* (*A*) Map showing the
697 sampling sites of six asexual (all-female) populations [Ashizuri (AS), Muroto (MR),
698 Tokushima (TK), Sata (ST), Toi (TI), and Saiki (SK)] and four sexual populations
699 [Kushimoto (KS), Amami-Oshima Island (AM), Okinawa Island (OK), Ogasawara Islands
700 (OG)] across Japan. (*B*) PCoA of 84 individuals from field colonies of asexual (ten or two
701 female workers from a field colony in each of six populations) and sexual (five male and five
702 female workers from a field colony in each of four populations) *G. nakajimai* based on
703 genetic distance calculated using 4,191 SNPs, resulting in three distinct groups: asexual
704 lineage 1 (AL1), asexual lineage 2 (AL2), and sexual lineage 1 (SL1). PC1 and PC2 are the
705 first and second principal coordinates, respectively, and the numbers in parentheses refer to
706 the proportion of variance explained by the principal coordinates. (*C*) Structure clustering of
707 the six asexual and four sexual populations using 4,191 SNP markers obtained for $K = 2$
708 (*top*) and $K = 10$ (*bottom*).
709
710 **Fig. 2.** Enhanced heterozygosity in males by male meiotic chromosome chain-formation, and
711 markedly higher heterozygosity in asexual females than males and sexual females in
712 *Glyptotermes nakajimai*. (*A*) Mitotic (*left*) and meiotic (*right*) chromosomes of a male from
713 the Okinawa Island population of the *G. nakajimai* sexual lineage 1 (SL1). A diploid
714 chromosome complement of 2n = 34 is seen in members of this and other populations of SL1
715 (ref. 22). Meiotic chromosomes show the characteristic chain formation of a subset of
716 chromosomes (arrow), as seen commonly in kalotermitid termites (refs. 20, 21, 30). The male
717 meiotic chromosome complement includes a chain of 30 chromosomes, which is predicted to
718 comprise 15 Y and neo-Y chromosomes and 15 X and neo-X chromosomes, plus 2 bivalents.
719 At the end of meiosis, all Y and neo-Y chromosomes are expected to be inherited together

into one gamete, while all X and neo-X chromosomes are expected to be inherited together into a separate gamete. Each gamete also inherits one copy of each non-sex-linked chromosome in a random fashion. (*B*) Comparison of the percentage of heterozygous SNP loci between males of SL1 (n = 20), females of SL1 (n = 20), females of the *G. nakajimai* asexual lineage 1 (AL1) (n = 33), and females of the *G. nakajimai* asexual lineage 2 (AL2) (n = 11). Values are mean ± SEM. Different letters on the bars indicate significant differences [$P < 0.001$, Tukey's HSD test following nested ANOVA (colony: $F_{12, 68} = 27.58$, $P < 0.0001$; subject: $F_{3, 68} = 12482$, $P < 0.0001$; nested ANOVA with colonies nested within subjects)].

Fig. 3. Contemporary gene flow and migration rates between populations of *Glyptotermes nakajimai* estimated from the SNP data using BayesAss. Arrows indicate direction of gene flow among populations. Values are mean rates. Only gene flows significantly greater than zero are shown. Distribution of the lineages was estimated by SNP genotyping. AL1, the *G. nakajimai* asexual lineage 1; AL2, the *G. nakajimai* asexual lineage 2; SL1, the *G. nakajimai* sexual lineage 1.

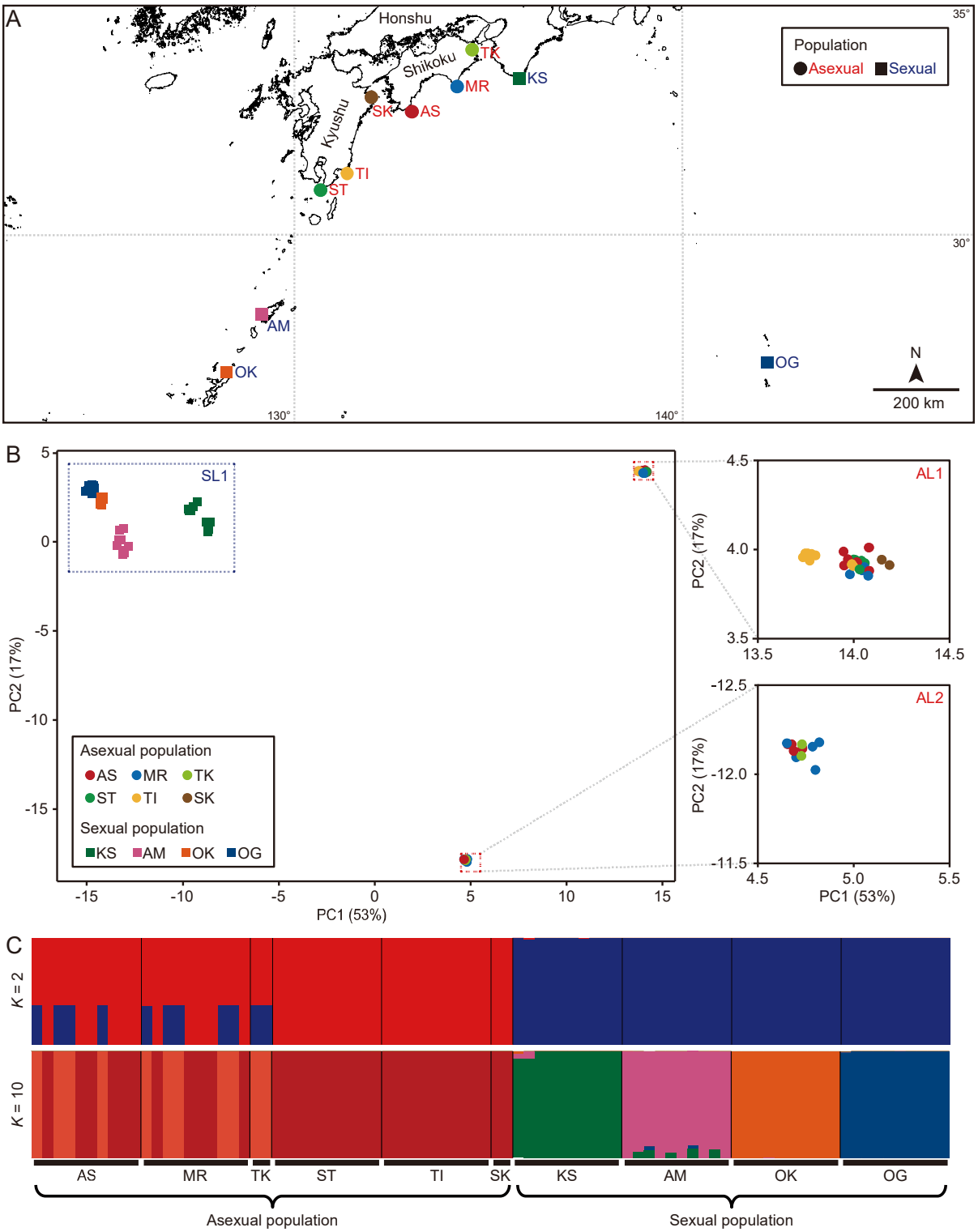

Fig. 1. Population genetic structure in *Glyptotermes nakajimai*. (*A*) Map showing the sampling sites of six asexual (all-female) populations [Ashizuri (AS), Muroto (MR), Tokushima (TK), Sata (ST), Toi (TI), and Saiki (SK)] and four sexual populations [Kushimoto (KS), Amami-Oshima Island (AM), Okinawa Island (OK), Ogasawara Islands

(OG)] across Japan. (*B*) PCoA of 84 individuals from field colonies of asexual (ten or two female workers from a field colony in each of six populations) and sexual (five male and five female workers from a field colony in each of four populations) *G. nakajimai* based on genetic distance calculated using 4,191 SNPs, resulting in three distinct groups: asexual lineage 1 (AL1), asexual lineage 2 (AL2), and sexual lineage 1 (SL1). PC1 and PC2 are the first and second principal coordinates, respectively, and the numbers in parentheses refer to the proportion of variance explained by the principal coordinates. (*C*) Structure clustering of the six asexual and four sexual populations using 4,191 SNP markers obtained for $K = 2$ (*top*) and $K = 10$ (*bottom*).

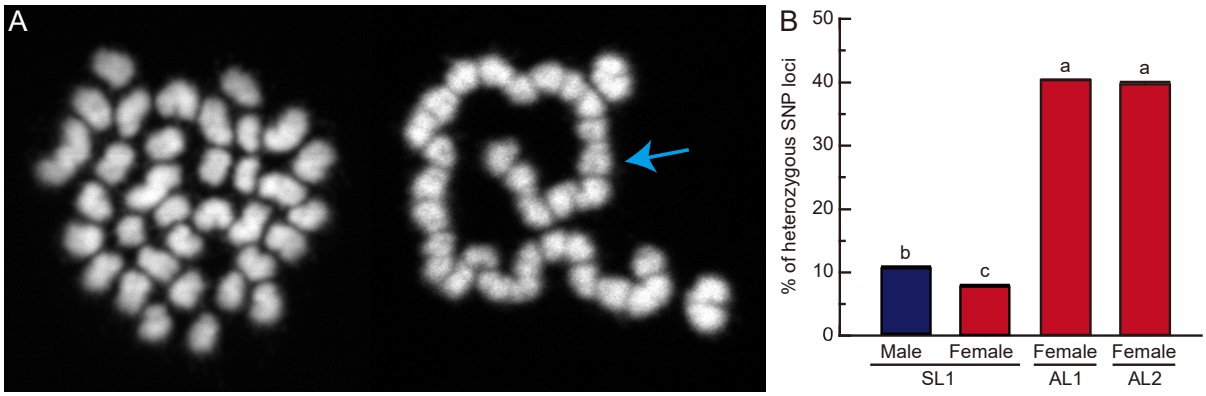

Fig. 2. Enhanced heterozygosity in males by male meiotic chromosome chain-formation, and markedly higher heterozygosity in asexual females than males and sexual females in *Glyptotermes nakajimai*. (*A*) Mitotic (*left*) and meiotic (*right*) chromosomes of a male from the Okinawa Island population of the *G. nakajimai* sexual lineage 1 (SL1). A diploid chromosome complement of 2n = 34 is seen in members of this and other populations of SL1 (ref. 22). Meiotic chromosomes show the characteristic chain formation of a subset of chromosomes (arrow), as seen commonly in kalotermitid termites (refs. 20, 21, 31). The male meiotic chromosome complement includes a chain of 30 chromosomes, which is predicted to comprise 15 Y and neo-Y chromosomes and 15 X and neo-X chromosomes, plus 2 bivalents. At the end of meiosis, all Y and neo-Y chromosomes are expected to be inherited together into one gamete, while all X and neo-X chromosomes are expected to be inherited together into a separate gamete. Each gamete also inherits one copy of each non-sex-linked chromosome in a random fashion. (*B*) Comparison of the percentage of heterozygous SNP loci between males of SL1 ($n = 20$), females of SL1 ($n = 20$), females of the *G. nakajimai* asexual lineage 1 (AL1) ($n = 33$), and females of the *G. nakajimai* asexual lineage 2 (AL2) ($n = 11$). Values are mean ± SEM. Different letters on the bars indicate significant differences [$P < 0.001$, Tukey's HSD test following nested ANOVA (colony: $F_{12, 68} = 27.58$, $P < 0.0001$; subject: $F_{3, 68} = 12482$, $P < 0.0001$; nested ANOVA with colonies nested within subjects)].

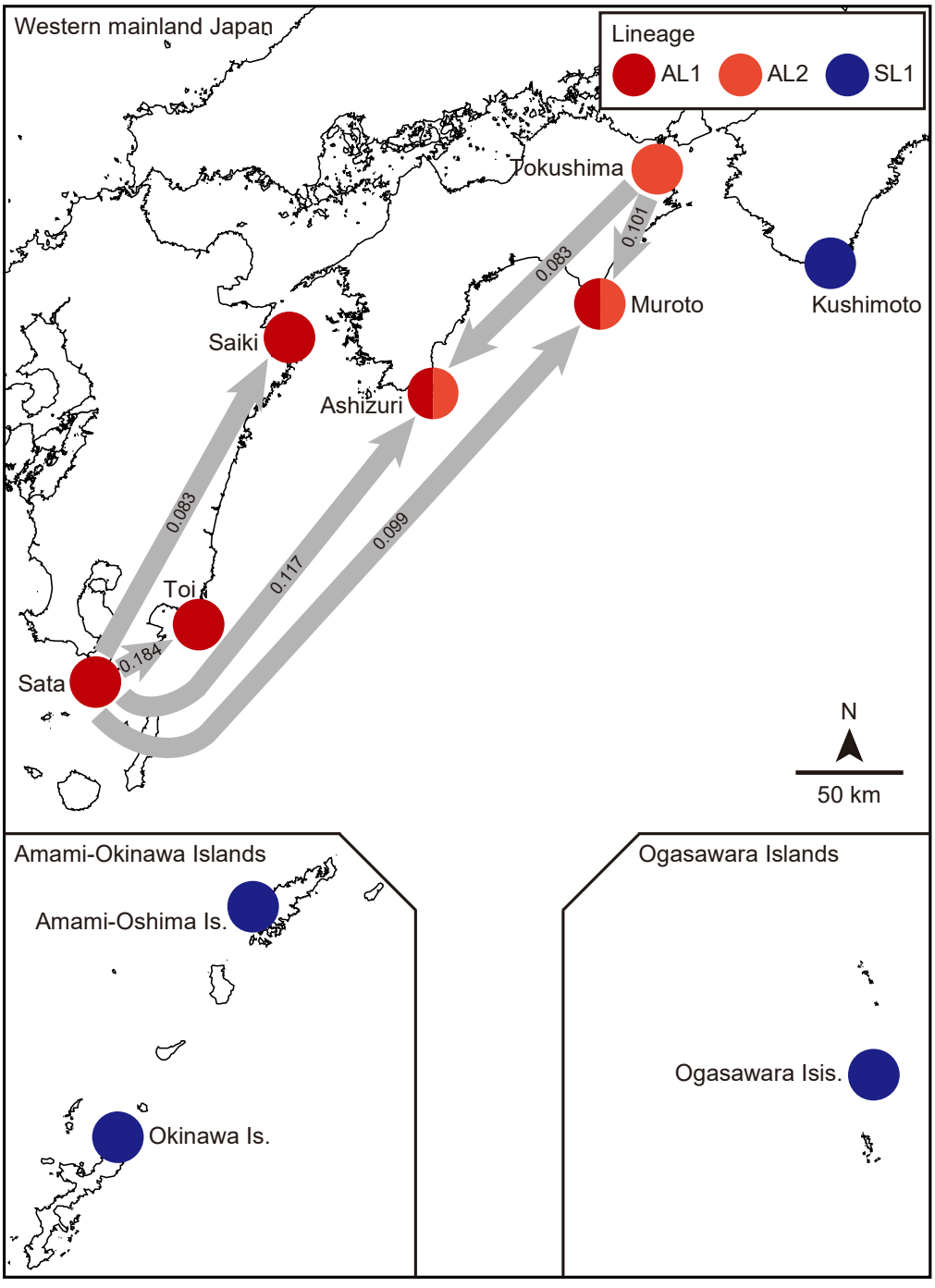

Fig. 3. Contemporary gene flow and migration rates between populations of *Glyptotermes nakajimai* estimated from the SNP data using BayesAss. Arrows indicate direction of gene flow among populations. Values are mean rates. Only gene flows significantly greater than zero are shown. Distribution of the lineages was estimated by SNP genotyping. AL1, the *G. nakajimai* asexual lineage 1; AL2, the *G. nakajimai* asexual lineage 2; SL1, the *G. nakajimai* sexual lineage 1.

Supporting Information for

Enhanced heterozygosity from male meiotic chromosome chains is superseded by hybrid female asexuality in termites

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This PDF file includes:

Figs S1 to S5
Tables S1 to S5

Other supplementary materials for this manuscript include the following:

Datasets S1

Fig. S1. Mitotic (*left*) and meiotic (*right*) chromosomes of a male from the Ogasawara Islands population of the *G. nakajimai* sexual lineage 1 (SL1). A diploid chromosome complement of 2n = 34 is seen in members of this and other populations of SL1 (ref. S1). Meiotic chromosomes show the characteristic chain formation of a subset of chromosomes (arrow), as seen commonly in kalotermitid termites (refs. S2–S4). The male meiotic chromosome complement includes a chain of 12 chromosomes, which is predicted to comprise 6 Y and neo-Y chromosomes and 6 X and neo-X chromosomes, plus 11 bivalents.

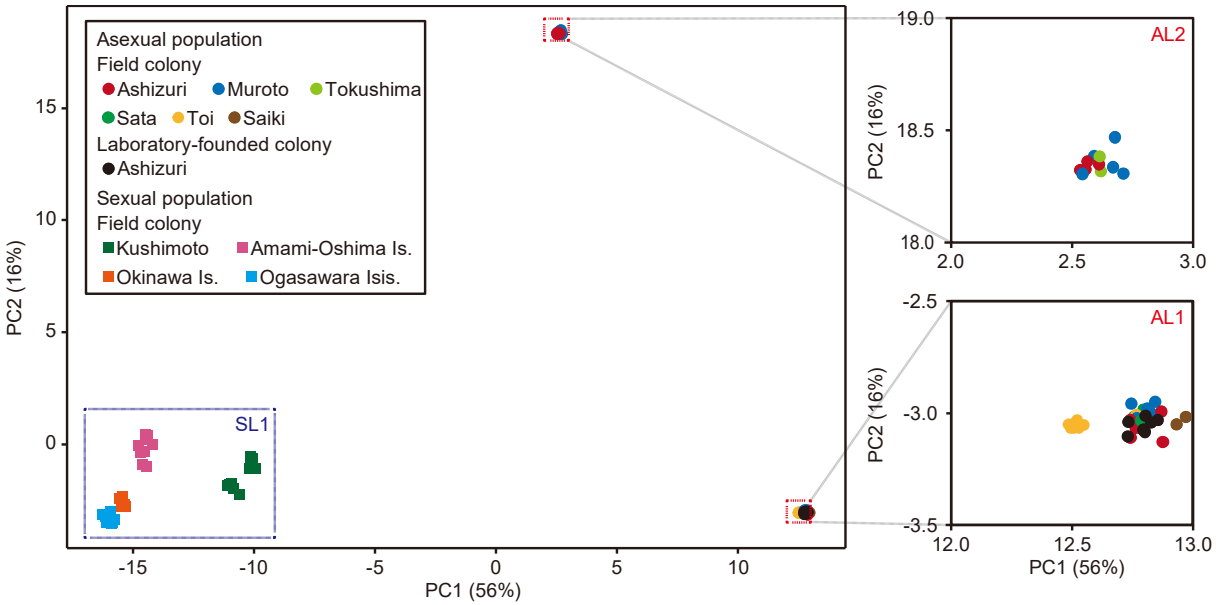

Fig. S2. PCoA of 93 *Glyptotermes nakajimai* individuals from field colonies of asexual and sexual populations and 9 individuals from laboratory-founded colonies whose natal colony had been collected from an asexual population (Ashizuri population) based on genetic distance calculated using 4,191 SNPs, resulting in three distinct groups: asexual lineage 1 (AL1), asexual lineage 2 (AL2), and sexual lineage 1 (SL1). PC1 and PC2 are the first and second principal coordinates, respectively, and the numbers in parentheses refer to the proportion of variance explained by the principal coordinates.

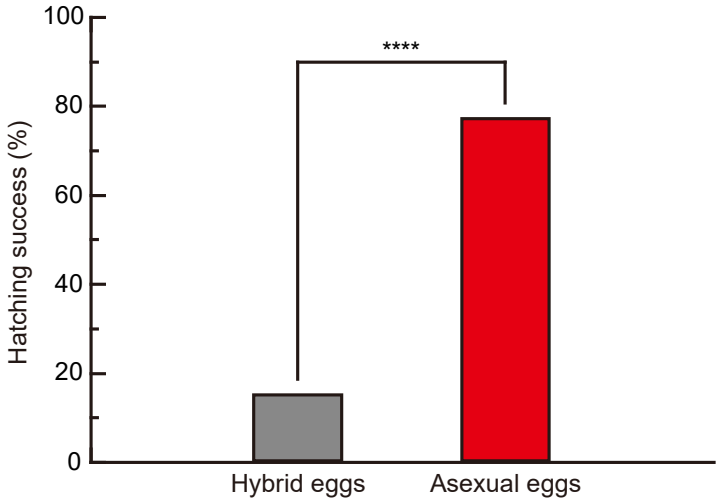

Fig. S3. Decreased hatching success of hybrid eggs of the asexual and sexual *Glyptotermes nakajimai*. Comparison of the percentage of eggs hatched within 100 days after colony foundation between hybrid eggs of the asexual and sexual *G. nakajimai* (n = 59) and unfertilized eggs of the asexual *G. nakajimai* (n = 57). ****, $P < 0.0001$ (Fisher's exact probability test).

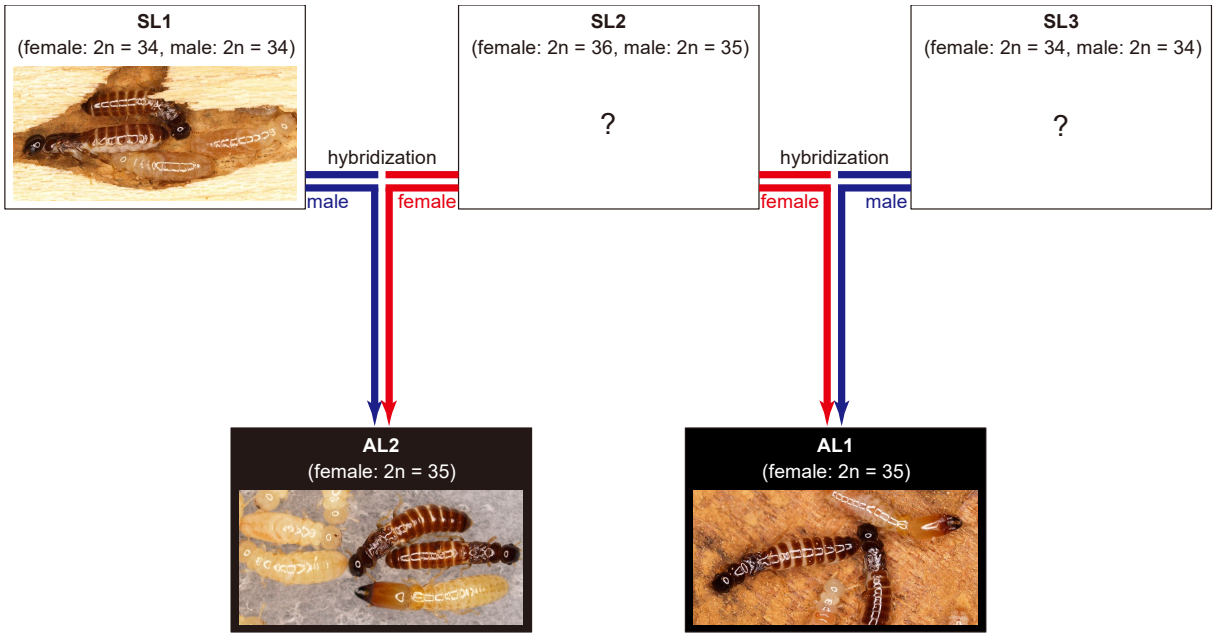

Fig. S4. Model for the evolutionary origin of the two asexual lineages of *Glyptotermes nakajimai*. Hybridization between the sexual lineage 1 (SL1) and the (unidentified) sexual lineage 2 (SL2) could have resulted in the asexual lineage 2 (AL2), and hybridization between the (unidentified) sexual lineage 2 (SL2) and the (unidentified) sexual lineage 3 (SL3) could have resulted in the asexual lineage 1 (AL1). As a possible scenario for the evolution of the asexual lineages, we hypothesize that maternal ancestors of AL1 and AL2 belonged to a lineage possessing 2n = 36 females and 2n = 35 males as described below. Under a neo-Y chromosome system in termites, centric fusions and fissions involving chromosomes forming chains (or rings) at male meiosis accelerate the differentiation of chromosome numbers (refs. S2–S4). In the case of a 2n = 34 lineage, such as SL1, males can produce n = 18 female-determining sperm and n = 17 male-determining sperm via a single centric fission of one of the neo-X chromosomes comprising a male meiotic chromosome chain, providing an opportunity for the evolution of a new lineage of 2n = 36 for females and 2n = 35 for males, such as SL2. These two lineages having different chromosome numbers would most likely be reproductively isolated due to chromosome pairing incompatibilities during meiosis in hybrid offspring, resulting opportunities for the evolution of asexual lineages possessing 2n = 35 chromosomes.

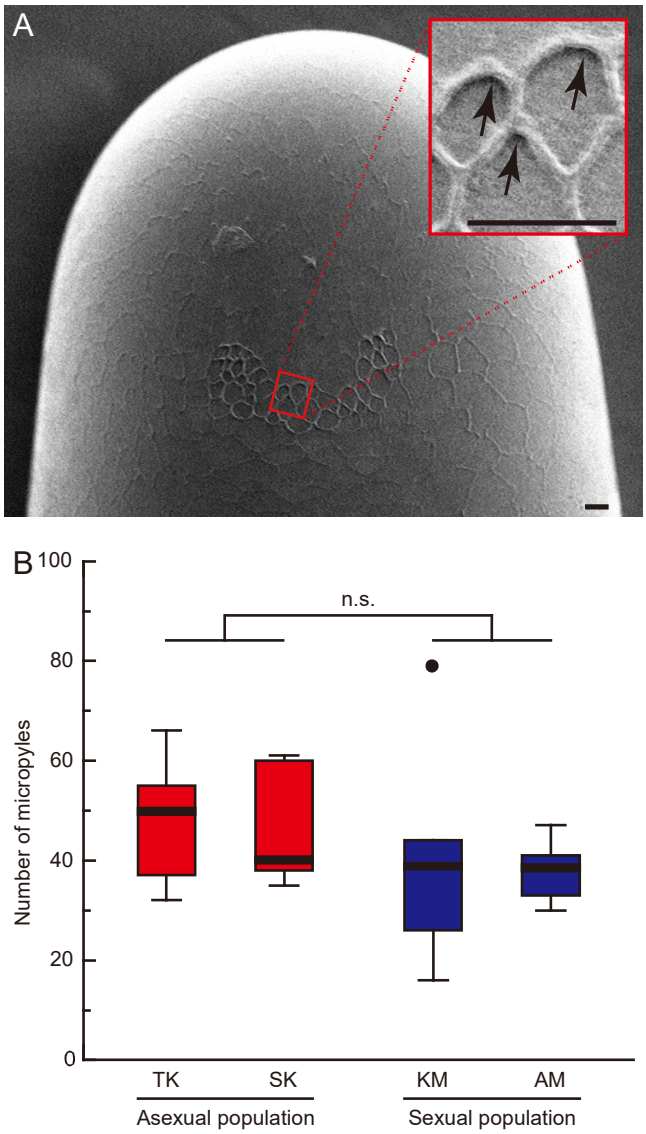

Fig. S5. Presence of micropyles in the eggs of the asexual *Glyptotermes nakajimai*. (*A*) Scanning electron microscope image of the posterior end of an egg (ventral view) and (*inset, right*) its micropyles of the asexual *G. nakajimai* (Scale bars: 20 μm.) (*B*) Comparison of the number of micropyles between eggs of asexual populations [Tokushima (TK): $n = 7$, Saiki (SK): $n = 8$] and those of sexual populations [Kushimoto (KM): $n = 5$, Amami-Oshima Is. (AM): $n = 10$]. Parameters of the box-and-whisker plots: line, median; box, first to third quartile; upper whisker, third quartile + 1.5 × interquartile range; lower whisker = first quartile − 1.5 × interquartile range; black dots, outliers. n.s., $P > 0.05$ (nested ANOVA).

Table S1. Pairwise population F_{ST} values for the *Glyptotermes nakajimai* sexual lineage 1 (SL1), the *G. nakajimai* asexual lineage 1 (AL1), and the *G. nakajimai* asexual lineage 2 (AL2) based on the SNP data

SL1	Kushimoto	Amami-Oshima Is.	Okinawa Is.
Kushimoto			
Amami-Oshima Is.	0.376***		
Okinawa Is.	0.490***	0.391***	
Ogasawara Isis.	0.548***	0.445***	0.517***

AL1	Ashizuri	Muroto	Sata	Toi
Ashizuri				
Muroto	0.007			
Sata	0.008	0.004		
Toi	0.014	0.008	0.009	
Saiki	0.007	0.008	0.012	0.019

AL2	Ashizuri	Muroto
Ashizuri		
Muroto	0.005	
Tokushima	0.007	0.000

***, $P < 0.001$ (AMOVA with 9,999 permutations).

Table S2. Mean F_{IS} values for sexual and asexual populations of *Glyptotermes nakajimai* based on the SNP data

Population	Mean F_{IS}
Sexual population (males + females)	
Kushimoto	-0.209
Amami-Oshima Is.	-0.100
Okinawa Is.	-0.228
Ogasawara Isis.	-0.031
Sexual population (males)	
Kushimoto	-0.452
Amami-Oshima Is.	-0.264
Okinawa Is.	-0.537
Ogasawara Isis.	-0.184
Sexual population (females)	
Kushimoto	-0.290
Amami-Oshima Is.	-0.233
Okinawa Is.	-0.408
Ogasawara Isis.	-0.064
Asexual population (females)	
Ashizuri	-0.471
Muroto	-0.458
Tokushima	-0.987
Sata	-0.976
Toi	-0.951
Saiki	-0.993

Table S3. Heterozygosity for males and females in each population of the *Glyptotermes nakajimai* sexual lineage 1 (SL1), and females in each population of the *G. nakajimai* asexual lineage 1 (AL1) and the *G. nakajimai* asexual lineage 2 (AL2) based on the SNP data

Population	Heterozygosity (Mean ± SD)
SL1 (males)	
Kushimoto	0.106 ± 0.005
Amami-Oshima Is.	0.113 ± 0.008
Okinawa Is.	0.121 ± 0.004
Ogasawara Isis.	0.081 ± 0.005
Total	0.105 ± 0.016
SL1 (females)	
Kushimoto	0.072 ± 0.007
Amami-Oshima Is.	0.095 ± 0.004
Okinawa Is.	0.082 ± 0.003
Ogasawara Isis.	0.063 ± 0.008
Total	0.078 ± 0.013
AL1 (females)	
Ashizuri	0.402 ± 0.003
Muroto	0.402 ± 0.004
Sata	0.408 ± 0.001
Toi	0.401 ± 0.003
Saiki	0.402 ± 0.000
Total	0.404 ± 0.004
AL2 (females)	
Ashizuri	0.404 ± 0.013
Muroto	0.391 ± 0.011
Tokushima	0.399 ± 0.004
Total	0.397 ± 0.012

Table S4. Migration rates among populations using BayesAss

Migration*	Migration rate [mean (95% confidence interval)]†
Asexual population → Asexual population	
Ashizuri → Muroto	0.017 (0.000–0.048)
Ashizuri → Tokushima	0.028 (0.000–0.078)
Ashizuri → Toi	0.017 (0.000–0.048)
Ashizuri → Sata	0.017 (0.000–0.049)
Ashizuri → Saiki	0.028 (0.000–0.079)
Muroto → Ashizuri	0.017 (0.000–0.048)
Muroto → Tokushima	0.028 (0.000–0.079)
Muroto → Toi	0.017 (0.000–0.047)
Muroto → Sata	0.017 (0.000–0.048)
Muroto → Saiki	0.028 (0.000–0.078)
Tokushima → Ashizuri	**0.083 (0.023-0.143)**
Tokushima → Muroto	**0.101 (0.035-0.167)**
Tokushima → Toi	0.016 (0.000–0.046)
Tokushima → Sata	0.016 (0.000–0.046)
Tokushima → Saiki	0.028 (0.000–0.077)
Toi → Ashizuri	0.017 (0.000–0.049)
Toi → Muroto	0.017 (0.000–0.047)
Toi → Tokushima	0.028 (0.000–0.078)
Toi → Sata	0.017 (0.000–0.048)
Toi → Saiki	0.028 (0.000–0.077)
Sata → Ashizuri	**0.117 (0.048-0.185)**
Sata → Muroto	**0.099 (0.035-0.163)**
Sata → Tokushima	0.027 (0.000–0.077)
Sata → Toi	**0.184 (0.112-0.256)**
Sata → Saiki	**0.083 (0.006-0.161)**
Saiki → Ashizuri	0.017 (0.000–0.048)
Saiki → Muroto	0.017 (0.000–0.048)
Saiki → Tokushima	0.028 (0.000–0.079)
Saiki → Toi	0.017 (0.000–0.049)
Saiki → Sata	0.017 (0.000–0.048)
Sexual population → Sexual population	
Kushimoto → Amami-Oshima Is.	0.016 (0.000–0.047)
Kushimoto → Okinawa Is.	0.017 (0.000–0.048)
Kushimoto → Ogasawara Isis.	0.017 (0.000–0.048)
Amami-Oshima Is. → Kushimoto	0.017 (0.000–0.047)
Amami-Oshima Is. → Okinawa Is.	0.017 (0.000–0.048)
Amami-Oshima Is. → Ogasawara Isis.	0.017 (0.000–0.047)
Okinawa Is. → Kushimoto	0.017 (0.000–0.048)
Okinawa Is. → Amami-Oshima Is.	0.017 (0.000–0.048)
Okinawa Is. → Ogasawara Isis.	0.017 (0.000–0.048)
Ogasawara Isis. → Kushimoto	0.017 (0.000–0.047)
Ogasawara Isis. → Amami-Oshima Is.	0.017 (0.000–0.047)
Ogasawara Isis. → Okinawa Is.	0.016 (0.000–0.047)
Asexual population → Sexual population	
Ashizuri → Kushimoto	0.017 (0.000–0.047)
Ashizuri → Amami-Oshima Is.	0.017 (0.000–0.048)
Ashizuri → Okinawa Is.	0.017 (0.000–0.048)
Ashizuri → Ogasawara Isis.	0.017 (0.000–0.047)
Muroto → Kushimoto	0.017 (0.000–0.048)
Muroto → Amami-Oshima Is.	0.017 (0.000–0.048)
Muroto → Okinawa Is.	0.017 (0.000–0.048)

Muroto → Ogasawara Isis.	0.017 (0.000–0.048)
Tokushima → Kushimoto	0.017 (0.000–0.048)
Tokushima → Amami-Oshima Is.	0.017 (0.000–0.048)
Tokushima → Okinawa Is.	0.017 (0.000–0.048)
Tokushima → Ogasawara Isis.	0.017 (0.000–0.048)
Toi → Kushimoto	0.017 (0.000–0.048)
Toi → Amami-Oshima Is.	0.017 (0.000–0.048)
Toi → Okinawa Is.	0.017 (0.000–0.047)
Toi → Ogasawara Isis.	0.017 (0.000–0.049)
Sata → Kushimoto	0.017 (0.000–0.047)
Sata → Amami-Oshima Is.	0.017 (0.000–0.047)
Sata → Okinawa Is.	0.017 (0.000–0.048)
Sata → Ogasawara Isis.	0.017 (0.000–0.048)
Saiki → Kushimoto	0.017 (0.000–0.049)
Saiki → Amami-Oshima Is.	0.017 (0.000–0.047)
Saiki → Okinawa Is.	0.016 (0.000–0.047)
Saiki → Ogasawara Isis.	0.017 (0.000–0.048)
Sexual population → Aexual population	
Kushimoto → Ashizuri	0.017 (0.000–0.047)
Kushimoto → Muroto	0.017 (0.000–0.048)
Kushimoto → Tokushima	0.027 (0.000–0.077)
Kushimoto → Toi	0.017 (0.000–0.047)
Kushimoto → Sata	0.017 (0.000–0.047)
Kushimoto →Saiki	0.028 (0.000–0.078)
Amami-Oshima Is. → Ashizuri	0.017 (0.000–0.048)
Amami-Oshima Is. → Muroto	0.017 (0.000–0.048)
Amami-Oshima Is. → Tokushima	0.028 (0.000–0.079)
Amami-Oshima Is. → Toi	0.017 (0.000–0.047)
Amami-Oshima Is. → Sata	0.017 (0.000–0.048)
Amami-Oshima Is. → Saiki	0.028 (0.000–0.078)
Okinawa Is. → Ashizuri	0.017 (0.000–0.048)
Okinawa Is. → Muroto	0.017 (0.000–0.048)
Okinawa Is. → Tokushima	0.028 (0.000–0.079)
Okinawa Is. → Toi	0.017 (0.000–0.047)
Okinawa Is. → Sata	0.017 (0.000–0.048)
Okinawa Is. → Saiki	0.027 (0.000–0.076)
Ogasawara Isis. → Ashizuri	0.017 (0.000–0.048)
Ogasawara Isis. → Muroto	0.017 (0.000–0.047)
Ogasawara Isis. → Tokushima	0.027 (0.000–0.076)
Ogasawara Isis. → Toi	0.017 (0.000–0.047)
Ogasawara Isis. → Sata	0.016 (0.000–0.047)
Ogasawara Isis. → Saiki	0.028 (0.000–0.077)

*Arrows indicate direction of movement.

†Migration rates significantly greater than zero are indicated in bold.

Table S5. Genotypes of primary queens (PQ), primary kings (PK), and larvae (L) in the laboratory-founded colonies of FM pairs at each of two microsatellite loci

	Locus		
Individual	*Gly8* (genotype)†	*Gly18* (genotype)†	S/A‡
Colony: $F_{MR150910D}M_{IZ150430A}$-1*			
PQ	314/314	422/422	
PK	**326**/**326**	**420**/**422**	
L-1	314/**326**	**420**/422	S
L-2	314/**326**	**420**/422	S
Colony: $F_{MR150910D}M_{IZ150430A}$-2*			
PQ	314/314	422/422	
PK	**326**/**326**	**420**/**420**	
L-1	314/**326**	**420**/422	S
L-2	314/**326**	**420**/422	S
L-3	314/**326**	**420**/422	S
L-4	314/**326**	**420**/422	S
Colony: $F_{MR150910D}M_{IZ150430A}$-3*			
PQ	314/314	422/422	
PK	**326**/**326**	**422**/**422**	
L-1	314/314	422/422	A
L-2	314/314	422/422	A
Colony: $F_{MR150910D}M_{IZ150430A}$-4*			
PQ	314/314	422/422	
PK	**326**/**326**	**420**/**422**	
L-1	314/**326**	422/422	S
Colony: $F_{MR150910D}M_{HH151016D}$-5*			
PQ	314/314	422/422	
PK	**314**/**326**	**422**/**422**	
L-1	314/**326**	422/422	S
L-2	314/**326**	422/422	S
Colony: $F_{ST160304C}M_{HH151016D}$-4*			
PQ	314/314	422/422	
PK	**326**/**326**	**422**/**422**	
L-1	314/314	422/422	A

S/A, sexual or asexual offspring.

*Subscripts in colony codes indicate natal colonies, see Materials and Methods, of female (F) and male (M) founders, respectively.

†Kings' alleles are indicated in bold.

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