**Supplementary materials of**

**Loss of pair formation predates the evolution of male-less society in *Glyptotermes* termites**

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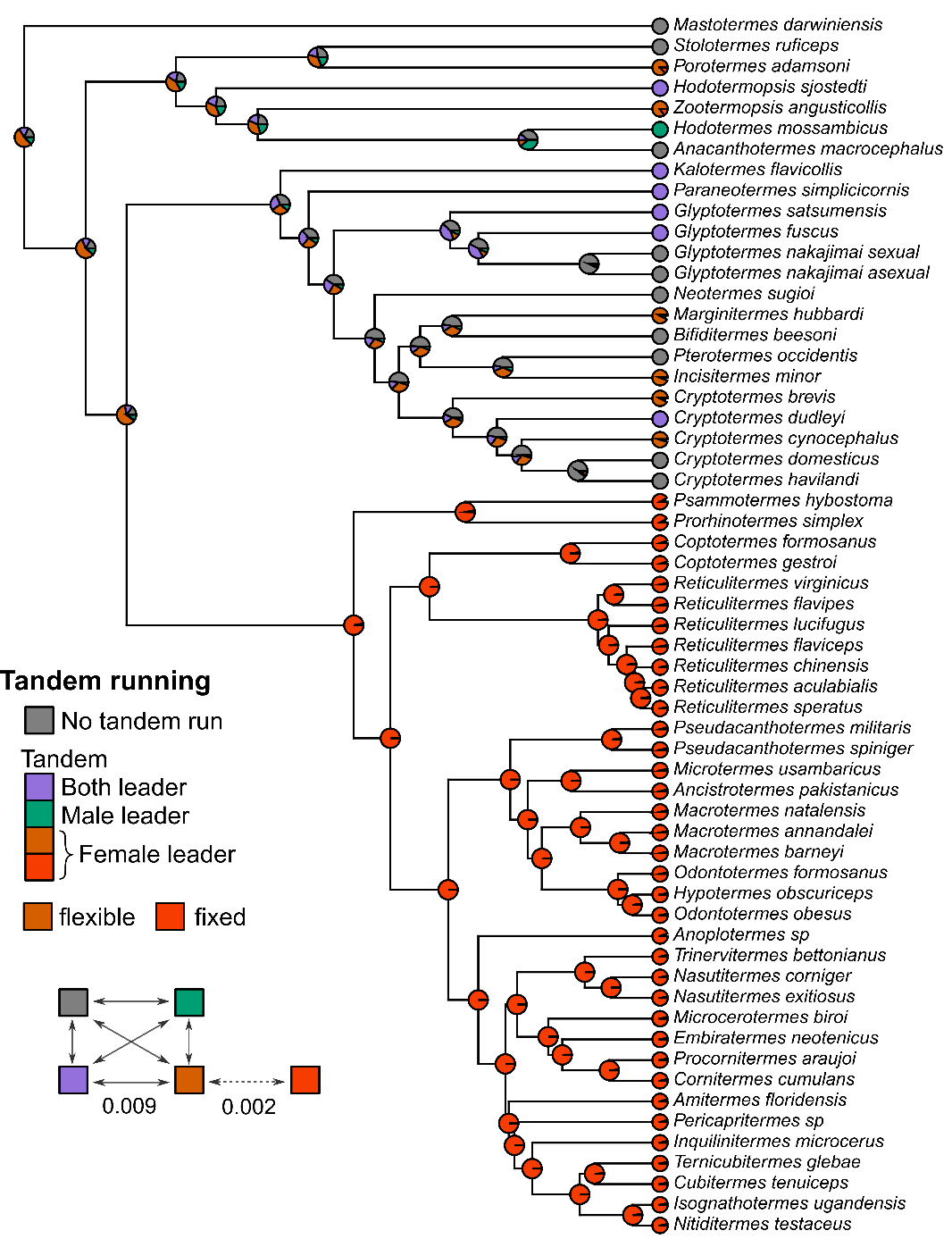
Email: [nzm0095@auburn.edu](mailto:nzm0095@auburn.edu)

This file includes

Figure S1

Table S1-S2

References for supplementary materials

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**Figure S1.** The full ancestral state reconstruction of tandem running behavior. We used the hidden rate model, where the female leader has two hidden states: a flexible state that can change to another state and a fixed state that cannot change to another state. For female-leader species, we estimated the state of female leaders of the extant species, too.

**Table 1. Composition of reproductives in *G. fuscus* and *G. satsumensis*.**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Mature colony |  |  |  |  |  |  |
| **Species** | **Colony** | **Location** | **PQ** | **PK** | **SQ** | **SK** |
| *Glyptotermes* *fuscus* | GPS253B | Ashizuri, Kochi | 0 | 1 | 1 | 0 |
|  | GPS329-1 | Toi, Miyazaki | 2 | 2 | 0 | 0 |
|  | GPS332 | Shibushi, Kagoshima | 2 | 1 | 0 | 0 |
|  | GPS313 | Issou, Yakushima, Kagoshima | 2 | 2 | 0 | 0 |
|  | GPS324 | Setouchi, Kagoshima | 2 | 2 | 0 | 0 |
|  | GPS063 | Setouchi, Kagoshima | 9 | 8 | 0 | 0 |
|  | GPS283-1 | Oku, Kunigami, Okinawa | 2 | 1 | 0 | 0 |
|  | GPS264 | Tanodake, Nago, Okinawa | 3 | 3 | 0 | 0 |
|  | GPS268 | Tanodake, Nago, Okinawa | 2 | 1 | 0 | 0 |
| *Glyptotermes* *satsumensis* | GPS253C | Ashizuri, Kochi | 2 | 3 | 0 | 0 |
|  |  |  |  |  |  |  |
| Incipient colony |  |  |  |  |  |  |
| **Species** | **Colony** | **Location** | **PQ** | **PK** | **SQ** | **SK** |
| *Glyptotermes* *fuscus* | GPS283-2 | Oku, Kunigami, Okinawa | 1 | 1 | 0 | 0 |
|  | GPS275 | Tanodake, Nago, Okinawa | 1 | 1 | 0 | 0 |
| *Glyptotermes* *satsumensis* | GPS329 | Toi, Miyazaki | 1 | 1 | 0 | 0 |
|  | GPS407-5 | Sata, Kagoshima | 1 | 1 | 0 | 0 |

PQ: primary queen, PK: primary king, SQ: secondary queen, SK: secondary king. Primary indicates alate derived. Secondary indicates neotenic.

**Table S2. Information on mating types in termites with available tandem running data.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Genus | Species | Incipient | Mature | Source | Ref |
| *Zootermopsis* | *angusticollis* | monogamous | monogamous | genetics | [1] |
| *Anacanthotermes* | *ochraceus* | NA | NA | NA | NA |
| *Anacanthotermes* | *macrocephalus* | multiple | NA | field | [2] |
| *Hodotermes* | *mossambicus* | monogamous | NA | behavior | [3] |
| *Porotermes* | *adamsoni* | monogamous | monogamous | field | [4] |
| *Stolotermes* | *ruficeps* | monogamous | monogamous | field | [5] |
| *Hodotermopsis* | *sjostedti* | monogamous | NA | field | NA1 |
| *Mastotermes* | *darwiniensis* | NA | NA | NA | NA |
| *Bifiditermes* | *beesoni* | NA | NA | NA | NA |
| *Cryptotermes* | *brevis* | monogamous2 | NA | behavior | [6] |
| *Cryptotermes* | *havilandi* | monogamous | NA | behavior | [7] |
| *Cryptotermes* | *domesticus* | NA | NA | NA | NA |
| *Cryptotermes* | *cynocephalus* | NA | NA | NA | NA |
| *Cryptotermes* | *dudleyi* | monogamous | NA | field | [8] |
| *Incisitermes* | *minor* | NA | monogamous | field | [9] |
| *Kalotermes* | *flavicollis* | NA | NA | NA | NA |
| *Marginitermes* | *hubbardi* | NA | monogamous | field | [9] |
| *Neotermes* | *sugioi* | monogamous | monogamous | field | [10] |
| *Paraneotermes* | *simplicicornis* | monogamous | monogamous | field/behavior | [11,12] |
| *Pterotermes* | *occidentis* | NA | monogamous | field | [9] |
| *Glyptotermes* | *satsumensis* | monogamous | multiple | field | Table S1 |
| *Glyptotermes* | *nakajimai\_sexual* | NA | multiple | field | [13] |
| *Glyptotermes* | *nakajimai\_asexual* | multiple | multiple | field | [13] |
| *Glyptotermes* | *fuscus* | monogamous | multiple | field | Table S1 |
| *Coptotermes* | *formosanus* | monogamous | monogamous | genetics | [14] |
| *Coptotermes* | *gestroi* | monogamous | monogamous | genetics | [14] |
| *Prorhinotermes* | *simplex* | NA | NA | NA | NA |
| *Psammotermes* | *hybostoma* | NA | NA | NA | NA |
| *Reticulitermes* | *speratus* | monogamous | monogamous | field | [15] |
| *Reticulitermes* | *flavipes* | monogamous | monogamous | genetics | [16] |
| *Reticulitermes* | *virginicus* | monogamous | monogamous | genetics | [16] |
| *Reticulitermes* | *flaviceps* | NA | NA | NA | NA |
| *Reticulitermes* | *chinensis* | monogamous | monogamous | genetics | [17] |
| *Reticulitermes* | *aculabialis* | monogamous | monogamous | genetics | [18] |
| *Reticulitermes* | *lucifugus* | monogamous | monogamous | genetics | [19] |
| *Ancistrotermes* | *dimorphus* | NA | monogamous/multiple | field | [20] |
| *Ancistrotermes* | *pakistanicus* | NA | NA | NA | NA |
| *Hypotermes* | *obscuriceps* | NA | NA | NA | NA |
| *Macrotermes* | *natalensis* | monogamous | NA | behavior | [21] |
| *Macrotermes* | *annandalei* | NA | NA | NA | NA |
| *Macrotermes* | *barneyi* | monogamous | monogamous/multiple | unclear3 | [22] |
| *Macrotermes* | *convulsionarius* | NA | NA | NA | NA |
| *Microtermes* | *usambaricus* | NA | NA | NA | NA |
| *Microtermes* | *unicolor* | NA | NA | NA | NA |
| *Odontotermes* | *formosanus* | monogamous/multiple | monogamous/multiple | field | [23] |
| *Odontotermes* | *obesus* | monogamous | Monogamous4 | field | [24] |
| *Odontotermes* | *distans* | NA | Monogamous2 | field | [25] |
| *Odontotermes* | *brunneus* | NA | monogamous | field | [26] |
| *Odontotermes* | *assmuthi* | NA | NA | NA | NA |
| *Pseudacanthotermes* | *spiniger* | NA | multiple2 | field | [27] |
| *Pseudacanthotermes* | *militaris* | NA | NA | NA | NA |
| *Anoplotermes* | *sp* | NA | monogamous | field | [28] |
| *Cubitermes* | *tenuiceps* | NA | NA | NA | NA |
| *Isognathotermes* | *ugandensis* | NA | NA | NA | NA |
| *Nitiditermes* | *testaceus* | NA | NA | NA | NA |
| *Ternicubitermes* | *glebae* | NA | NA | NA | NA |
| *Cornitermes* | *bequarerti* | NA | NA | NA | NA |
| *Cornitermes* | *cumulans* | monogamous | monogamous5 | field | [29] |
| *Embiratermes* | *neotenicus* | monogamous | monogamous | genetics | [30] |
| *Procornitermes* | *araujoi* | NA | NA | NA | NA |
| *Nasutitermes* | *corniger* | monogamous | monogamous/multiple | genetics | [31] |
| *Nasutitermes* | *nigriceps* | monogamous | monogamous | field/genetics | [32,33] |
| *Nasutitermes* | *ephratae* | NA | monogamous | field | [34] |
| *Nasutitermes* | *costalis* | NA | monogamous/multiple | field | [35,36] |
| *Nasutitermes* | *exitiosus* | NA | monogamous/multiple | field/genetics | [37,38] |
| *Trinervitermes* | *suspensus* | NA | NA | NA | NA |
| *Trinervitermes* | *bettonianus* | NA | NA | NA | NA |
| *Amitermes* | *floridensis* | NA | NA | NA | NA |
| *Amitermes* | *atlanticus* | monogamous | monogamous | field | [39] |
| *Amitermes* | *wheeleri* | NA | NA | NA | NA |
| *Inquilinitermes* | *microcerus* | NA | NA | NA | NA |
| *Microcerotermes* | *biroi* | NA | monogamous | field | [40] |
| *Microcerotermes* | *edentatus* | NA | NA | NA | NA |
| *Pericapritermes* | *sp* | NA | onogamous2,6 | field | [41] |

1 NM personal observations;

2 limited sample size;

3 based on the citation of a previous study, “Li, G.X., Z.R. Dai & D. Li 1989. Termite and Its Control in China. Science Press, Beijing (in Chinese)”, which observed the reproductive composition. But we could not locate the original article.

4 but exceptionally multiple reproductives, see [42].

5 occasionally multiple reproductives

6 *nitobei*

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