



## Supporting Online Material for

### **Queen Succession Through Asexual Reproduction in Termites**

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## Supporting Online Material

# Queen Succession through Asexual Reproduction in Termites

Kenji Matsuura<sup>1\*</sup>, Edward L. Vargo<sup>2</sup>, Kazutaka Kawatsu<sup>3</sup>, Paul E. Labadie<sup>2</sup>, Hiroko Nakano<sup>1</sup>, Toshihisa Yashiro<sup>1</sup>, Kazuki Tsuji<sup>4</sup>

**The evolution and maintenance of sexual reproduction may involve important tradeoffs as asexual reproduction can double an individual's contribution to the gene pool but reduces diversity. Moreover, in social insects the maintenance of genetic diversity among workers may be important for colony growth and survival. We discovered a termite breeding system in which both parthenogenesis and sexual reproduction are conditionally used. Queens produce their replacements asexually but use normal sexual reproduction to produce other colony members. These findings show how eusociality can lead to extraordinary reproductive systems providing important new insights into the advantages and disadvantages of sex.**

## Methods

### Sampling

Nests of *R. speratus* were collected at five field sites: Yoshidayama (35°01'35"N, 135°47'13"E), Uryuyama (35°02'23"N 135°48'09"E), Takaragaike (35°03'30"N, 135°46'55"E), Kamigamo (35°03'56"N, 135°45'38"E) and Iwakura (35°05'30"N, 135°46'22"E) in Kyoto, Japan in summer 1998, 1999, 2000, 2001, 2006, 2007 and 2008. Like most subterranean termites, *Reticulitermes* termites have cryptic nesting habits with transient, hidden royal chambers underground or deep inside wood, making it difficult to collect reproductive individuals reliably (S1, S2). To obtain reproductives from a sufficient number of natural colonies, we collected more than 600 nests in the field. We successfully found the royal chambers, where reproductives and young brood were protected, from 30 colonies (Table S1). We removed the parts of the nest wood containing the royal chambers using a chain saw, and then brought them into the laboratory for further dismantling. All reproductives were sampled by cutting the wood into ca. 15-cm-thick cross sections, and carefully splitting the wood along the growth rings to expose termites inside. The reproductives from each colony were immediately preserved in 100% ethanol with nestmate workers and nymphs in a vial for subsequent genetic analysis. We distinguished primary reproductives (alate-derived) from secondary reproductives (neotenics) on the basis of the fully melanised body color and the presence of wing scales. Sex was determined from the configuration of the caudal sternites under a stereoscope. Neotenic reproductives were investigated for the presence or absence of wing pads to separate into nymphoid (nymph-derived) and ergatoid (worker-derived) reproductives.

### Microsatellite analysis.

We used seven field colonies collected at Yoshidayama (colony A, B and C), Uryuyama (D and E) and Takaragaike (F and G) for detailed genetic analysis. We genotyped the primary king, 20 secondary queens, 20 workers and 20 alate nymphs for

each of the seven field colonies. In colony F, the primary king had been replaced by a single secondary king which was genotyped.

In colony E, the primary queen was also present and genotyped. In colony G, only 15 secondary queens were collected and all of them were genotyped. Alate nymphs were available only for colonies D and G. All of these nymphs were last instars (alate nymphs) with fully developed wing pads, which are destined to develop into alates without the option of being neotenic reproductives at this stage. Heads of individual termites were ground in Bio-Rad Chelex 100 resin solution (Bio-Rad Laboratories, Hercules, CA). DNA was extracted and purified in accordance with standard Chelex-based protocols (S3). The individuals were genotyped at five highly polymorphic microsatellite loci (*Rf24-2*, *Rf21-1*, *Rs10*, *Rs15* and *Rf6-1*: see protocol in S4) exhibiting 7, 10, 6, 4, and 6 alleles, respectively, across all colonies (Table S2-8). *F*-statistics and heterozygosities were calculated with FSTAT (S5); relatedness coefficients were estimated with the program Relatedness 5.0.8 (S6). The observed heterozygosity for overall loci ( $H_o$ ) was compared with the expected heterozygosity ( $H_e$ ) for outcrossing of the primary king and the primary queen by means of a *G*-test (S7).

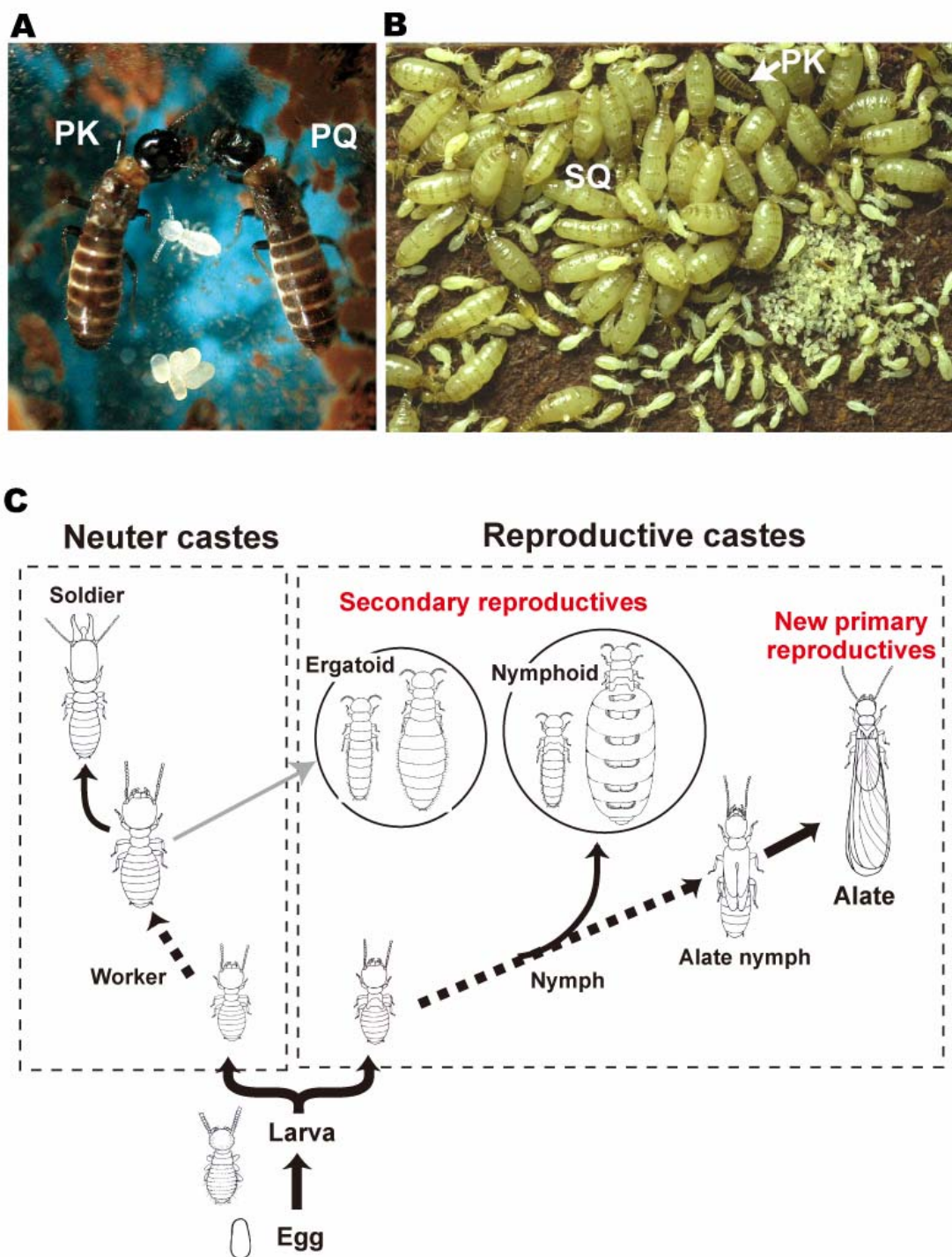
### Supplementary discussion

In automictic parthenogenesis with terminal fusion, two haploid pronuclei that divide at meiosis II fuse. Thus offspring are homozygous for a single maternal allele at all loci that did not cross over, whereas offspring have the same genotype as their mother at loci where crossing-over occurred (S3). As expected from automictic parthenogenesis with terminal fusion, 98.4% (129 of 131) of the parthenogenetic secondary queens were homozygous for one of the two maternal alleles at all five loci. We found only a single case of crossing-over in colony A, in which a secondary queen had the same heterozygous genotype as the primary queen at the locus *Rf21-1* (Table S2). The single exception was a secondary queen in colony B, which had a unique allele at *Rf21-1* that neither the king nor queen had and may have arisen *de novo* through mutation, as the other four loci indicated that this was a parthenogen of the primary queen (Table S3).

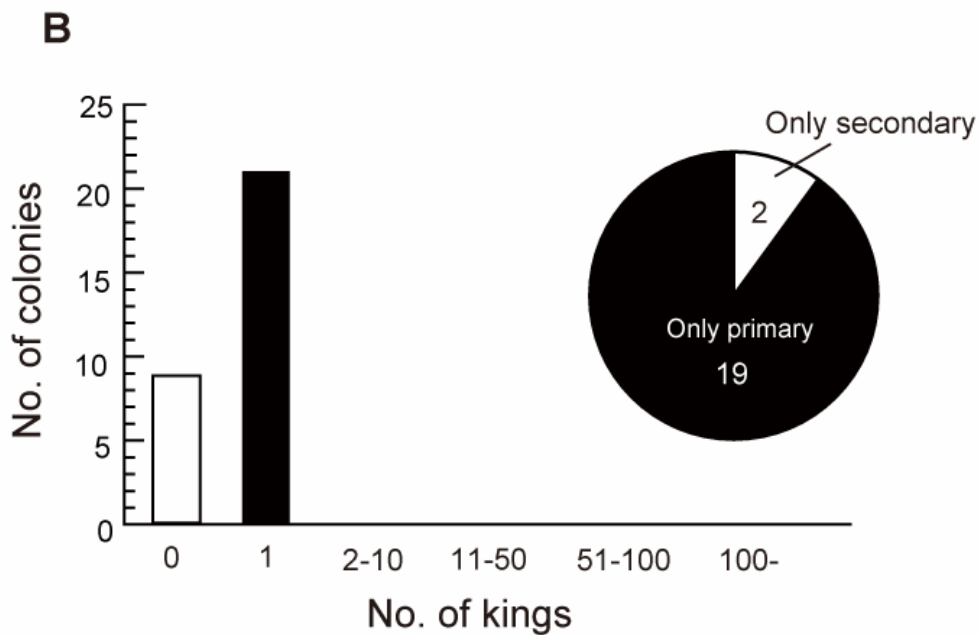
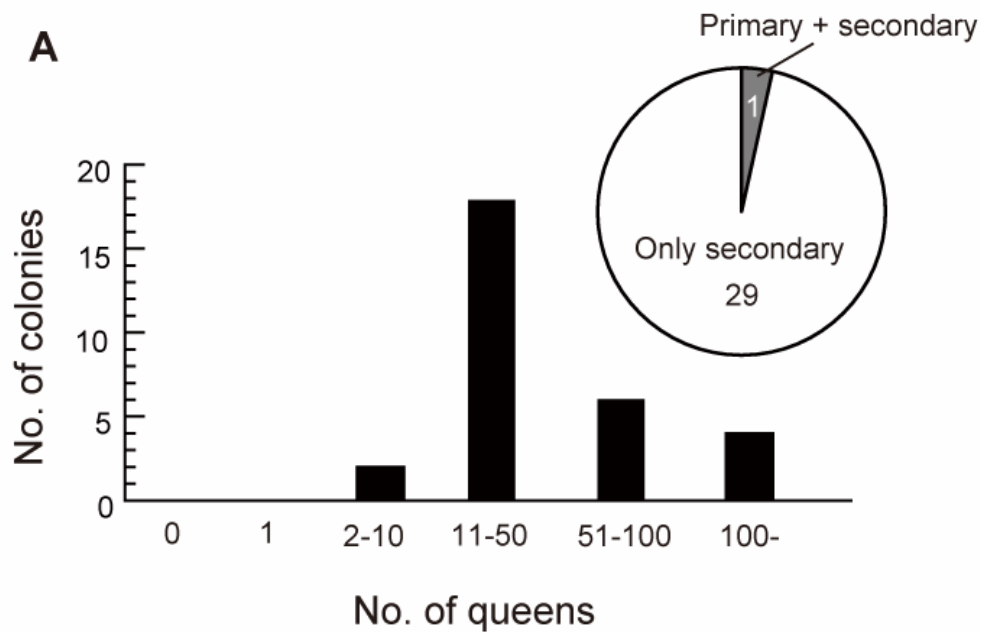
On the basis of our and previous data (S3, 8, 9), we inferred the following life history for *R. speratus*. Colonies are founded by monogamous pairs of primary kings and queens who join together during mating flights. The primary queen in each colony dies at an early stage in the life of the colony, and is replaced by dozens of parthenogenetic daughters who become secondary queens. The secondary queens mate with the king to produce workers and alates through sexual reproduction. Because the reproductive output of the many secondary queens is greater than that of a single primary queen, the presence of the secondary queens allows the colony to reach a larger size than with a single queen. This may then accelerate colony growth, depending on when secondary queens arise in the life of the colony. Thus, production of secondary queens through conditional parthenogenesis effectively extends the reproductive life of the primary queen and expands her reproductive capacity. This process of queen succession allows the colony to boost its size and possibly its growth rate without suffering a loss of genetic diversity or diminishing the transmission rate of the queen's genes to her grand offspring, feats that would not be possible if secondary queens were produced by normal sexual reproduction.

In this study, all of the 30 natural colonies of *R. speratus* had only nymphoid reproductives. However, an earlier paper reported the occurrence of ergatoid

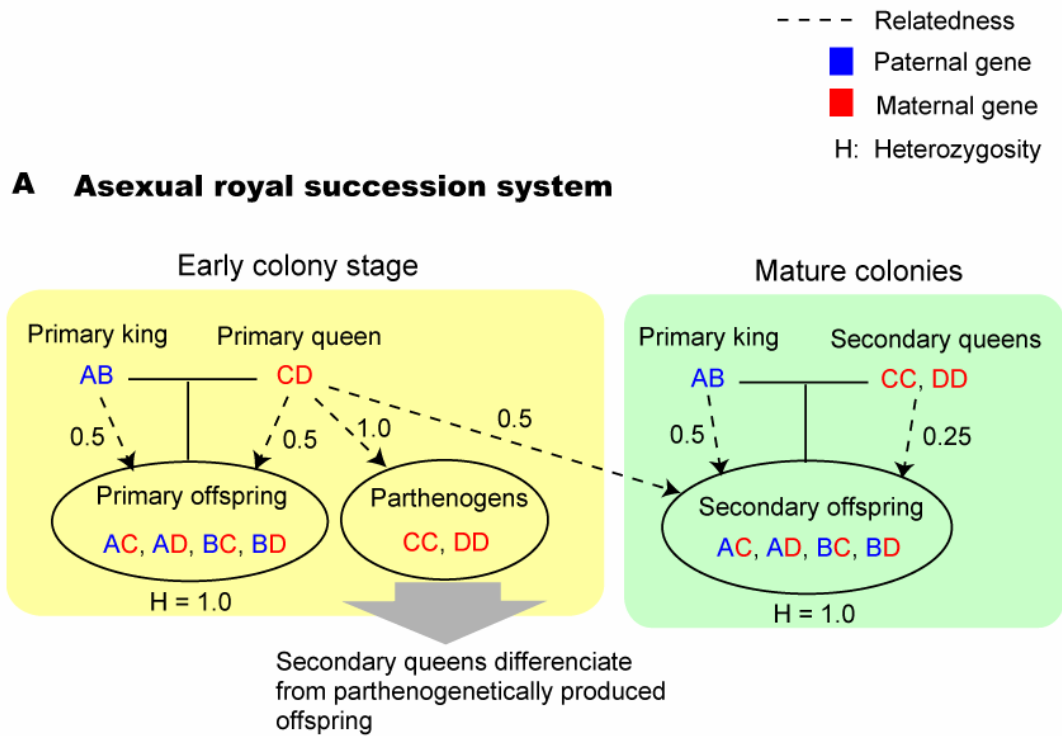
reproductives, i.e. neotenic reproductives derived from workers, in natural colonies in other population (*S10*). If the breeding system of *R. speratus* truly exhibits such inter-population variability, this variation could provide an opportunity to investigate the evolutionary causes and consequences of termite breeding systems. Moreover, a strong genetic influence on caste determination was demonstrated in *R. speratus*, and an X-linked, one-locus- two-allele model was hypothesized (*S11*). Thus *R. speratus* appears to be an excellent subject for elucidating several key components of termite sociality. Future comparative studies of termite breeding systems in natural colonies in conjunction with studies of the genetic and physiological mechanisms determining caste differentiation could reveal how diverse the breeding systems of termites are as well as the proximate and ultimate factors driving this diversity.



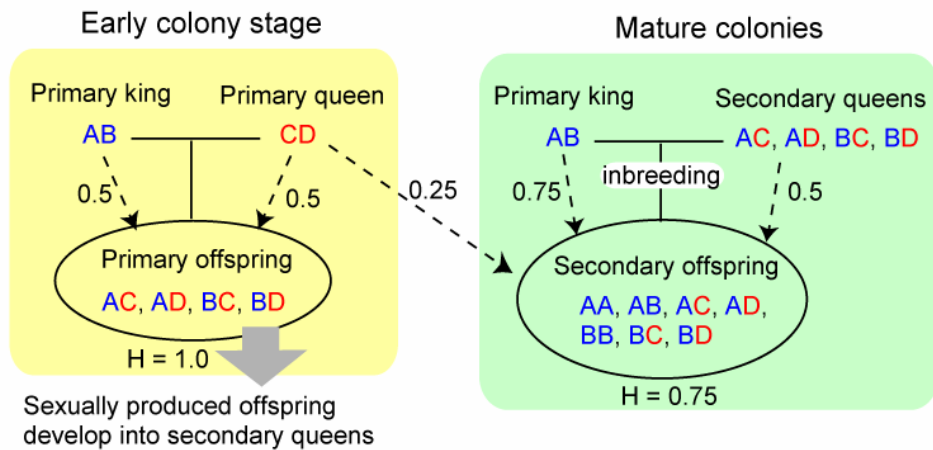
**Fig. S1.** Primary and secondary reproductives, and their differentiation pathways in *Reticulitermes speratus*. **A**, Colony foundation by a primary king (PK) and queen (PQ). **B**, Reproductives in a mature colony. The primary king (PK) was present but the primary queen had been replaced by many secondary queens (SQ). **C**, Differentiation pathways of primary and secondary reproductives. In nature, secondary reproductives always differentiate from nymphs but not from workers.



**Fig. S2.** Number of reproductives in field colonies. **A**, Number of queens in a colony. The pie chart shows that primary queens had been replaced by secondary queens in nearly all field colonies (29 of 30 colonies). **B**, Number of kings in a colony. Primary kings were present in most colonies (19 of 21 colonies). The white bar indicates the number of colonies in which we did not recover kings, although we found royal chambers.



**B King-daughter inbreeding system**



**Fig. S3.** Proposed scheme for asexual queen succession in termites compared to the scheme sexually produced replacement queens. **A**, Asexual royal succession. This breeding system enables the primary queen to maintain her full genetic contribution to the next generation of primary reproductives (alates) while avoiding any loss in genetic diversity from inbreeding. Regression relatedness is shown in the figure. Life for life relatedness of the primary queen to parthenogens is 0.5. **B**, Sexual royal succession with king-daughter inbreeding. Under this system, heterozygosity of colony members

decreases after queen replacement. The primary queen does not retain her full genetic contribution to descendants after she is replaced.

Table S1 | Royal composition of mature field colonies.

Nym.: nymphoid, Erg.: ergatoid. \*: King was not collected most likely due to collection error.

| Colony code | King    |           |      | Queen |         |           |      |       |
|-------------|---------|-----------|------|-------|---------|-----------|------|-------|
|             | Primary | Secondary |      | Total | Primary | Secondary |      | Total |
|             |         | Nym.      | Erg. |       |         | Nym.      | Erg. |       |
| TA980526    | 1       | 0         | 0    | 1     | 0       | 13        | 0    | 13    |
| TA980604    | 1       | 0         | 0    | 1     | 0       | 22        | 0    | 22    |
| TA980809    | 1       | 0         | 0    | 1     | 0       | 21        | 0    | 21    |
| TB991016A   | 1       | 0         | 0    | 1     | 0       | 18        | 0    | 18    |
| TA000619    | 1       | 0         | 0    | 1     | 0       | 74        | 0    | 74    |
| KB000904A   | 1       | 0         | 0    | 1     | 0       | 25        | 0    | 25    |
| KB000907    | 1       | 0         | 0    | 1     | 0       | 31        | 0    | 31    |
| KB010703    | 1       | 0         | 0    | 1     | 0       | 67        | 0    | 67    |
| KB010725    | 1       | 0         | 0    | 1     | 0       | 109       | 0    | 109   |
| KB010728    | 0       | 0         | 0    | 0*    | 0       | 15        | 0    | 15    |
| IW010730    | 1       | 0         | 0    | 1     | 0       | 15        | 0    | 15    |
| U060712     | 1       | 0         | 0    | 1     | 1       | 128       | 0    | 129   |
| U060719     | 0       | 0         | 0    | 0*    | 0       | 45        | 0    | 45    |
| Y060730A    | 0       | 0         | 0    | 0*    | 0       | 37        | 0    | 37    |
| Y060730B    | 0       | 0         | 0    | 0*    | 0       | 25        | 0    | 25    |
| Y060731     | 1       | 0         | 0    | 1     | 0       | 29        | 0    | 29    |
| Y060802A    | 1       | 0         | 0    | 1     | 0       | 37        | 0    | 37    |
| Y060802B    | 1       | 0         | 0    | 1     | 0       | 38        | 0    | 38    |
| T0608007    | 0       | 0         | 0    | 0*    | 0       | 10        | 0    | 10    |
| Y060810B    | 1       | 0         | 0    | 1     | 0       | 80        | 0    | 80    |
| U060814     | 0       | 0         | 0    | 0*    | 0       | 4         | 0    | 4     |
| Y060815     | 0       | 0         | 0    | 0*    | 0       | 13        | 0    | 13    |
| Y060816     | 0       | 0         | 0    | 0*    | 0       | 365       | 0    | 365   |
| Y060901B    | 1       | 0         | 0    | 1     | 0       | 131       | 0    | 131   |
| T060905B    | 1       | 0         | 0    | 1     | 0       | 15        | 0    | 15    |
| U060912     | 1       | 0         | 0    | 1     | 0       | 70        | 0    | 70    |
| Y060930     | 1       | 0         | 0    | 1     | 0       | 49        | 0    | 49    |
| T070515     | 0       | 0         | 0    | 0*    | 0       | 68        | 0    | 68    |
| T070528A    | 0       | 1         | 0    | 1     | 0       | 28        | 0    | 28    |
| T080520A    | 0       | 1         | 0    | 1     | 0       | 78        | 0    | 78    |
| Total       | 19      | 2         | 0    | 21    | 1       | 1660      | 0    | 1661  |



Table S2 | Genotypes of the primary king (PK), the primary queen (PQ), secondary queens (SQ), and workers (W) in colony A at each of the five microsatellite loci.

\*P/S: Parthenogenetically produced (P) or sexually produced (S). \*\*Genotype of the primary queen was determined from genotype of offspring since the primary queen had been replaced by secondary queens.

| Individual | Rf.24.2 |     | Rf.21.1 |     | Rs.10 |     | Rs.15 |     | Rf.6.1 |     | P/S* | Sex |
|------------|---------|-----|---------|-----|-------|-----|-------|-----|--------|-----|------|-----|
| PK         | 090     | 087 | 223     | 184 | 197   | 143 | 279   | 279 | 167    | 158 | S    | M   |
| PQ**       | 105     | 078 | 223     | 196 | 158   | 143 | 279   | 270 | 161    | 161 | S    | F   |
| SQ-1       | 078     | 078 | 223     | 223 | 143   | 143 | 279   | 279 | 161    | 161 | P    | F   |
| SQ-2       | 105     | 105 | 223     | 223 | 143   | 143 | 279   | 279 | 161    | 161 | P    | F   |
| SQ-3       | 078     | 078 | 196     | 196 | 158   | 158 | 279   | 279 | 161    | 161 | P    | F   |
| SQ-4       | 078     | 078 | 223     | 223 | 143   | 143 | 270   | 270 | 161    | 161 | P    | F   |
| SQ-5       | 078     | 078 | 196     | 196 | 143   | 143 | 279   | 279 | 161    | 161 | P    | F   |
| SQ-6       | 078     | 078 | 196     | 196 | 158   | 158 | 270   | 270 | 161    | 161 | P    | F   |
| SQ-7       | 078     | 078 | 196     | 196 | 158   | 158 | 270   | 270 | 161    | 161 | P    | F   |
| SQ-8       | 078     | 078 | 196     | 196 | 143   | 143 | 279   | 279 | 161    | 161 | P    | F   |
| SQ-9       | 078     | 078 | 196     | 196 | 143   | 143 | 270   | 270 | 161    | 161 | P    | F   |
| SQ-10      | 078     | 078 | 196     | 196 | 143   | 143 | 279   | 279 | 161    | 161 | P    | F   |
| SQ-11      | 105     | 105 | 196     | 196 | 158   | 158 | 270   | 270 | 161    | 161 | P    | F   |
| SQ-12      | 105     | 105 | 223     | 223 | 143   | 143 | 279   | 279 | 161    | 161 | P    | F   |
| SQ-13      | 105     | 105 | 223     | 223 | 158   | 158 | 270   | 270 | 161    | 161 | P    | F   |
| SQ-14      | 105     | 105 | 196     | 196 | 158   | 158 | 279   | 279 | 161    | 161 | P    | F   |
| SQ-15      | 078     | 078 | 196     | 196 | 158   | 158 | 270   | 270 | 161    | 161 | P    | F   |
| SQ-16      | 105     | 105 | 223     | 196 | 158   | 158 | 270   | 270 | 161    | 161 | P    | F   |
| SQ-17      | 105     | 105 | 223     | 223 | 143   | 143 | 279   | 279 | 161    | 161 | P    | F   |
| SQ-18      | 078     | 078 | 223     | 223 | 158   | 158 | 270   | 270 | 161    | 161 | P    | F   |
| SQ-19      | 105     | 105 | 196     | 196 | 158   | 158 | 270   | 270 | 161    | 161 | P    | F   |
| SQ-20      | 078     | 078 | 196     | 196 | 143   | 143 | 279   | 279 | 161    | 161 | P    | F   |
| W-1        | 087     | 078 | 223     | 184 | 143   | 143 | 279   | 279 | 167    | 161 | S    | M   |
| W-2        | 105     | 087 | 184     | 184 | 158   | 143 | 279   | 270 | 161    | 158 | S    | F   |
| W-3        | 090     | 078 | 223     | 196 | 197   | 158 | 279   | 270 | 167    | 161 | S    | F   |
| W-4        | 090     | 078 | 223     | 196 | 197   | 143 | 279   | 270 | 167    | 161 | S    | M   |
| W-5        | 105     | 087 | 196     | 184 | 143   | 143 | 279   | 270 | 161    | 158 | S    | F   |
| W-6        | 087     | 078 | 223     | 223 | 197   | 158 | 279   | 279 | 167    | 161 | S    | F   |
| W-7        | 090     | 078 | 196     | 184 | 197   | 158 | 279   | 270 | 167    | 161 | S    | M   |
| W-8        | 105     | 090 | 223     | 223 | 143   | 143 | 279   | 279 | 167    | 161 | S    | F   |
| W-9        | 090     | 078 | 223     | 196 | 197   | 143 | 279   | 270 | 161    | 158 | S    | F   |
| W-10       | 090     | 078 | 223     | 223 | 143   | 143 | 279   | 279 | 167    | 161 | S    | M   |
| W-11       | 105     | 087 | 223     | 223 | 197   | 158 | 279   | 270 | 167    | 161 | S    | F   |
| W-12       | 087     | 078 | 223     | 196 | 197   | 143 | 279   | 279 | 161    | 158 | S    | M   |
| W-13       | 087     | 078 | 223     | 196 | 158   | 143 | 279   | 279 | 161    | 158 | S    | M   |
| W-14       | 090     | 078 | 196     | 184 | 197   | 143 | 279   | 279 | 161    | 158 | S    | M   |
| W-15       | 087     | 078 | 223     | 196 | 197   | 143 | 279   | 279 | 161    | 158 | S    | F   |
| W-16       | 087     | 078 | 223     | 223 | 143   | 143 | 279   | 279 | 161    | 158 | S    | F   |
| W-17       | 087     | 078 | 196     | 184 | 197   | 158 | 279   | 279 | 167    | 161 | S    | M   |
| W-18       | 105     | 090 | 196     | 184 | 197   | 143 | 279   | 270 | 167    | 161 | S    | M   |
| W-19       | 090     | 078 | 223     | 223 | 197   | 143 | 279   | 279 | 167    | 161 | S    | M   |
| W-20       | 087     | 078 | 223     | 196 | 143   | 143 | 279   | 279 | 161    | 158 | S    | F   |

Table S3 | Genotypes of the primary king (PK), the primary queen (PQ), secondary queens (SQ), and workers (W) in colony B.

\*P/S: Parthenogenetically produced (P) or sexually produced (S). \*\*Genotype of the primary queen was determined from genotype of offspring since the primary queen had been replaced by secondary queens.

| Individual | Rf.24.2 |     | Rf.21.1 |     | Rs.10 |     | Rs.15 |     | Rf.6.1 |     | P/S* | Sex |
|------------|---------|-----|---------|-----|-------|-----|-------|-----|--------|-----|------|-----|
| PK         | 105     | 105 | 208     | 196 | 176   | 143 | 279   | 273 | 167    | 152 | S    | M   |
| PQ**       | 114     | 078 | 241     | 196 | 197   | 143 | 279   | 279 | 164    | 152 | S    | F   |
| SQ-1       | 078     | 078 | 241     | 241 | 197   | 197 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-2       | 078     | 078 | 241     | 241 | 143   | 143 | 279   | 279 | 152    | 152 | P    | F   |
| SQ-3       | 078     | 078 | 196     | 196 | 197   | 197 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-4       | 078     | 078 | 241     | 241 | 197   | 197 | 279   | 279 | 152    | 152 | P    | F   |
| SQ-5       | 078     | 078 | 241     | 241 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-6       | 078     | 078 | 223     | 196 | 197   | 197 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-7       | 114     | 114 | 241     | 241 | 143   | 143 | 279   | 279 | 152    | 152 | P    | F   |
| SQ-8       | 078     | 078 | 241     | 241 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-9       | 078     | 078 | 241     | 241 | 197   | 197 | 279   | 279 | 152    | 152 | P    | F   |
| SQ-10      | 114     | 114 | 241     | 241 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-11      | 078     | 078 | 241     | 241 | 143   | 143 | 279   | 279 | 152    | 152 | P    | F   |
| SQ-12      | 078     | 078 | 196     | 196 | 197   | 197 | 279   | 279 | 152    | 152 | P    | F   |
| SQ-13      | 078     | 078 | 241     | 241 | 197   | 197 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-14      | 078     | 078 | 241     | 241 | 197   | 197 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-15      | 078     | 078 | 241     | 241 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-16      | 078     | 078 | 241     | 241 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-17      | 114     | 114 | 196     | 196 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-18      | 078     | 078 | 196     | 196 | 143   | 143 | 279   | 279 | 152    | 152 | P    | F   |
| SQ-19      | 078     | 078 | 241     | 241 | 197   | 197 | 279   | 279 | 152    | 152 | P    | F   |
| SQ-20      | 114     | 114 | 241     | 241 | 143   | 143 | 279   | 279 | 152    | 152 | P    | F   |
| W-1        | 105     | 078 | 241     | 208 | 197   | 143 | 279   | 279 | 167    | 152 | S    | F   |
| W-2        | 105     | 078 | 241     | 208 | 143   | 143 | 279   | 279 | 167    | 152 | S    | F   |
| W-3        | 114     | 105 | 208     | 208 | 143   | 143 | 279   | 279 | 164    | 152 | S    | F   |
| W-4        | 114     | 105 | 208     | 196 | 197   | 176 | 279   | 273 | 167    | 152 | S    | F   |
| W-5        | 105     | 078 | 241     | 196 | 143   | 143 | 279   | 279 | 167    | 152 | S    | M   |
| W-6        | 105     | 078 | 241     | 196 | 143   | 143 | 279   | 279 | 152    | 152 | S    | F   |
| W-7        | 105     | 078 | 241     | 208 | 197   | 176 | 279   | 273 | 164    | 152 | S    | F   |
| W-8        | 105     | 078 | 208     | 196 | 197   | 143 | 279   | 279 | 164    | 152 | S    | F   |
| W-9        | 105     | 078 | 241     | 196 | 143   | 143 | 279   | 279 | 152    | 152 | S    | F   |
| W-10       | 114     | 105 | 208     | 208 | 143   | 143 | 279   | 279 | 152    | 152 | S    | M   |
| W-11       | 105     | 078 | 241     | 196 | 176   | 143 | 279   | 273 | 167    | 164 | S    | F   |
| W-12       | 105     | 078 | 208     | 196 | 176   | 143 | 279   | 273 | 167    | 152 | S    | F   |
| W-13       | 114     | 105 | 196     | 196 | 197   | 143 | 279   | 279 | 164    | 152 | S    | F   |
| W-14       | 105     | 078 | 241     | 196 | 143   | 143 | 279   | 279 | 167    | 152 | S    | M   |
| W-15       | 105     | 078 | 196     | 196 | 197   | 176 | 279   | 273 | 167    | 152 | S    | F   |
| W-16       | 105     | 078 | 196     | 196 | 176   | 143 | 279   | 273 | 152    | 152 | S    | M   |
| W-17       | 114     | 105 | 241     | 208 | 143   | 143 | 279   | 279 | 152    | 152 | S    | F   |
| W-18       | 105     | 078 | 208     | 196 | 143   | 143 | 279   | 279 | 167    | 164 | S    | F   |
| W-19       | 105     | 078 | 208     | 196 | 176   | 143 | 279   | 273 | 164    | 152 | S    | M   |
| W-20       | 105     | 078 | 241     | 196 | 197   | 143 | 279   | 279 | 167    | 152 | S    | F   |

Table S4 | Genotypes of the primary king (PK), the primary queen (PQ), secondary queens (SQ), and workers (W) in colony C.

\*P/S: Parthenogenetically produced (P) or sexually produced (S). \*\*Genotype of the primary queen was determined from genotype of offspring since the primary queen had been replaced by secondary queens.

| Individual | Rf.24.2 |     | Rf.21.1 |     | Rs.10 |     | Rs.15 |     | Rf.6.1 |     | P/S* | Sex |
|------------|---------|-----|---------|-----|-------|-----|-------|-----|--------|-----|------|-----|
| PK         | 105     | 078 | 208     | 196 | 176   | 143 | 279   | 270 | 167    | 158 | S    | M   |
| PQ**       | 105     | 087 | 223     | 196 | 143   | 143 | 273   | 273 | 164    | 152 | S    | F   |
| SQ-1       | 105     | 105 | 196     | 196 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-2       | 105     | 105 | 196     | 196 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-3       | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-4       | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-5       | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-6       | 105     | 105 | 196     | 196 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-7       | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-8       | 105     | 105 | 196     | 196 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-9       | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-10      | 105     | 105 | 196     | 196 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-11      | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-12      | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-13      | 105     | 105 | 196     | 196 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-14      | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-15      | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-16      | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-17      | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-18      | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-19      | 105     | 105 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-20      | 087     | 087 | 223     | 223 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| W-1        | 105     | 087 | 223     | 208 | 143   | 143 | 279   | 273 | 164    | 158 | S    | F   |
| W-2        | 087     | 078 | 223     | 196 | 143   | 143 | 279   | 273 | 164    | 158 | S    | M   |
| W-3        | 087     | 078 | 223     | 196 | 143   | 143 | 279   | 273 | 167    | 164 | S    | F   |
| W-4        | 105     | 087 | 223     | 208 | 143   | 143 | 279   | 273 | 167    | 164 | S    | M   |
| W-5        | 105     | 078 | 208     | 196 | 176   | 143 | 273   | 270 | 167    | 164 | S    | M   |
| W-6        | 105     | 087 | 223     | 208 | 143   | 143 | 279   | 273 | 164    | 158 | S    | M   |
| W-7        | 105     | 078 | 196     | 196 | 176   | 143 | 273   | 270 | 167    | 164 | S    | M   |
| W-8        | 087     | 078 | 223     | 208 | 143   | 143 | 279   | 273 | 164    | 158 | S    | F   |
| W-9        | 105     | 087 | 223     | 208 | 176   | 143 | 273   | 270 | 167    | 164 | S    | F   |
| W-10       | 087     | 078 | 223     | 196 | 143   | 143 | 279   | 273 | 164    | 158 | S    | M   |
| W-11       | 105     | 087 | 223     | 208 | 176   | 143 | 273   | 270 | 167    | 164 | S    | M   |
| W-12       | 087     | 078 | 223     | 208 | 143   | 143 | 279   | 273 | 164    | 158 | S    | M   |
| W-13       | 105     | 087 | 223     | 208 | 176   | 143 | 273   | 270 | 164    | 158 | S    | F   |
| W-14       | 087     | 078 | 223     | 208 | 143   | 143 | 279   | 273 | 158    | 152 | S    | M   |
| W-15       | 087     | 078 | 223     | 208 | 143   | 143 | 279   | 273 | 164    | 158 | S    | F   |
| W-16       | 105     | 087 | 223     | 208 | 176   | 143 | 273   | 270 | 158    | 152 | S    | F   |
| W-17       | 087     | 078 | 223     | 196 | 176   | 143 | 273   | 270 | 164    | 158 | S    | M   |
| W-18       | 087     | 078 | 223     | 196 | 176   | 143 | 273   | 270 | 158    | 152 | S    | M   |
| W-19       | 087     | 078 | 223     | 196 | 143   | 143 | 279   | 273 | 167    | 164 | S    | F   |
| W-20       | 105     | 105 | 208     | 196 | 176   | 143 | 273   | 270 | 164    | 158 | S    | F   |

Table S5 | Genotypes of the primary king (PK), the primary queen (PQ), secondary queens (SQ), workers (W), and nymphs (N) in colony D.

| Individual | Rf.24.2 |     | Rf.21.1 |     | Rs.10 |     | Rs.15 |     | Rf.6.1 |     | P/S* | Sex |
|------------|---------|-----|---------|-----|-------|-----|-------|-----|--------|-----|------|-----|
| PK         | 090     | 078 | 238     | 223 | 143   | 143 | 279   | 273 | 164    | 164 | S    | M   |
| PQ**       | 105     | 087 | 223     | 184 | 176   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| SQ-1       | 087     | 087 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-2       | 087     | 087 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-3       | 087     | 087 | 184     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-4       | 087     | 087 | 184     | 184 | 176   | 176 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-5       | 105     | 105 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-6       | 087     | 087 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-7       | 087     | 087 | 184     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-8       | 105     | 105 | 184     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-9       | 105     | 105 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-10      | 105     | 105 | 184     | 184 | 176   | 176 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-11      | 087     | 087 | 184     | 184 | 176   | 176 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-12      | 105     | 105 | 184     | 184 | 176   | 176 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-13      | 105     | 105 | 184     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-14      | 105     | 105 | 223     | 223 | 176   | 176 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-15      | 105     | 105 | 184     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-16      | 105     | 105 | 223     | 223 | 176   | 176 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-17      | 105     | 105 | 223     | 223 | 176   | 176 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-18      | 087     | 087 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-19      | 087     | 087 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| SQ-20      | 105     | 105 | 184     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | P    | F   |
| W-1        | 087     | 078 | 238     | 184 | 176   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| W-2        | 105     | 090 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | S    | M   |
| W-3        | 105     | 078 | 238     | 223 | 176   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| W-4        | 105     | 078 | 238     | 184 | 176   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| W-5        | 105     | 090 | 238     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| W-6        | 087     | 078 | 223     | 184 | 143   | 143 | 279   | 273 | 164    | 164 | S    | M   |
| W-7        | 090     | 087 | 223     | 223 | 143   | 143 | 279   | 273 | 164    | 164 | S    | M   |
| W-8        | 087     | 078 | 238     | 184 | 143   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| W-9        | 087     | 078 | 238     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| W-10       | 090     | 087 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| W-11       | 105     | 078 | 238     | 184 | 176   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| W-12       | 105     | 078 | 238     | 184 | 176   | 143 | 279   | 273 | 164    | 164 | S    | M   |
| W-13       | 105     | 090 | 223     | 223 | 176   | 143 | 279   | 273 | 164    | 164 | S    | M   |
| W-14       | 105     | 090 | 238     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | S    | M   |
| W-15       | 087     | 078 | 238     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| W-16       | 105     | 090 | 223     | 223 | 143   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| W-17       | 090     | 087 | 223     | 184 | 176   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| W-18       | 087     | 078 | 238     | 184 | 176   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| W-19       | 087     | 078 | 223     | 184 | 176   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| W-20       | 105     | 078 | 223     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| N-1        | 105     | 090 | 238     | 184 | 143   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| N-2        | 105     | 078 | 223     | 184 | 176   | 143 | 279   | 279 | 164    | 164 | S    | M   |
| N-3        | 105     | 090 | 238     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| N-4        | 105     | 078 | 238     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | S    | M   |
| N-5        | 090     | 087 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| N-6        | 105     | 090 | 223     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | S    | M   |
| N-7        | 105     | 090 | 223     | 184 | 176   | 143 | 279   | 273 | 164    | 164 | S    | M   |
| N-8        | 105     | 078 | 238     | 184 | 176   | 143 | 279   | 273 | 164    | 164 | S    | M   |
| N-9        | 090     | 087 | 223     | 223 | 143   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| N-10       | 105     | 090 | 238     | 184 | 176   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| N-11       | 087     | 078 | 238     | 223 | 143   | 143 | 279   | 273 | 164    | 164 | S    | M   |
| N-12       | 090     | 087 | 238     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| N-13       | 105     | 078 | 238     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| N-14       | 105     | 090 | 238     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| N-15       | 105     | 090 | 238     | 184 | 143   | 143 | 279   | 273 | 164    | 164 | S    | M   |
| N-16       | 090     | 087 | 238     | 223 | 143   | 143 | 279   | 279 | 164    | 164 | S    | F   |
| N-17       | 105     | 105 | 184     | 184 | 176   | 176 | 279   | 279 | 164    | 164 | P    | F   |
| N-18       | 090     | 087 | 238     | 223 | 143   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| N-19       | 105     | 090 | 223     | 184 | 143   | 143 | 279   | 279 | 164    | 164 | S    | M   |
| N-20       | 087     | 078 | 238     | 223 | 176   | 143 | 279   | 273 | 164    | 164 | S    | F   |

Table S6 | Genotypes of the primary king (PK), the primary queen (PQ), secondary queens (SQ), and workers (W) in colony E.

\*P/S: Parthenogenetically produced (P) or sexually produced (S). The primary queen was present.

| Individual | Rf.24.2 |     | Rf.21.1 |     | Rs.10 |     | Rs.15 |     | Rf.6.1 |     | P/S* | Sex |
|------------|---------|-----|---------|-----|-------|-----|-------|-----|--------|-----|------|-----|
| PK         | 114     | 090 | 223     | 205 | 143   | 143 | 270   | 270 | 164    | 164 | S    | M   |
| PQ         | 126     | 105 | 205     | 205 | 155   | 143 | 282   | 279 | 167    | 167 | S    | F   |
| SQ-1       | 126     | 126 | 205     | 205 | 143   | 143 | 279   | 279 | 167    | 167 | P    | F   |
| SQ-2       | 126     | 126 | 205     | 205 | 143   | 143 | 279   | 279 | 167    | 167 | P    | F   |
| SQ-3       | 126     | 126 | 205     | 205 | 155   | 155 | 282   | 282 | 167    | 167 | P    | F   |
| SQ-4       | 126     | 126 | 205     | 205 | 143   | 143 | 282   | 282 | 167    | 167 | P    | F   |
| SQ-5       | 126     | 126 | 205     | 205 | 155   | 155 | 279   | 279 | 167    | 167 | P    | F   |
| SQ-6       | 126     | 126 | 205     | 205 | 143   | 143 | 282   | 282 | 167    | 167 | P    | F   |
| SQ-7       | 126     | 126 | 205     | 205 | 155   | 155 | 279   | 279 | 167    | 167 | P    | F   |
| SQ-8       | 126     | 126 | 205     | 205 | 155   | 155 | 279   | 279 | 167    | 167 | P    | F   |
| SQ-9       | 105     | 105 | 205     | 205 | 143   | 143 | 282   | 282 | 167    | 167 | P    | F   |
| SQ-10      | 126     | 126 | 205     | 205 | 143   | 143 | 279   | 279 | 167    | 167 | P    | F   |
| SQ-11      | 105     | 105 | 205     | 205 | 143   | 143 | 282   | 282 | 167    | 167 | P    | F   |
| SQ-12      | 105     | 105 | 205     | 205 | 155   | 155 | 282   | 282 | 167    | 167 | P    | F   |
| SQ-13      | 126     | 126 | 205     | 205 | 155   | 155 | 279   | 279 | 167    | 167 | P    | F   |
| SQ-14      | 126     | 126 | 205     | 205 | 143   | 143 | 282   | 282 | 167    | 167 | P    | F   |
| SQ-15      | 126     | 126 | 205     | 205 | 155   | 155 | 279   | 279 | 167    | 167 | P    | F   |
| SQ-16      | 105     | 105 | 205     | 205 | 143   | 143 | 282   | 282 | 167    | 167 | P    | F   |
| SQ-17      | 126     | 126 | 205     | 205 | 143   | 143 | 282   | 282 | 167    | 167 | P    | F   |
| SQ-18      | 126     | 126 | 205     | 205 | 155   | 155 | 279   | 279 | 167    | 167 | P    | F   |
| SQ-19      | 105     | 105 | 205     | 205 | 143   | 143 | 279   | 279 | 167    | 167 | P    | F   |
| SQ-20      | 126     | 126 | 205     | 205 | 155   | 155 | 279   | 279 | 167    | 167 | P    | F   |
| W-1        | 126     | 114 | 223     | 205 | 155   | 143 | 279   | 270 | 167    | 164 | S    | F   |
| W-2        | 126     | 090 | 205     | 205 | 155   | 143 | 279   | 270 | 167    | 164 | S    | M   |
| W-3        | 126     | 114 | 223     | 205 | 143   | 143 | 282   | 270 | 167    | 164 | S    | F   |
| W-4        | 105     | 090 | 223     | 205 | 143   | 143 | 279   | 270 | 167    | 164 | S    | F   |
| W-5        | 126     | 090 | 205     | 205 | 155   | 143 | 279   | 270 | 167    | 164 | S    | F   |
| W-6        | 105     | 090 | 223     | 205 | 143   | 143 | 282   | 270 | 167    | 164 | S    | F   |
| W-7        | 126     | 114 | 205     | 205 | 155   | 143 | 279   | 270 | 167    | 164 | S    | F   |
| W-8        | 126     | 090 | 223     | 205 | 155   | 143 | 279   | 270 | 167    | 164 | S    | M   |
| W-9        | 105     | 090 | 205     | 205 | 143   | 143 | 282   | 270 | 167    | 164 | S    | M   |
| W-10       | 126     | 090 | 223     | 205 | 143   | 143 | 279   | 270 | 167    | 164 | S    | M   |
| W-11       | 126     | 090 | 205     | 205 | 143   | 143 | 282   | 270 | 167    | 164 | S    | M   |
| W-12       | 114     | 105 | 223     | 205 | 155   | 143 | 282   | 270 | 167    | 164 | S    | F   |
| W-13       | 114     | 105 | 223     | 205 | 143   | 143 | 282   | 270 | 167    | 164 | S    | M   |
| W-14       | 105     | 090 | 205     | 205 | 143   | 143 | 282   | 270 | 167    | 164 | S    | M   |
| W-15       | 114     | 105 | 223     | 205 | 155   | 143 | 279   | 270 | 167    | 164 | S    | F   |
| W-16       | 114     | 105 | 223     | 205 | 143   | 143 | 282   | 270 | 167    | 164 | S    | M   |
| W-17       | 105     | 090 | 223     | 205 | 143   | 143 | 279   | 270 | 167    | 164 | S    | M   |
| W-18       | 126     | 114 | 205     | 205 | 155   | 143 | 279   | 270 | 167    | 164 | S    | M   |
| W-19       | 105     | 090 | 223     | 205 | 143   | 143 | 282   | 270 | 167    | 164 | S    | F   |
| W-20       | 114     | 105 | 223     | 205 | 155   | 143 | 279   | 270 | 167    | 164 | S    | F   |

Table S7 | Genotypes of the primary king (PK), secondary king (SK), the primary queen (PQ), secondary queens (SQ), and workers (W) in colony F.

\*P/S: Parthenogenetically produced (P) or sexually produced (S). \*\*Genotypes of the primary king and queen were determined from genotype of offspring since the primary king and queen had been replaced by secondary kings and queens.

| Individual | Rf.24.2 |     | Rf.21.1 |     | Rs.10 |     | Rs.15 |     | Rf.6.1 |     | P/S* | Sex |
|------------|---------|-----|---------|-----|-------|-----|-------|-----|--------|-----|------|-----|
| PK**       | 105     | 081 | 274     | 226 | 143   | 143 | 279   | 273 | 164    | 152 | S    | M   |
| SK         | 114     | 105 | 274     | 271 | 164   | 143 | 273   | 273 | 164    | 152 | S    | M   |
| PQ**       | 114     | 105 | 271     | 271 | 164   | 143 | 273   | 270 | 164    | 164 | S    | F   |
| SQ-1       | 105     | 105 | 271     | 271 | 164   | 164 | 270   | 270 | 164    | 164 | P    | F   |
| SQ-2       | 114     | 114 | 271     | 271 | 164   | 164 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-3       | 114     | 114 | 271     | 271 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-4       | 114     | 114 | 271     | 271 | 164   | 164 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-5       | 105     | 105 | 271     | 271 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-6       | 114     | 114 | 271     | 271 | 164   | 164 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-7       | 114     | 114 | 271     | 271 | 143   | 143 | 270   | 270 | 164    | 164 | P    | F   |
| SQ-8       | 114     | 114 | 271     | 271 | 164   | 164 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-9       | 105     | 105 | 271     | 271 | 164   | 164 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-10      | 114     | 114 | 271     | 271 | 164   | 164 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-11      | 114     | 114 | 271     | 271 | 143   | 143 | 270   | 270 | 164    | 164 | P    | F   |
| SQ-12      | 105     | 105 | 271     | 271 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-13      | 114     | 114 | 271     | 271 | 164   | 164 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-14      | 114     | 114 | 271     | 271 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-15      | 114     | 114 | 271     | 271 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-16      | 114     | 114 | 271     | 271 | 164   | 164 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-17      | 105     | 105 | 271     | 271 | 143   | 143 | 270   | 270 | 164    | 164 | P    | F   |
| SQ-18      | 105     | 105 | 271     | 271 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-19      | 114     | 114 | 271     | 271 | 143   | 143 | 273   | 273 | 164    | 164 | P    | F   |
| SQ-20      | 114     | 114 | 271     | 271 | 164   | 164 | 273   | 273 | 164    | 164 | P    | F   |
| W-1        | 105     | 105 | 271     | 226 | 164   | 143 | 279   | 273 | 164    | 152 | S    | M   |
| W-2        | 114     | 081 | 271     | 226 | 164   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| W-3        | 105     | 105 | 271     | 226 | 143   | 143 | 273   | 270 | 164    | 164 | S    | M   |
| W-4        | 114     | 105 | 271     | 226 | 143   | 143 | 279   | 270 | 164    | 152 | S    | M   |
| W-5        | 114     | 081 | 271     | 226 | 143   | 143 | 279   | 270 | 164    | 152 | S    | F   |
| W-6        | 114     | 081 | 271     | 226 | 164   | 143 | 273   | 273 | 164    | 152 | S    | M   |
| W-7        | 105     | 105 | 271     | 226 | 164   | 143 | 273   | 270 | 164    | 152 | S    | F   |
| W-8        | 114     | 105 | 274     | 271 | 143   | 143 | 273   | 270 | 164    | 164 | S    | M   |
| W-9        | 105     | 081 | 274     | 271 | 143   | 143 | 279   | 270 | 164    | 164 | S    | F   |
| W-10       | 114     | 081 | 271     | 226 | 164   | 143 | 273   | 270 | 164    | 164 | S    | M   |
| W-11       | 105     | 081 | 271     | 226 | 143   | 143 | 279   | 270 | 164    | 164 | S    | M   |
| W-12       | 114     | 105 | 271     | 226 | 143   | 143 | 273   | 270 | 164    | 152 | S    | M   |
| W-13       | 105     | 105 | 271     | 226 | 143   | 143 | 279   | 270 | 164    | 152 | S    | M   |
| W-14       | 105     | 105 | 274     | 271 | 143   | 143 | 279   | 270 | 164    | 152 | S    | F   |
| W-15       | 114     | 105 | 274     | 271 | 164   | 143 | 273   | 273 | 164    | 152 | S    | M   |
| W-16       | 105     | 081 | 271     | 226 | 143   | 143 | 273   | 270 | 164    | 152 | S    | M   |
| W-17       | 114     | 105 | 274     | 271 | 164   | 143 | 279   | 273 | 164    | 164 | S    | F   |
| W-18       | 114     | 081 | 274     | 271 | 164   | 143 | 279   | 273 | 164    | 152 | S    | F   |
| W-19       | 114     | 105 | 271     | 226 | 143   | 143 | 273   | 270 | 164    | 152 | S    | M   |
| W-20       | 114     | 105 | 274     | 271 | 164   | 143 | 279   | 273 | 164    | 164 | S    | F   |

Table S8 | Genotypes of the primary king (PK), the primary queen (PQ), secondary queens (SQ), workers (W), and nymphs (N) in colony G.

\*P/S: Parthenogenetically produced (P) or sexually produced (S). \*\*Genotype of the primary queen was determined from genotype of offspring since the primary queen had been replaced by secondary queens.

| Individual | Rf.24.2 |     | Rf.21.1 |     | Rs.10 |     | Rs.15 |     | Rf.6.1 |     | P/S* | Sex |
|------------|---------|-----|---------|-----|-------|-----|-------|-----|--------|-----|------|-----|
| PK         | 105     | 090 | 205     | 196 | 155   | 143 | 279   | 279 | 167    | 158 | S    | M   |
| PQ**       | 126     | 081 | 223     | 184 | 176   | 143 | 279   | 279 | 170    | 152 | S    | F   |
| SQ-1       | 126     | 126 | 184     | 184 | 176   | 176 | 279   | 279 | 170    | 170 | P    | F   |
| SQ-2       | 126     | 126 | 223     | 223 | 143   | 143 | 279   | 279 | 170    | 170 | P    | F   |
| SQ-3       | 081     | 081 | 223     | 223 | 143   | 143 | 279   | 279 | 170    | 170 | P    | F   |
| SQ-4       | 081     | 081 | 184     | 184 | 176   | 176 | 279   | 279 | 152    | 152 | P    | F   |
| SQ-5       | 126     | 105 | 223     | 205 | 143   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| SQ-6       | 126     | 090 | 223     | 196 | 143   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| SQ-7       | 126     | 126 | 223     | 223 | 143   | 143 | 279   | 279 | 170    | 170 | P    | F   |
| SQ-8       | 126     | 105 | 223     | 205 | 143   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| SQ-9       | 126     | 126 | 223     | 223 | 143   | 143 | 279   | 279 | 170    | 170 | P    | F   |
| SQ-10      | 126     | 126 | 223     | 223 | 143   | 143 | 279   | 279 | 170    | 170 | P    | F   |
| SQ-11      | 126     | 126 | 223     | 223 | 143   | 143 | 279   | 279 | 170    | 170 | P    | F   |
| SQ-12      | 126     | 126 | 223     | 223 | 143   | 143 | 279   | 279 | 170    | 170 | P    | F   |
| SQ-13      | 126     | 126 | 223     | 223 | 143   | 143 | 279   | 279 | 170    | 170 | P    | F   |
| SQ-14      | 126     | 126 | 184     | 184 | 176   | 176 | 279   | 279 | 170    | 170 | P    | F   |
| SQ-15      | 126     | 105 | 223     | 196 | 143   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| W-1        | 126     | 090 | 223     | 205 | 143   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| W-2        | 126     | 105 | 223     | 205 | 143   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| W-3        | 126     | 090 | 205     | 184 | 176   | 143 | 279   | 279 | 170    | 167 | S    | M   |
| W-4        | 126     | 105 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 158 | S    | M   |
| W-5        | 126     | 105 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 167 | S    | M   |
| W-6        | 126     | 105 | 223     | 205 | 155   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| W-7        | 126     | 105 | 223     | 205 | 155   | 143 | 279   | 279 | 170    | 167 | S    | M   |
| W-8        | 090     | 081 | 223     | 205 | 155   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| W-9        | 126     | 105 | 205     | 205 | 143   | 143 | 279   | 279 | 158    | 158 | S    | M   |
| W-10       | 126     | 090 | 223     | 205 | 155   | 143 | 279   | 279 | 170    | 167 | S    | M   |
| W-11       | 126     | 090 | 223     | 205 | 155   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| W-12       | 126     | 090 | 196     | 184 | 176   | 155 | 279   | 279 | 170    | 167 | S    | M   |
| W-13       | 126     | 105 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 167 | S    | M   |
| W-14       | 126     | 090 | 205     | 184 | 155   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| W-15       | 126     | 105 | 205     | 184 | 143   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| W-16       | 126     | 090 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| W-17       | 126     | 090 | 196     | 184 | 155   | 143 | 279   | 279 | 170    | 167 | S    | M   |
| W-18       | 090     | 081 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 158 | S    | M   |
| W-19       | 126     | 105 | 196     | 184 | 176   | 143 | 279   | 279 | 167    | 152 | S    | M   |
| W-20       | 126     | 090 | 196     | 184 | 143   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| N-1        | 105     | 081 | 196     | 184 | 176   | 155 | 279   | 279 | 167    | 152 | S    | F   |
| N-2        | 126     | 090 | 205     | 205 | 155   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| N-3        | 126     | 105 | 223     | 205 | 143   | 143 | 279   | 279 | 170    | 167 | S    | M   |
| N-4        | 126     | 090 | 223     | 196 | 143   | 143 | 279   | 279 | 170    | 167 | S    | M   |
| N-5        | 105     | 081 | 223     | 196 | 143   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| N-6        | 126     | 105 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| N-7        | 126     | 105 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| N-8        | 126     | 090 | 223     | 205 | 155   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| N-9        | 126     | 105 | 223     | 196 | 143   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| N-10       | 126     | 090 | 196     | 184 | 176   | 155 | 279   | 279 | 170    | 158 | S    | F   |
| N-11       | 090     | 090 | 205     | 205 | 143   | 143 | 279   | 279 | 170    | 170 | P    | F   |
| N-12       | 126     | 105 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 158 | S    | M   |
| N-13       | 126     | 105 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| N-14       | 126     | 090 | 223     | 205 | 143   | 143 | 279   | 279 | 170    | 158 | S    | F   |
| N-15       | 126     | 105 | 223     | 205 | 143   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| N-16       | 105     | 090 | 223     | 205 | 155   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| N-17       | 126     | 105 | 205     | 184 | 176   | 155 | 279   | 279 | 170    | 167 | S    | F   |
| N-18       | 126     | 105 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 167 | S    | F   |
| N-19       | 126     | 090 | 223     | 196 | 155   | 143 | 279   | 279 | 170    | 158 | S    | M   |
| N-20       | 126     | 105 | 223     | 205 | 143   | 143 | 279   | 279 | 170    | 158 | S    | F   |

### Supplementary References

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