Supporting Information

Yashiro and Matsuura 10.1073/pnas.1412481111

SI Text

Study System.
A novel breeding system called asexual queen succession (AQS) has been described in three species of subterranean termite *Reticulitermes speratus* in Japan (1), *R. virginicus* in the United States (2), and *R. lucifugus* in Italy (3). In AQS species, queens produce their neotenic replacement reproductives asexually but use sexual reproduction to produce other colony members (1–3). These species undergo typical colony founding by a pair of primary reproductives. As the primary queen senesces and thus, its egg production become insufficient for the colony, secondary queens (neotenic queens) differentiate within the colony and supplement egg production, and then, they replace the primary queen. These secondary queens are produced asexually by the primary queen (Fig. 1). In termites, parthenogenesis produces only females (i.e., thelytokous), whereby ploidy restoration is accomplished by automixis with terminal fusion, yielding almost completely homozygous offspring (4). These neotenic queens mate with the primary king and produce workers, soldiers, and new primary reproductives through sexual reproduction. Primary kings live much longer than primary queens. This AQS system enables founding queens to increase their reproductive output while retaining the same transmission rate of their genes to future generations. Because of the complete avoidance of inbreeding, colonies maintain heterozygosity in worker and alate populations (Fig. 1). Therefore, the founder queen can be considered genetically immortal until the colony dies, because female neotenics are themselves replaced by new cohorts of parthenogenetically produced neotenics.

Methods.
Collection of reproductives. Like many other subterranean termites, *Reticulitermes* termites have cryptic nesting habits with transient, hidden royal chambers underground or deep inside wood, making the collection of reproductive individuals reliably very difficult (5, 6). We collected more than 1,000 nests in the field to obtain reproductives from a sufficient number of natural *R. speratus* colonies. We successfully found the royal chambers, where reproductives and young brood were protected, in 54 colonies collected in Kyoto, Shiga, Wakayama, and Okayama Prefectures, western Honshu, Japan from 1998 to 2014. After finding young larvae and eggs, which indicate the presence of royal chambers nearby, we removed the parts of the nest wood containing the royal chambers using a chain saw and brought them into the laboratory for further dismantling. All reproductives were sampled by cutting the wood into ∼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 99.5% (vol/vol) ethanol with nestmate workers and nymphs in a vial. We distinguished primary reproductives (alate-derived) from secondary reproductives (neotenics) on the basis of the fully melanized body color and the presence of wing scales. Sex was determined from the configuration of the caudal sternites under a stereomicroscope (SZX7; Olympus). Neotenic reproductives were investigated for the presence or absence of wing pads to separate them into nymphoid (nymph-derived) and ergatoid (worker-derived) reproductives.
Detection of sperm in queen spermathecae. We used three polygynous colonies collected in Okayama (colony TA090620A), Wakayama (colony GB130502C), and Shiga (colony ZE130827B) for microsatellite analysis of spermathecae. Twenty secondary (neotenic) queens randomly chosen from each colony were dissected, and their spermathecae were removed under a stereomicroscope (SZX7; Olympus). We also genotyped the dissected queens and the single primary king from each colony (Table S3 shows the royal composition of each colony). Whole spermathecae or heads of individual termites were ground in Chelex-100 resin solution (Bio-Rad). DNA was extracted and purified in accordance with standard Chelex-based protocols (1). The samples were genotyped at five highly polymorphic microsatellite loci: *Rf6-1*, *Rf21-1*, *Rf24-2* (7), *Rs10*, and *Rs15* (8). The PCR conditions are described in previous studies (7, 8), and fluorescently labeled PCR products were analyzed in a 3500 Genetic Analyzer (Applied Biosystems) with the internal GeneScan-600 LIZ size standard (Applied Biosystems). Allele sizes were determined using GeneMapper 5 (Applied Biosystems). The spermathecae were determined with (both with paternal and maternal alleles) or without sperm (only with maternal alleles) based on the genotypes of five microsatellite loci.

Results and Discussion.
Micropyles of young primary queen eggs. No micropyleless eggs were observed in the eggs laid by young primary queens. The average numbers of micropyles of the eggs laid by the queens from the natal colonies UR140513F, OO140531A, and OO140603A were 10.57 ± 0.31 (range = 4–23), 9.91 ± 0.28 (range = 4–17), and 9.92 ± 0.28 (range = 4–21), respectively (mean ± SEM, $n = 100$) (Fig. S3). There was no significant difference in the number of micropyles among natal colonies ($F_{2,\ 297} = 1.69$, $P = 0.19$; one-way ANOVA). We detected a significant difference in the number of micropyles between young primary queens and young secondary queens (colony: $F_{3,\ 495} = 6.75$, $P < 0.001$; queen type: $F_{1,\ 495} = 10.24$, $P < 0.01$; nested ANOVA), where the eggs laid by young primary queens had significantly larger numbers of micropyles than those laid by young secondary queens ($P < 0.01$; Tukey's HSD). This result implies that primary queens might live longer until replacement than secondary queens.
Priority of asexually produced offspring to be secondary queens. The AQS system can work only if asexually produced daughters have priority to become secondary queens. Why is it that nymphs produced by parthenogenesis can exclusively differentiate into secondary queens when there are numerous sexually produced nymphs at the same time? Genetic influences on queen–worker differentiation seem essential to maintain the AQS system. In AQS species, asexually produced offspring are strongly biased to develop into secondary queens, suggesting that differentiation into this caste is genetically influenced, possibly by whether individuals are heterozygous or homozygous at certain loci (9). In addition, a stable AQS system requires the genetic advantage to be determined by an independent multilocus genotype. A single locus system cannot discriminate asexually produced offspring and sexually produced offspring, and thus, AQS is impossible if the founding pair had the same allele at the locus (especially in sibling pairs). A multilocus system provides a rigorous mechanism by which only parthenogens can develop into secondary queens, because terminal fusion yields progeny of near-total homozygosity.

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9. Yamamoto Y, Matsuura K (2012) Genetic influence on caste determination underlying the asexual queen succession system in a termite. *Behav Ecol Sociobiol* 66(1):39–46.

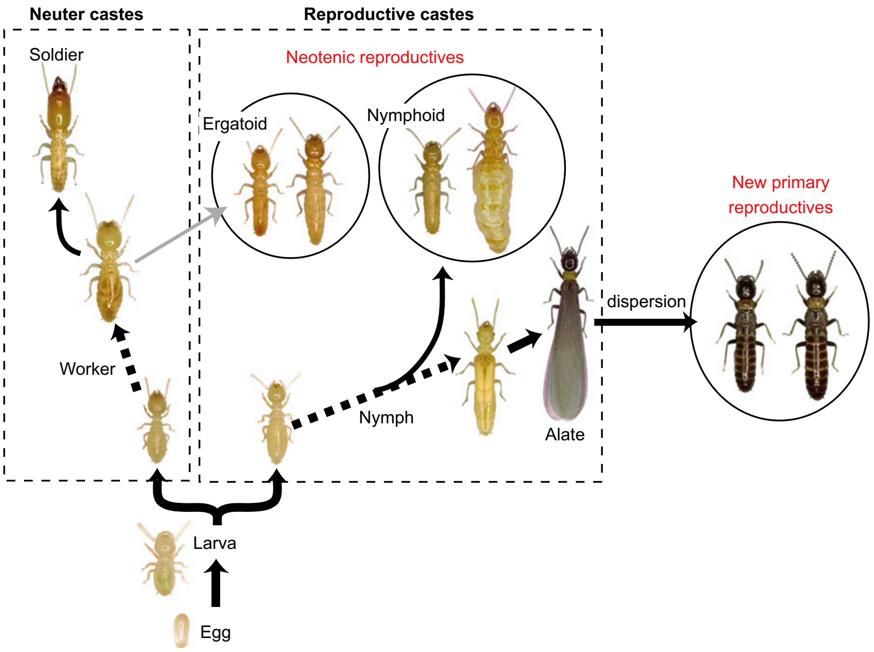

Fig. S1. Differentiation pathways of primary and secondary (neotenic) reproductives in *Reticulitermes* termites. Primary reproductives (queens and kings) derived from alates found new colonies. Ergatoids are neotenic reproductives differentiated from workers, and nymphoids are neotenic reproductives differentiated from nymphs.

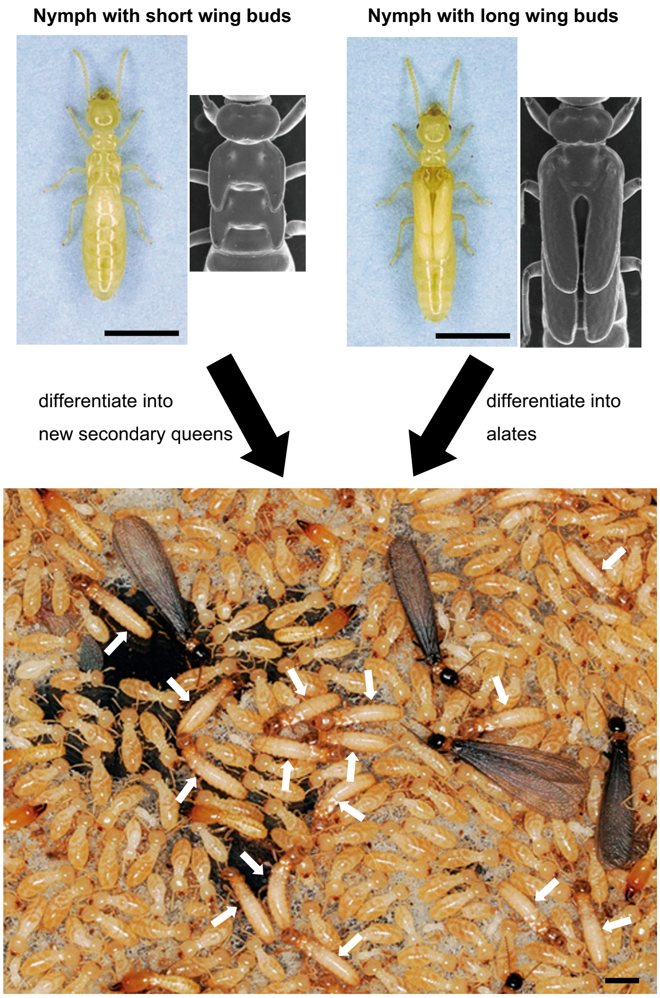

Fig. S2. Simultaneous differentiation of neotenic queens and alates of the termite *R. speratus* in mid-May just before swarming. Nymphs with short wing buds molt into neotenic queens (indicated by white arrows), and nymphs with long wing buds molt into alates. (Scale bars: 2 mm.)

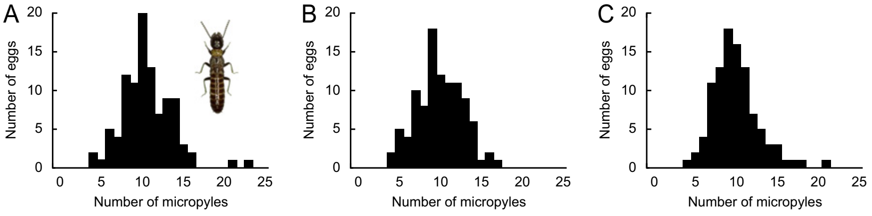

Fig. S3. Frequency distribution of the number of micropyles of the eggs laid by young primary queens in the first year of incipient colonies. Data for the young primary queens that originated from natal colonies (*A*) UR140513F, (*B*) OO140531A, and (*C*) OO140603A are shown.

Table S1. Numbers of micropyles of eggs in field colonies

Colony code*	Location	Date collected	No. of micropyles: Range	No. of micropyles: Mean ± SEM
YO120508A	Mount Yoshidayama, Kyoto, Kyoto	May 8, 2012	0–12	6.33 ± 0.30
MI120903A	Saga Mizuo, Kyoto, Kyoto	September 3, 2012	4–19	9.94 ± 0.30
MI120903B	Saga Mizuo, Kyoto, Kyoto	September 3, 2012	4–16	9.40 ± 0.26
MI120903C	Saga Mizuo, Kyoto, Kyoto	September 3, 2012	4–17	9.46 ± 0.24
MI120903D	Saga Mizuo, Kyoto, Kyoto	September 3, 2012	4–17	10.93 ± 0.27
TO120903C	Takao, Kyoto, Kyoto	September 3, 2012	3–15	9.14 ± 0.26
TO120903F	Takao, Kyoto, Kyoto	September 3, 2012	4–18	9.53 ± 0.27
TO120903G	Takao, Kyoto, Kyoto	September 3, 2012	2–14	6.99 ± 0.23
GB130502C	Enjugahama, Mihama, Wakayama	May 2, 2013	0–22	9.19 ± 0.45
HI130508E	Kitashirakawa Kogamedani, Kyoto, Kyoto	May 8, 2013	3–14	8.39 ± 0.26
OO130531C	Oomione, Kyoto, Kyoto	May 31, 2013	0–24	9.27 ± 0.40
YO130613B	Mount Yoshidayama, Kyoto, Kyoto	June 13, 2013	3–14	8.30 ± 0.24
GB130618M	Enjugahama, Mihama, Wakayama	June 18, 2013	4–21	10.52 ± 0.33
GB130618N	Enjugahama, Mihama, Wakayama	June 18, 2013	3–23	10.09 ± 0.37
GB130618O	Enjugahama, Mihama, Wakayama	June 18, 2013	5–21	12.43 ± 0.31
GB130618P	Enjugahama, Mihama, Wakayama	June 18, 2013	3–20	10.00 ± 0.33
GB130618Q	Enjugahama, Mihama, Wakayama	June 18, 2013	4–15	8.64 ± 0.21
GB130618R	Enjugahama, Mihama, Wakayama	June 18, 2013	5–21	10.74 ± 0.31
GB130618S	Enjugahama, Mihama, Wakayama	June 18, 2013	4–19	11.15 ± 0.30
GB130618T	Enjugahama, Mihama, Wakayama	June 18, 2013	4–17	10.17 ± 0.29
GB130618U	Enjugahama, Mihama, Wakayama	June 18, 2013	6–21	11.12 ± 0.28
OO130701B	Oomione, Kyoto, Kyoto	July 1, 2013	2–23	9.39 ± 0.37
OO130701C	Oomione, Kyoto, Kyoto	July 1, 2013	5–29	12.56 ± 0.34
SY130701A	Mount Shakadaniyama, Kyoto, Kyoto	July 1, 2013	2–17	8.56 ± 0.29
SY130701D	Mount Shakadaniyama, Kyoto, Kyoto	July 1, 2013	6–18	11.18 ± 0.28
SY130701F	Mount Shakadaniyama, Kyoto, Kyoto	July 1, 2013	4–19	10.42 ± 0.26
GB130709A	Enjugahama, Mihama, Wakayama	July 9, 2013	5–18	9.27 ± 0.23
GB130709C	Enjugahama, Mihama, Wakayama	July 9, 2013	4–20	9.62 ± 0.27
UR130711B	Mount Uryuzan, Kyoto, Kyoto	July 11, 2013	4–19	8.29 ± 0.25
HI130717A	Kitashirakawa Kogamedani, Kyoto, Kyoto	July 17, 2013	4–14	9.03 ± 0.22
HI130717B	Kitashirakawa Kogamedani, Kyoto, Kyoto	July 17, 2013	5–15	9.95 ± 0.21
HI130717C	Kitashirakawa Kogamedani, Kyoto, Kyoto	July 17, 2013	3–20	9.91 ± 0.35
HI130717D	Kitashirakawa Kogamedani, Kyoto, Kyoto	July 17, 2013	2–17	9.70 ± 0.30
HI130717E	Kitashirakawa Kogamedani, Kyoto, Kyoto	July 17, 2013	1–17	8.09 ± 0.29
HI130717F	Kitashirakawa Kogamedani, Kyoto, Kyoto	July 17, 2013	6–22	12.09 ± 0.29
HI130717G	Kitashirakawa Kogamedani, Kyoto, Kyoto	July 17, 2013	3–21	9.89 ± 0.27
YO130803A	Mount Yoshidayama, Kyoto, Kyoto	August 3, 2013	5–18	9.42 ± 0.23
UR130806A	Mount Uryuzan, Kyoto, Kyoto	August 6, 2013	3–20	8.64 ± 0.27
UR130806B	Mount Uryuzan, Kyoto, Kyoto	August 6, 2013	2–15	9.22 ± 0.24
UR130806C	Mount Uryuzan, Kyoto, Kyoto	August 6, 2013	4–20	9.21 ± 0.28
YO130823A	Mount Yoshidayama, Kyoto, Kyoto	August 23, 2013	3–17	8.30 ± 0.25
YO130826B	Mount Yoshidayama, Kyoto, Kyoto	August 26, 2013	4–14	8.57 ± 0.22
ZE130827B	Zeze Hibarigaoka, Otsu, Shiga	August 27, 2013	0–13	5.86 ± 0.23
ZE130827C	Zeze Hibarigaoka, Otsu, Shiga	August 27, 2013	4–19	8.74 ± 0.25
ZE130827H	Zeze Hibarigaoka, Otsu, Shiga	August 27, 2013	5–13	8.35 ± 0.18
ZE130827I	Zeze Hibarigaoka, Otsu, Shiga	August 27, 2013	4–16	8.80 ± 0.23
OG130912A	Kamigamo Okamotoguchi, Kyoto, Kyoto	September 12, 2013	2–13	7.35 ± 0.21
IW130922A	Iwakura Kino, Kyoto, Kyoto	September 22, 2013	6–16	10.45 ± 0.23
TA130924A	Takaragaike Park, Kyoto, Kyoto	September 24, 2013	3–16	9.65 ± 0.25
TA131002A	Takaragaike Park, Kyoto, Kyoto	October 2, 2013	4–14	8.99 ± 0.21
GB131002A	Enjugahama, Mihama, Wakayama	October 2, 2013	5–17	10.71 ± 0.27
GB131002B	Enjugahama, Mihama, Wakayama	October 2, 2013	3–17	8.69 ± 0.27
SY131010A	Mount Shakadaniyama, Kyoto, Kyoto	October 10, 2013	2–15	8.05 ± 0.27
SY131010C	Mount Shakadaniyama, Kyoto, Kyoto	October 10, 2013	4–13	7.87 ± 0.18
YO131010A	Mount Yoshidayama, Kyoto, Kyoto	October 10, 2013	1–15	7.10 ± 0.23
AO140511A	Shimoshirogane, Hirosaki, Aomori	May 11, 2014	0–25	9.42 ± 0.37
DA140528A	Mount Daimonjiyama, Kyoto, Kyoto	May 28, 2014	5–16	9.62 ± 0.24
OO140529B	Oomione, Kyoto, Kyoto	May 29, 2014	4–16	8.36 ± 0.22
OO140529D	Oomione, Kyoto, Kyoto	May 29, 2014	0–25	12.54 ± 0.45
KW140531A	Hanase Bessho, Kyoto, Kyoto	May 31, 2014	0–33	14.93 ± 0.80
Total			0–33	9.48 ± 0.04

*Numbers in colony codes indicate the dates when the colonies were collected. For example, colony YO120508A was collected on May 8, 2012.

Table S2. Genotypes of embryos of the eggs with or without micropyles, primary kings, primary queens, and secondary (neotenic) queens in colonies KW140531A and AO140511A at each of five microsatellite loci

Individual	*Rf24-2*	*Rf21-1*	*Rs10*	*Rs15*	*Rf6-1*	P/S*
Colony KW140531A						
Embryo (no. of MP)						
E-1 (0)	111† 111†	196 196	182† 182†	273† 273†	164† 164†	P
E-2 (0)	111† 111†	196 196	182† 182†	273† 273†	164† 164†	P
E-3 (0)	123† 123†	223 223	182† 182†	273† 273†	164† 164†	P
E-4 (1)	111† 102‡	223 196	140 140	276‡ 273†	167 158‡	S
E-5 (1)	123† 102‡	223 223	182† 140	279‡ 270†	167 167	S
E-6 (1)	123† 87‡	196 196	140 140	276‡ 270†	164† 158‡	S
E-7 (2)	111† 87‡	196 196	140 140	279‡ 270†	167 158‡	S
E-8 (2)	123† 102‡	196 196	182† 140	279‡ 270†	167 158‡	S
E-9 (4)	111† 102‡	196 196	140 140	279‡ 270†	167 167	S
E-10 (5)	111† 102‡	223 223	140 140	276‡ 273†	167 158‡	S
E-11 (5)	123† 102‡	196 196	140 140	276‡ 273†	167 158‡	S
E-12 (5)	123† 102‡	223 196	158‡ 140	276‡ 270†	167 158‡	S
PK§	102‡ 87‡	223‡ 196‡	158‡ 140‡	279‡ 276‡	167‡ 158‡	
PQ§	123† 111†	223† 196†	182† 140†	273† 270†	167† 164†	
SQ						
SQ-1	111† 111†	223† 223†	182† 182†	273† 273†	164† 164†	
SQ-2	123† 123†	196† 196†	182† 182†	273† 273†	164† 164†	
SQ-3	123† 123†	196† 196†	182† 182†	273† 273†	164† 164†	
SQ-4	123† 123†	223† 223†	182† 182†	273† 273†	164† 164†	
SQ-5	111† 111†	196† 196†	182† 182†	273† 273†	164† 164†	
SQ-6	111† 111†	196† 196†	140† 140†	270† 270†	164† 164†	
SQ-7	111† 111†	223† 223†	140† 140†	270† 270†	164† 164†	
SQ-8	123† 123†	196† 196†	140† 140†	270† 270†	164† 164†	
SQ-9	111† 111†	196† 196†	140† 140†	270† 270†	167† 167†	
SQ-10	123† 123†	223† 223†	140† 140†	273† 273†	164† 164†	
Colony AO140511A						
Embryo (no. of MP)						
E-1 (0)	114 114	205† 205†	140 140	276 276	158 158	P
E-2 (1)	114 114	226‡ 211†	176‡ 158†	276 276	164 158	S
E-3 (3)	114 114	226‡ 205†	158† 140	300 276	158 158	S
E-4 (4)	114 114	226‡ 211†	158† 140	300 300	164 164	S
E-5 (4)	114 114	229‡ 205†	140 140	276 276	164 158	S
E-6 (4)	114 114	229‡ 205†	140 140	276 276	164 158	S
E-7 (5)	114 114	226‡ 211†	158† 140	276 276	164 158	S
E-8 (5)	114 114	226‡ 211†	176‡ 158†	276 276	158 158	S
E-9 (5)	114 114	229‡ 205†	140 140	276 276	158 158	S
PK§	114 114	229‡ 226‡	176‡ 140‡	300‡ 276‡	164‡ 158‡	
PQ§	114† 114†	211† 205†	158† 140†	300† 276†	164† 158†	
SQ						
SQ-1	114† 114†	205† 205†	158† 158†	300† 300†	158† 158†	
SQ-2	114† 114†	205† 205†	140† 140†	276† 276†	164† 164†	
SQ-3	114† 114†	205† 205†	158† 158†	276† 276†	158† 158†	
SQ-4	114† 114†	211† 211†	140† 140†	276† 276†	158† 158†	
SQ-5	114† 114†	205† 205†	140† 140†	276† 276†	164† 164†	
SQ-6	114† 114†	205† 205†	158† 158†	300† 300†	158† 158†	
SQ-7	114† 114†	205† 205†	158† 158†	276† 276†	164† 164†	
SQ-8	114† 114†	211† 211†	140† 140†	276† 276†	164† 164†	
SQ-9	114† 114†	205† 205†	158† 158†	276† 276†	158† 158†	
SQ-10	114† 114†	205† 205†	140† 140†	276† 276†	158† 158†	

E, embryo; MP, micropyle; PK, primary king; PQ, primary queen; SQ, secondary (neotenic) queen.

*P/S indicates parthenogenetically developed (P) or sexually developed (S).

†Maternal alleles.

‡Paternal alleles.

§The genotype of primary queens was determined from the genotype of offspring, because primary queens had been replaced by parthenogenetically produced secondary queens.

Table S3. List of royal composition of mature field colonies

	No. of kings				No. of queens			
		Secondary				Secondary		
Colony code*	Primary	Nym.	Erg.	Total	Primary	Nym.	Erg.	Total
TA980526A	1	0	0	1	0	13	0	13
TA980604A	1	0	0	1	0	22	0	22
TA980809A	1	0	0	1	0	21	0	21
TB991016A	1	0	0	1	0	18	0	18
TA000619A	1	0	0	1	0	74	0	74
KB000904A	1	0	0	1	0	25	0	25
KB000907A	1	0	0	1	0	31	0	31
KB010703A	1	0	0	1	0	67	0	67
KB010725A	1	0	0	1	0	109	0	109
IW010730A	1	0	0	1	0	15	0	15
UR060712A	1	0	0	1	1	128	0	129
YO060731A	1	0	0	1	0	29	0	29
YO060802A	1	0	0	1	0	37	0	37
YO060802B	1	0	0	1	0	38	0	38
YO060810B	1	0	0	1	0	80	0	80
YO060901B	1	0	0	1	0	131	0	131
TA060905B	1	0	0	1	0	15	0	15
UR060912A	1	0	0	1	0	70	0	70
YO060930A	1	0	0	1	0	49	0	49
TA070528A	0	1	0	1	0	28	0	28
TA080520A	0	1	0	1	0	78	0	78
TA090501A	1	0	0	1	0	21	0	21
OA090504A	1	0	0	1	0	15	0	15
OA090610H	1	0	0	1	0	25	0	25
OU090619A	1	0	0	1	0	61	0	61
TA090620A	1	0	0	1	0	676	0	676
TA090620B	1	0	0	1	0	5	0	5
TA090623B	1	0	0	1	0	73	0	73
TK090624A	1	0	0	1	0	208	0	208
TK090624B	1	0	0	1	0	36	0	36
HA090626A	1	0	0	1	0	40	0	40
SU090707A	1	0	0	1	0	14	0	14
SU090710C	1	0	0	1	0	10	0	10
NI090722A	1	0	0	1	0	39	0	39
SU090727B	1	0	0	1	0	16	0	16
U.K.090730A	1	0	0	1	0	15	0	15
GI090730B	1	0	0	1	0	103	0	103
SU090902A	1	0	0	1	0	51	0	51
KY091004B	1	0	0	1	0	24	0	24
TA100714A	1	0	0	1	1	3	0	4
HI121110A	1	0	0	1	0	120	0	120
GB130502C	1	0	0	1	0	27	0	27
OO130531B	0	4	0	4	0	31	0	31
OO130701B	1	0	0	1	0	52	0	52
HI130717B	1	0	0	1	0	79	0	79
YO130803A	1	0	0	1	0	30	1	31
ZE130827B	1	0	0	1	0	25	0	25
ZE130827H	1	0	0	1	0	26	0	26
UT140416E	1	0	0	1	0	19	0	19
UR140513C	1	0	0	1	0	70	0	70
UR140524A	1	0	0	1	0	8	0	8
OO140529A	1	0	0	1	0	63	0	63
MO140602A	1	0	0	1	0	36	0	36
UR140610A	1	0	0	1	0	10	0	10
Total	51	6	0	57	2	3,109	1	3,112
Mean (SE)	0.94 (0.03)	0.11 (0.08)	0 (0)	1.06 (0.06)	0.04 (0.03)	57.57 (12.84)	0.02 (0.02)	57.63 (12.84)

The colonies GB130502C and ZE130827B were used for the microsatellite analysis of embryos. The colonies TA090620A, GB130502C, and ZE130827B were used for the microsatellite analysis of queen spermathecae. Erg., ergatoid; Nym., nymphoid.

*Numbers in colony codes indicate the dates when the colonies were collected. For example, colony TA980526A was collected on May 26, 1998.

Table S4. Microsatellite profiles for spermathecae of secondary (neotenic) queens in colony TA090620A at each of five microsatellite loci

Individual	*Rf24-2* (genotype*)	*Rf21-1* (genotype*)	*Rs10* (genotype*)	*Rs15* (genotype*)	*Rf6-1* (genotype*)	W/O
SQ-1	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-2	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-3	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-4	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-5	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-6	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-7	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-8	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-9	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-10	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-11	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-12	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-13	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-14	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-15	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-16	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-17	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-18	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-19	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
SQ-20	81 (81 81)	**226** 205 **184** (205 205)	**185 176** 161 (161 161)	276 **270 246** (276 276)	**170** 167 (167 167)	W
PK	**81 81**	**226 184**	**185 176**	**270 246**	**170 167**	

The royal composition of this colony is listed in Table S3. Sperm alleles (i.e., kings' alleles) are indicated in bold. PK, primary king; SQ, secondary queen; W/O, with or without sperm in spermatheca.

*The heads were used for genotyping of individuals.

Table S5. Microsatellite profiles for spermathecae of secondary (neotenic) queens in colony GB130502C at each of five microsatellite loci

Individual	*Rf24-2* (genotype*)	*Rf21-1* (genotype*)	*Rs10* (genotype*)	*Rs15* (genotype*)	*Rf6-1* (genotype*)	W/O
SQ-1	102 **87** (102 102)	**265 241** 196 (196 196)	**161 155** 152 (152 152)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-2	102 **87** (102 102)	**265 241** 196 (196 196)	**161 155** 152 (152 152)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-3	102 **87** (102 102)	**265 241** 196 (196 196)	**161 155** 140 (140 140)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-4	102 **87** (102 102)	**265 241** 202 (202 202)	**161 155** 140 (140 140)	291 **279 273** (291 291)	170 **161 152** (170 170)	W
SQ-5	**102** 99 **87** (99 99)	**265 241** 202 (202 202)	**161 155** 140 (140 140)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-6	102 **87** (102 102)	**265 241** 196 (196 196)	**161 155** 140 (140 140)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-7	**102** 99 **87** (99 99)	**265 241** 196 (196 196)	**161 155** 140 (140 140)	291 **279 273** (291 291)	170 **161 152** (170 170)	W
SQ-8	**102** 99 **87** (99 99)	**265 241** 202 (202 202)	**161 155** 152 (152 152)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-9	**102** 99 **87** (99 99)	**265 241** 196 (196 196)	**161 155** 140 (140 140)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-10	102 **87** (102 102)	**265 241** 196 (196 196)	**161 155** 152 (152 152)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-11	**102** 99 **87** (99 99)	**265 241** 202 (202 202)	**161 155** 152 (152 152)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-12	**102** 99 **87** (99 99)	**265 241** 202 (202 202)	**161 155** 152 (152 152)	291 **279 273** (291 291)	170 **161 152** (170 170)	W
SQ-13	**102** 99 **87** (99 99)	**265 241** 196 (196 196)	**161 155** 152 (152 152)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-14	102 **87** (102 102)	**265 241** 196 (196 196)	**161 155** 152 (152 152)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-15	**102** 99 **87** (99 99)	**265 241** 202 (202 202)	**161 155** 140 (140 140)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-16	102 **87** (102 102)	**265 241** 196 (196 196)	**161 155** 140 (140 140)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-17	**102** 99 **87** (99 99)	**265 241** 202 (202 202)	**161 155** 152 (152 152)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-18	**102** 99 **87** (99 99)	**265 241** 196 (196 196)	**161 155** 140 (140 140)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-19	102 **87** (102 102)	**265 241** 196 (196 196)	**161 155** 152 (152 152)	291 **279 273** (291 291)	161 **152** (161 161)	W
SQ-20	102 **87** (102 102)	**265 241** 196 (196 196)	**161 155** 152 (152 152)	291 **279 273** (291 291)	161 **152** (161 161)	W
PK	**102 87**	**265 241**	**161 155**	**279 273**	**161 152**	

The royal composition of this colony is listed in Table S3. Sperm alleles (i.e., kings' alleles) are indicated in bold. PK, primary king; SQ, secondary queen; W/O, with or without sperm in spermatheca.

*The heads were used for genotyping of individuals.

Table S6. Microsatellite profiles for spermathecae of secondary (neotenic) queens in colony ZE130827B at each of five microsatellite loci

Individual	*Rf24-2* (genotype*)	*Rf21-1* (genotype*)	*Rs10* (genotype*)	*Rs15* (genotype*)	*Rf6-1* (genotype*)	W/O
SQ-1	**114** 105 **87** (105 105)	223 **205** (223 223)	**188** 164 **140** (164 164)	**273** 270 (270 270)	167 (167 167)	W
SQ-2	**114** 105 **87** (105 105)	223 **205** (223 223)	**188** 140 (140 140)	**273** 270 (270 270)	**167** 152 (152 152)	W
SQ-3	**114** 90 **87** (90 90)	223 **205** (223 223)	**188** 140 (140 140)	**273** 270 (270 270)	**167** 152 (152 152)	W
SQ-4	**114** 105 **87** (105 105)	226 **223 205** (226 226)	**188** 140 (140 140)	**273 270** 255 (255 255)	**167** 152 (152 152)	W
SQ-5	**114** 105 **87** (105 105)	223 **205** (223 223)	**188** 140 (140 140)	**273** 270 (270 270)	**167** 152 (152 152)	W
SQ-6	**114** 90 **87** (90 90)	226 **223 205** (226 226)	**188** 140 (140 140)	**273** 270 (270 270)	167 (167 167)	W
SQ-7	**114** 105 **87** (105 105)	226 **223 205** (226 226)	**188** 140 (140 140)	**273** 270 (270 270)	**167** 152 (152 152)	W
SQ-8	**114** 105 **87** (105 105)	226 **223 205** (226 226)	**188** 140 (140 140)	**273** 270 (270 270)	**167** 152 (152 152)	W
SQ-9	**114** 90 **87** (90 90)	223 **205** (223 223)	**188** 140 (140 140)	**273** 270 (270 270)	**167** 152 (152 152)	W
SQ-10	**114** 105 **87** (105 105)	226 **223 205** (226 226)	**188** 140 (140 140)	**273** 270 (270 270)	**167** 152 (152 152)	W
SQ-11	**114** 90 **87** (90 90)	223 **205** (223 223)	**188** 140 (140 140)	**273 270** 255 (255 255)	**167** 152 (152 152)	W
SQ-12	**114** 90 **87** (90 90)	226 **223 205** (226 226)	**188** 164 **140** (164 164)	**273** 270 (270 270)	167 (167 167)	W
SQ-13	**114** 105 **87** (105 105)	226 **223 205** (226 226)	**188** 140 (140 140)	**273** 270 (270 270)	**167** 152 (152 152)	W
SQ-14	**114** 105 **87** (105 105)	226 **223 205** (226 226)	**188** 140 (140 140)	**273 270** 255 (255 255)	**167** 152 (152 152)	W
SQ-15	**114** 90 **87** (90 90)	223 **205** (223 223)	**188** 140 (140 140)	**273 270** 255 (255 255)	**167** 152 (152 152)	W
SQ-16	**114** 90 **87** (90 90)	226 **223 205** (226 226)	**188** 140 (140 140)	**273** 270 (270 270)	167 (167 167)	W
SQ-17	**114** 90 **87** (90 90)	223 **205** (223 223)	**188** 140 (140 140)	**273 270** 255 (255 255)	**167** 152 (152 152)	W
SQ-18	**114** 90 **87** (90 90)	223 **205** (223 223)	**188** 164 **140** (164 164)	**273 270** 255 (255 255)	167 (167 167)	W
SQ-19	**114** 105 **87** (105 105)	226 **223 205** (226 226)	**188** 140 (140 140)	**273** 270 (270 270)	**167** 152 (152 152)	W
SQ-20	**114** 90 **87** (90 90)	226 **223 205** (226 226)	**188** 140 (140 140)	**273** 270 (270 270)	167 (167 167)	W
PK	**114 87**	**223 205**	**188 140**	**273 270**	**167 167**	

The royal composition of this colony is listed in Table S3. Sperm alleles (i.e., kings' alleles) are indicated in bold. PK, primary king; SQ, secondary queen; W/O, with or without sperm in spermatheca.

*The heads were used for genotyping of individuals.