JOINT EVOLUTION OF CLONAL QUEEN PRODUCTION AND ALTERNATIVE SOCIAL ORGANIZATION IN THE TROPICAL FIRE ANT *SOLENOPSIS GEMINATA*

by

KIP DAVID LACY

(Under the Direction of Kenneth G. Ross)

ABSTRACT

Biological complexity evolves via major evolutionary transitions featuring the onset of cooperative reproduction by formerly independent reproducers. Examples include the transition from asexual to sexual reproduction and from solitary to social life. An analogous transition exists in ants, from monogyne (single reproductive queen) to polygyne (multiple reproductive queens) colonies. We investigated this transition in the fire ant *Solenopsis geminata* and show: 1) polygyne colonies employ a peculiar reproductive mode with queens produced asexually and workers sexually, while monogyne colonies display the typical reproductive mode of Hymenoptera; 2) the polygyne form exploits gene flow from the monogyne form to produce workers and, hence, viable colonies; and 3) two divergent polygyne subpopulations arose independently from a neighboring monogyne population. These instances of an ostensible reduction in complexity on one vector (sexual to asexual reproduction) coupled with an increase in complexity on another (monogyny to polygyny) highlight underappreciated interactions between different biological transitions.

INDEX WORDS: Major Transitions, Solenopsis geminata, Fire Ant, Social Evolution, Parthenogenesis, Clonal, Asexual, Polygyne, Monogyne,

JOINT EVOLUTION OF CLONAL QUEEN PRODUCTION AND ALTERNATIVE SOCIAL ORGANIZATION IN THE TROPICAL FIRE ANT *SOLENOPSIS GEMINATA*

by

KIP DAVID LACY

B.S., The University of Georgia, 2016

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial
Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2018

JOINT EVOLUTION OF CLONAL QUEEN PRODUCTION AND ALTERNATIVE SOCIAL ORGANIZATION IN THE TROPICAL FIRE ANT *SOLENOPSIS GEMINATA*

by

KIP DAVID LACY

Major Professor:	Kenneth G. Ross
Committee:	Brendan G. Hunt D. DeWayne Shoemaker

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2018

DEDICATION

I dedicate this thesis to my parents, for encouraging me in all endeavors, infusing me with love, and teaching me more than I know; to my brother, for demonstrating care, compassion, and the virtue of productivity; to my girlfriend Molly, for filling my life with love, my heart with joy, and my belly with food; to all of my friends, lab-mates, teachers, advisors and mentors who have helped, challenged, and encouraged me along the way; and finally to Ken, for his superlative mentorship, his infectious enthusiasm for science, and for four years of good times.

ACKNOWLEDGEMENTS

I thank Eileen Carroll for assistance in the field and the laboratory, as well as Sanford Porter, Travis Davis, Joan King, Horace Zeng and Emma Pletcher for help with colony collection and/or maintenance, and Brendan Hunt, DeWayne Shoemaker, Samuel Arsenault, and Horace Zeng for valuable comments that greatly improved this thesis.

TABLE OF CONTENTS

Page

CHAPTER

APPENDICES

LIST OF FIGURES

CHAPTER ONE

INTRODUCTION

Biological complexity evolves through major evolutionary transitions, each featuring the advent of cooperative reproduction by previously independent reproducers. Examples include the transition from asexual to sexual reproduction and the transition from solitary to social life[1]. While most species occupy one side of a transition, some straddle the threshold. For example, many species have sexually reproducing as well as asexual populations[2-7], and some arthropod species have both solitary and social populations[8-12]. Some ant species straddle an analogous transition, including both monogyne colonies, which contain a single reproductive queen, and polygyne colonies, which contain multiple cooperatively reproducing queens[13,14]. In such ants, the two social forms are thought to represent alternative evolutionary strategies, with each adapted to different environmental conditions and featuring different colony dispersal tactics and life histories, thereby differing in their ecological and economic impact[14-21]. Studying the causes of differentiation between social forms is important for understanding the processes of social evolution as well as informing the broader issue of the origin of complexity.

Polymorphism in colony social form has been well studied in the fire ant *Solenopsis invicta*. Early studies demonstrated that social form was completely associated with genotype at a Mendelian element marked by the gene *Gp-9*, with genotypes of queens from polygyne colonies, but not monogyne colonies, containing alleles of a specific lineage[22,23], which have since been shown to mark a large supergene (cluster of loci in tight genetic linkage) on what has been termed the “social chromosome”[24]. This supergene is also linked with social form in the closest relatives of *S. invicta*[25], suggesting identical genetic control of colony queen number throughout the clade of socially polymorphic South American fire ants.

Other species likely have alternate determinants of similar social polymorphisms[16,18], and comparative study of these promises to yield insights into the factors influencing transitions in social organization. The socially polymorphic fire ant *S. geminata*, a relative of *S. invicta* placed in a different species-complex[26], is well suited for such study. Alternate social forms exist in sympatry, but this variation is not associated with *Gp-9* genotype. Specifically, a previous genetic survey of a northern Florida population revealed not only that the two forms of *S. geminata* featured an identical *Gp-9* sequence, but that the sequence does not code for diagnostic amino acid residues associated with polygyny in *S. invicta* and its closest relatives[25,27,28]. Moreover, whole genome sequence analyses show that neither form of *S. geminata* from this population possesses a supergene homologous to the social supergene in *S. invicta* (unpublished data). To further explore the underpinnings of social polymorphism in *S. geminata*, we sampled ants from a large number of nests of both forms in northern Florida and subjected them to genetic, behavioral, and phenotypic analyses. Our data demonstrate a remarkable alternate reproductive mode in the polygyne form, thus revealing unexpected connections between transitions at different levels of complexity in this ant.

CHAPTER TWO

METHODS

Collection and Sampling

Monogyne and polygyne *Solenopsis geminata* nests were collected from multiple locations in Gainesville, Alachua Co. in northern Florida, US in the spring and early summers of 2014-2017. Polygyne nests were collected from two sites approximately 19km apart: Parker Road (PR) [29°34'02.2"N 82°28'28.1"W] and Boulware Springs (BS) [29°37'11.8"N 82°17'22.5"W]. Monogyne nests were collected from PR, BS, and a third location in Alachua Co.: Kanapaha Oaks (KO) [29°33'45.6"N 82°27'23.6"W]. Ants were reared and maintained in the laboratory as described by Ross[32]. A mean of 11.5 workers (pupae or adults) was sampled from each nest, along with reproductive (dealate, inseminated) queens (from 16 monogyne and 25 polygyne nests), daughter (winged virgin) queens (from 22 monogyne and 23 polygyne nests), and males (from 14 monogyne and 20 polygyne nests), and sperm was extracted from the spermathecae of 56 reproductive queens (from 17 polygyne nests) (see Appendix A for complete sample sizes).

To obtain known offspring of individual queens from polygyne nests, we established colony fragments with single queens isolated from field-collected nests from both sites housed individually in small trays with a few thousand workers and brood. Because some of the initial brood were offspring of queens from the polygyne source nest other than the isolated queen, we waited for all such brood to eclose as adults before sampling pupae. We genotyped individuals from 44 fragments, including reproductive queens heading 36 fragments, daughter queens from nine fragments (88 total; mean=9.8 per fragment), workers from 25 fragments (294 total; mean=11.8 per fragment), and males from seven fragments (21 total; mean=3.0 per fragment), and we dissected spermathecae from 23 queens (See Appendix A for complete sample sizes).

Microsatellite Genotyping

We genotyped all samples (2277 individuals from 73 monogyne and 42 polygyne nests) at nine previously described polymorphic microsatellite loci (*Bertha, Sdag_C294, Sdag_C536, Sol_i114, Sol_i120, Sol_i126, Sol_i129, Sol-42f, and Sol-49*, diversity data in Appendix B)[81]. A subset of sampled individuals (one individual from each of 30 monogyne nests and 43 polygyne queens; 13 from 7 nests at site BS and 30 from 15 nests at site PR) also was genotyped at 26 additional microsatellite loci (*Jackstraw, Jerry_Garcia*, *Sdag_C1*, *Sdag_C121*, *Sdag_C185*, *Sdag_C204*, *Sdag_C216*, *Sdag_C234*, *Sdag_C264*, *Sdag_C278*, *Sdag_C316*, *Sdag_C334*, *Sdag_C368*, *Sdag_C485*, *Sol_i113*, *Sol_i125*, *Sol_i127*, *Sol_i136*, *Sol-6*, *Sol-20*, *Sol-55*, *Sol-J1*, *Sol-M2*, *Sol-M3*, *Sol-M5*, and *Wharf_Rat*; diversity data in Appendix C)[81]. Total genomic DNA was extracted from each individual (or spermatheca) using the PureGene Core Kit A (Qiagen) and following the manufacturer's instructions. Microsatellites were amplified using previously described methods[81]. PCR amplicons were diluted (34:1 or 45:1, depending on the locus) and pooled before 1.5 µL was added to a 96-well plate. Liz 600 size standard (0.1 µL) and formamide were added to all dilutions before analysis on an ABI-3730XL-96 capillary sequencer (Applied Biosystems) at the Georgia Genomics and Bioinformatics Core at the University of Georgia. Microsatellite genotypes initially were scored using GeneMarker (SoftGenetics; *https://softgenetics.com/GeneMarker.php*) then were manually confirmed. We inspected microsatellite data for quality both manually and with Microchecker[82] , and found no evidence of genotype miscalling due to stutter, large allele dropout, or presence of null alleles.

Nuclear Genotypic Analyses

The genotype to individual ratio (G:N)[39] and observed heterozygosity (H_o) were calculated by randomly resampling a single individual from each nest and calculating these statistics for each of 10,000 iterations; this was done in order to avoid the problem of non-independence of nestmate genotypes. G:N was calculated as the number of unique genotypes divided by the total number of individuals (nests), and H_o was calculated for each locus by dividing the total number of heterozygotes by the total number of individuals.

To identify paternal contributions by social form to worker genotypes, multilocus genotypes of workers and sperm were analyzed to determine whether they contained paternal alleles found within either the “parthenogen gene pool” comprising the complete set of alleles represented in the parthenogenetic multilocus genotypes of polygyne reproductive queens, or the “monogyne gene pool” comprising the complete set of alleles represented in monogyne worker multilocus genotypes.

Discriminant analysis of principal components (DAPC) using nuclear genotypic data with the R package adegenet[83] (https://github.com/thibautjombart/adegenet) was conducted to identify clusters of genetically similar individuals, which were naively identified using the find.cluster() function. We used the dapc() function to construct preliminary DAPCs retaining all principal components (PCs) and discriminant functions, used these DAPCs to generate the optimal number of PCs using the optim.a.score() function, and then conducted a final DAPC retaining only this optimum number. We visualized all pairwise relationships for the first three PCs using scatterplots with superimposed minimum spanning networks connecting cluster centroids. For investigation of the origin of the polygyne subpopulations from the local *S. geminata* population, we used data from 30 polygyne queens from site PR, 13 polygyne queens from site BS, and 30 monogyne females, along with five previously published MLGs of *S. geminata* individuals from Mexico[46] as an outgroup, employing 32 microsatellite loci. For investigation of an interspecific hybrid origin, we used other members of the North American fire ant clade[46] along with Florida *S. geminata*, again employing 32 loci. For investigation of a hybrid origin involving other nominal *S. geminata* populations, we surveyed 23 microsatellite loci in Florida *S. geminata* and other nominal conspecific populations from around the world[50].

mtDNA Sequence Analyses

We sequenced a 784bp portion of the *mitochondrial cytochrome oxidase subunit 1* (*COI*) gene for a subset of monogyne and polygyne individuals. Sequences were aligned manually with Sequencher (https://www.genecodes.com/) and Geneious (https://www.geneious.com/). A minimum spanning network was constructed in PopART[84] (http://popart.otago.ac.nz/index.shtml). A maximum parsimony phylogeny was constructed using TNT[85] (http://www.lillo.org.ar/phylogeny/tnt/).

Endosymbiont Screening

PCR amplification of *Wolbachia* DNA was attempted in four monogyne and 12 polygyne daughter queens (six from each polygyne site) by using primers *Wsp*81F and

*Wsp*691R[51,86]. A portion of the nuclear gene EF1α was amplified as a positive control. While *Wolbachia* previously has been shown to infect all body regions of other fire ant species[51], it may be restricted to reproductive tissues in some hosts[87]. For this reason we screened genomic DNA isolated from portions of queen bodies containing the gaster (which contains the ovaries) when possible. PCR reaction mixes, primer sequences, and thermal cycling profiles are described elsewhere[88].

Dissections of Male Reproductive Tracts

Two to five adult males were sampled randomly from each of 11 nests of each social form (mean = 4.05 males/nest). These males were dissected and scored in random order, with the investigator blind to social form of origin. Male reproductive tract development was scored on a discrete scale from 1 to 4, according to the following scoring scheme: ‘1’ –reproductive tract not easily visible, very small and deflated, translucent; ‘2’ –reproductive tract more easily visible, small and deflated, typically with a faint white coloration; ‘3’ –reproductive tract easily visible, moderate size and turgidity, often with creamy white coloration; ‘4’ –reproductive tract easily visible, enlarged and turgid, with creamy white coloration.

Statistics

To test whether genotypes in the Alachua Co. monogyne form conformed to Hardy-Weinberg expectations, we conducted exact probability tests using single resampled workers per nest with Genepop on the Web[89,90] (http://genepop.curtin.edu.au/) and with the default Markov chain parameter values. To determine statistical significance of H_o values, we used the non-parametric Kruskal-Wallis test followed by a Dunn’s Multiple Comparisons Test with the Benjamini-

Hochberg method. Significant differences in G:N values between groups were judged by the non-overlap of 95% confidence intervals derived from resampling analyses.

We used non-parametric Mann-Whitney U-Tests to determine whether the distributions of reproductive development scores for males of the two forms differed significantly. Because of the small number of discrete values and non-independence of nestmate males, a randomization test was performed as well—a single male's score was randomly selected from each nest then randomly assigned to one of two 11-nest pools; means were calculated for each pool and the differences between the means over 10,000 replicates were sorted. Statistically significant differences between the forms were inferred by determining if the mean differences between the actual scores of single males per nest of the two forms fell within the 2.5% tails of the score-difference distributions from randomized males.

Queen Introduction Assays

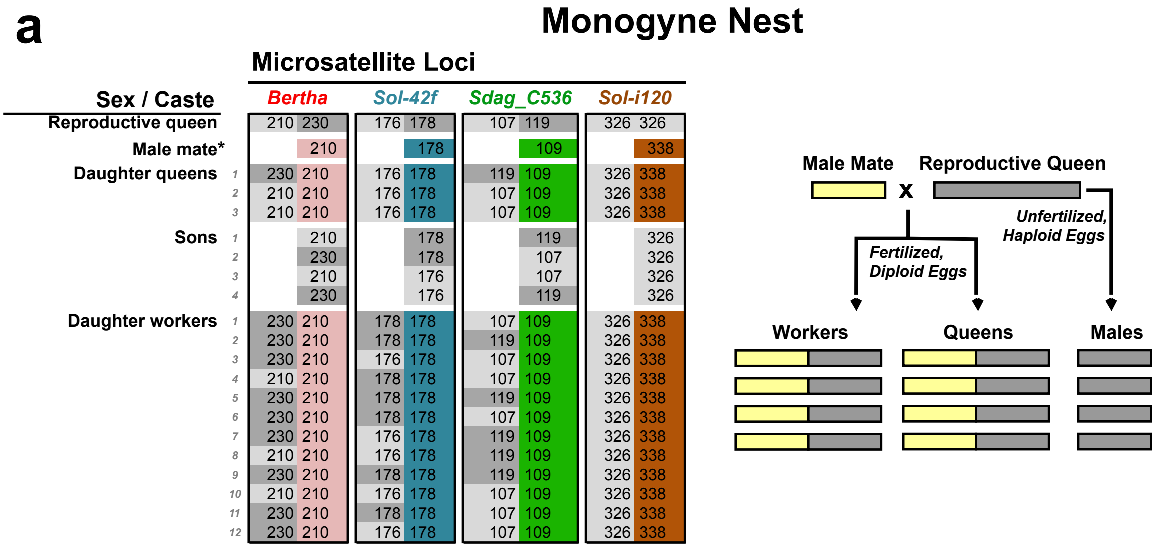
Fragments of workers were held queenless overnight, and a single live reproductive queen was introduced into each and monitored over the ensuing six-hour period, following procedures for the assays performed by Trible and Ross[45]. Results of assays were scored as "accepted" (queen alive in nest at end of six-hour assay) or "rejected" (queen dead). Queens and worker fragments were never re-used within each treatment category.

CHAPTER THREE

RESULTS

We collected 73 monogyne and 42 polygyne nests of *S. geminata* in Alachua County, Florida, sampling workers from each nest as well as reproductive queens, daughter (winged virgin) queens, and males when available (see Appendix A for sample sizes). All sampled individuals were genotyped at nine polymorphic microsatellite loci (diversity data in Appendix B). We noticed striking differences between the social forms in their patterns of genotypic variation within nests. Distributions of multilocus genotypes (MLGs) among nestmates from all monogyne nests reflected the standard reproductive mode of Hymenoptera—a single diploid mother queen, singly mated to a haploid male, producing all daughters (queens and workers) from fertilized diploid eggs and males from unfertilized haploid eggs (see also ref. [29]). All daughters in such nests have equal maternal and paternal genetic contributions from the same two parents, while sons possess only maternal alleles (Figure 1a, Appendix D).

Figure 1 / Genotype distributions at four microsatellite loci and pedigrees showing reproductive mode for representative *Solenopsis geminata* nests/nest fragments. Tables of multilocus genotypes (left) and pedigrees with colored bars characterizing genotypic compositions (right) are shown for individuals of a **(a)** monogyne nest, **(b)** polygyne nest, and **(c)** nest fragment containing a single polygyne reproductive queen and her progeny. For each panel, unique colors represent different maternal or paternal alleles (left) or genotypic contributions (right). Diploid genotypes of multilocus genotypes (left) comprise pairs of three-digit alleles separated by a space. Maternal alleles or genotypic contributions are shaded in gray, while paternal alleles/contributions are shaded in other colors. Solid lines in pedigrees represent known reproductive modes, while dashed lines represent putative alternative modes of production of males. The lighter shade of grey and smaller font size (left) or dashed lines (right; **b** and **c**) reflect the fact that polygyne males can be either haploid or diploid. * Male mate multilocus haplotype inferred from mother and daughter genotypes. ^Male mate multilocus haplotype determined from extracted DNA of pooled sperm from queen spermatheca.

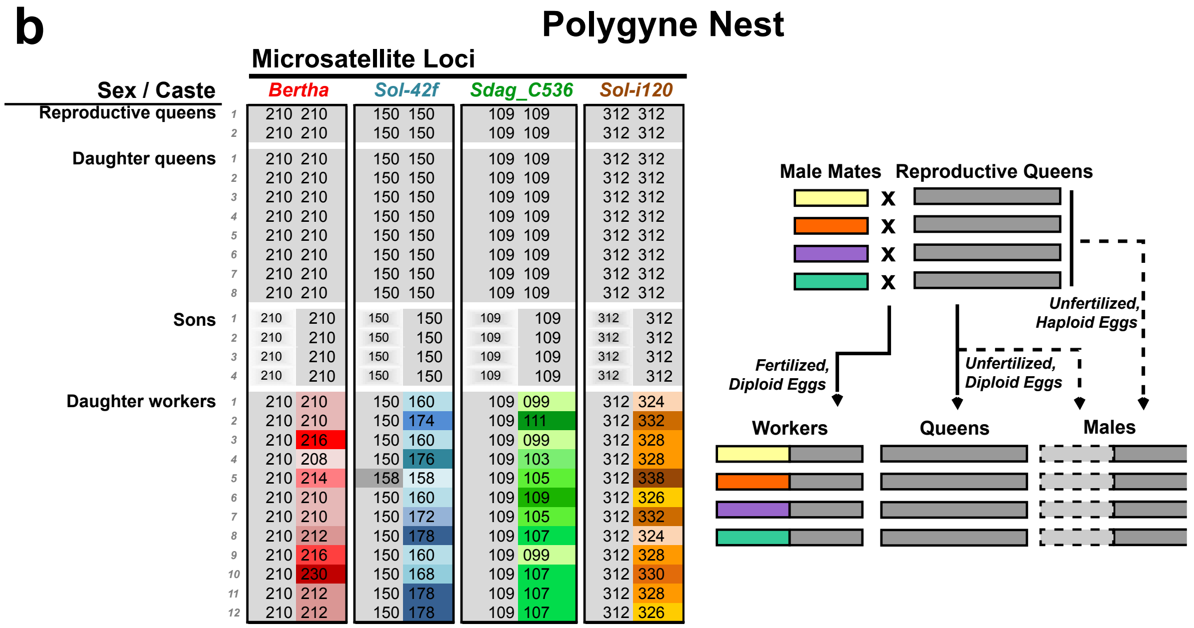


a

Monogyne Nest

Microsatellite Loci

Sex / Caste		*Bertha*	*Sol-42f*	*Sdag_C536*	*Sol-i120*
Reproductive queen		210 230	176 178	107 119	326 326
Male mate*		210	178	109	338
Daughter queens	1	230 210	176 178	119 109	326 338
	2	210 210	176 178	107 109	326 338
	3	210 210	176 178	107 109	326 338
Sons	1	210	178	119	326
	2	230	178	107	326
	3	210	176	107	326
	4	230	176	119	326
Daughter workers	1	230 210	178 178	107 109	326 338
	2	230 210	178 178	119 109	326 338
	3	230 210	176 178	107 109	326 338
	4	210 210	178 178	107 109	326 338
	5	230 210	178 178	119 109	326 338
	6	230 210	178 178	107 109	326 338
	7	230 210	176 178	119 109	326 338
	8	210 210	176 178	119 109	326 338
	9	230 210	178 178	119 109	326 338
	10	210 210	176 178	107 109	326 338
	11	230 210	178 178	107 109	326 338
	12	230 210	176 178	107 109	326 338

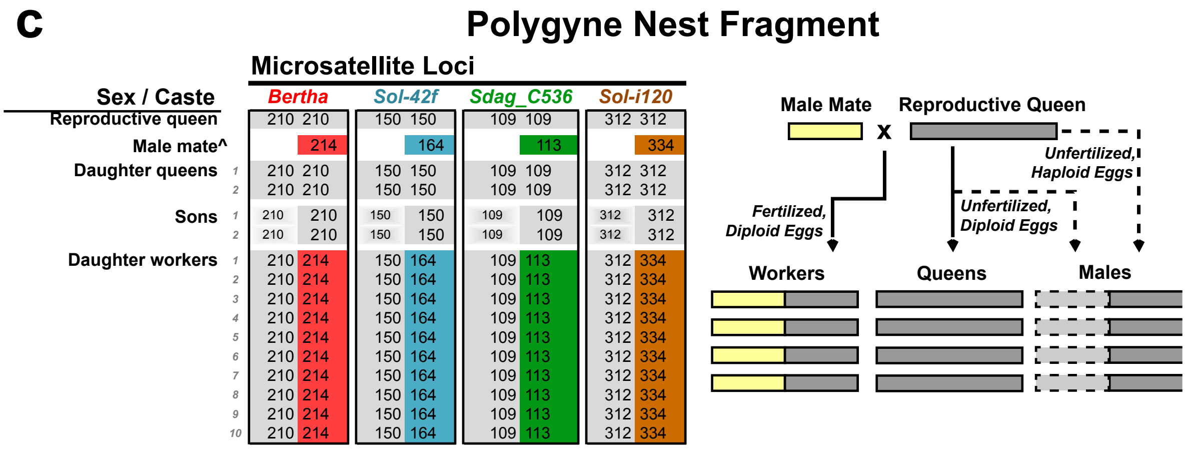


b

Polygyne Nest

Microsatellite Loci

Sex / Caste		*Bertha*	*Sol-42f*	*Sdag_C536*	*Sol-i120*
Reproductive queens	1	210 210	150 150	109 109	312 312
	2	210 210	150 150	109 109	312 312
Daughter queens	1	210 210	150 150	109 109	312 312
	2	210 210	150 150	109 109	312 312
	3	210 210	150 150	109 109	312 312
	4	210 210	150 150	109 109	312 312
	5	210 210	150 150	109 109	312 312
	6	210 210	150 150	109 109	312 312
	7	210 210	150 150	109 109	312 312
	8	210 210	150 150	109 109	312 312
Sons	1	210 210	150 150	109 109	312 312
	2	210 210	150 150	109 109	312 312
	3	210 210	150 150	109 109	312 312
	4	210 210	150 150	109 109	312 312
Daughter workers	1	210 210	150 160	109 099	312 324
	2	210 210	150 174	109 111	312 332
	3	210 216	150 160	109 099	312 328
	4	210 208	150 176	109 103	312 328
	5	210 214	158 158	109 105	312 338
	6	210 210	150 160	109 109	312 326
	7	210 210	150 172	109 105	312 332
	8	210 212	150 178	109 107	312 324
	9	210 216	150 160	109 099	312 328
	10	210 230	150 168	109 107	312 330
	11	210 212	150 178	109 107	312 328
	12	210 212	150 178	109 107	312 326



c

Polygyne Nest Fragment

Microsatellite Loci

Sex / Caste		*Bertha*	*Sol-42f*	*Sdag_C536*	*Sol-i120*
Reproductive queen		210 210	150 150	109 109	312 312
Male mate^		214	164	113	334
Daughter queens	1	210 210	150 150	109 109	312 312
	2	210 210	150 150	109 109	312 312
Sons	1	210 210	150 150	109 109	312 312
	2	210 210	150 150	109 109	312 312
Daughter workers	1	210 214	150 164	109 113	312 334
	2	210 214	150 164	109 113	312 334
	3	210 214	150 164	109 113	312 334
	4	210 214	150 164	109 113	312 334
	5	210 214	150 164	109 113	312 334
	6	210 214	150 164	109 113	312 334
	7	210 214	150 164	109 113	312 334
	8	210 214	150 164	109 113	312 334
	9	210 214	150 164	109 113	312 334
	10	210 214	150 164	109 113	312 334

We expected reproductive structure in polygyne *S. geminata* nests to mirror those typical of polygyne *S. invicta*[29-33]—multiple diploid reproductive queens, each typically mated singly to a different haploid male, producing all daughters via sexual reproduction. Such nests typically contain diverse multilocus genotypes across sampled daughters of both castes. Sampled polygyne *S. geminata* nests differed strikingly from this pattern. Daughter queen MLGs typically contained only alleles found also in the MLGs of reproductive queens from the same nest, and both daughter and reproductive queen MLGs generally were homozygous at all loci (Figure 1b, Appendix E). At each locus, workers bore one allele found in nestmate queens and a second allele typically absent from these queens, with the second allele often different from the analogous alleles found in nestmate workers. The rare exceptions to these patterns were a small proportion (0.04) of daughter queens with MLGs similar to those of nestmate workers. Importantly, no workers were found with MLGs similar to those of queens, and no reproductive queens possessed worker-like genotypes.

A likely explanation for the observed genotypic differences between female castes in polygyne *S. geminata* is that daughter queens and workers are produced via different reproductive mechanisms, as has been observed occasionally in other ants[34-38]. To explore this possibility, we isolated individual reproductive queens in fragments of their original polygyne nest and genotyped their known progeny. MLGs of all but one of the resulting daughter queens (nine fragments, mean 9.8 per fragment) were identical to their mothers' MLGs, and worker MLGs (25 fragments, mean 11.8 per fragment) invariably contained non-maternal alleles (Figure 1c, Appendix F). The single exceptional daughter queen (GPR16-1-Q1-fp20) had a worker-like genotype and came from a fragment yielding 35 genotyped daughter queens; thus the mother queen evidently produced queen offspring with a worker-like genotype at a low frequency (0.03), similar to that inferred for the population (0.04). We also genotyped the contents of the spermatheca (sperm storage organ) of 23 isolated queens. The haplotypes of pooled sperm extracts from each queen always featured a single allele at each locus, consistent with single matings to haploid males, and in every case the sperm multilocus haplotype matched the non-maternal allele found in worker offspring of the queen (Figure 1c, Appendix F).

These data support the specific hypothesis that polygyne queens at our study sites produce daughter workers sexually and daughter queens asexually (via thelytokous parthenogenesis). Importantly, this exceptional reproductive strategy is associated with a lack of genotypic diversity in polygyne queens. We calculated the genotype to individual ratio (G:N)—the number of distinct MLGs in a sample divided by the number of individuals in that sample[39]—as well as the observed heterozygosity (H_o) by randomly resampling a single individual of each caste from each nest. The median G:N values for all groups of monogyne females, as well as for polygyne workers, were 1.0, demonstrating that MLGs from different nests rarely, if ever, were identical (Figure 2a). G:N values for polygyne reproductive and daughter queens were much lower (median=0.33 and 0.37, respectively, with overlapping 95% confidence intervals that do not, however, overlap those of other groups; Figure 2a). This latter result reflects the surprising fact that queens from different polygyne nests at the same site often share identical MLGs (Appendix G). A similar trend was observed for H_o, with all monogyne females and polygyne workers having similarly high mean values (0.62–0.67) and polygyne queens having exceptionally low mean values (0.01– 0.05) (Figure 2b). Low heterozygosity of polygyne queens was observed also at an additional 26 microsatellite loci (mean H_o=0.03 for all queens at both sites, diversity data in Appendix C). Together, these statistics reflect exceptionally low genetic variation in polygyne queens, both at individual loci and among MLGs of queens from different nests. Indeed, we observed only ten MLGs at our primary loci among 126 polygyne reproductive queens from 25 nests (Appendix G), compared with 16 MLGs among 16 monogyne reproductive queens. Eight of the ten polygyne queen MLGs were homozygous at all loci, with each of the remaining two heterozygous at just a single locus.

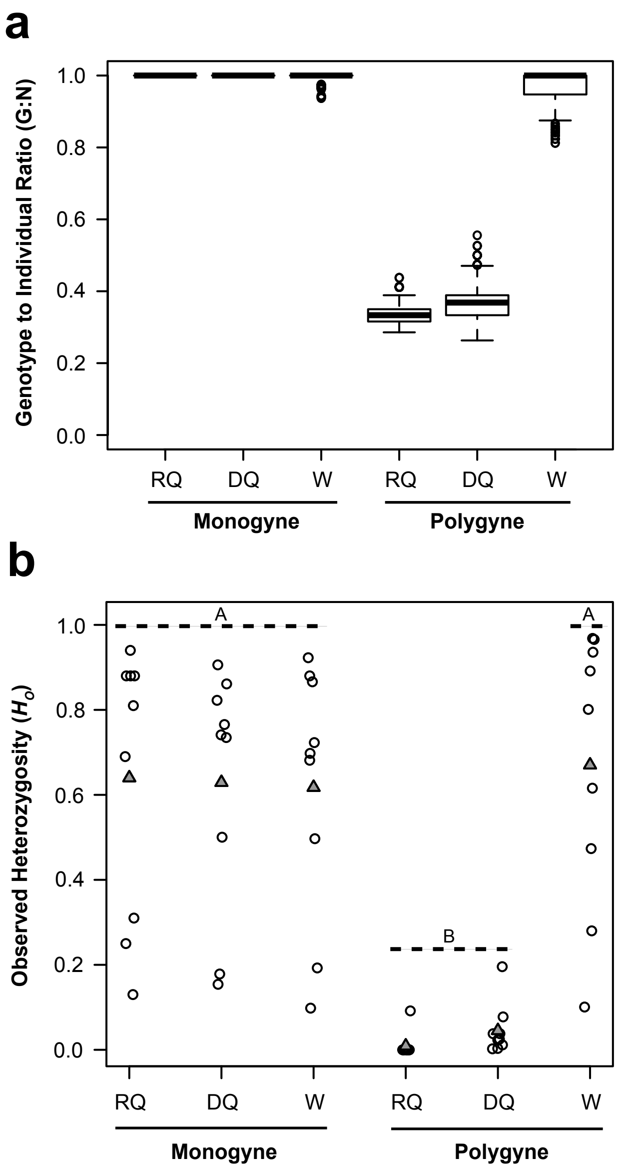


Figure 2 / Patterns of genetic variation for different classes of females in monogyne and polygyne *S. geminata*. Statistics are shown for reproductive queens (RQ), daughter queens (DQ), and workers (W). **(a)** Box-and-whisker plots showing G:N values calculated from the nine primary study loci using 10,000 random subsamples of a single individual per nest (thus reflecting among-nest diversity). Plots comprise the median (horizontal line), interquartile range (IQR, box), plus/minus 1.5·IQR (whiskers), and outliers (circles). **(b)** Mean H_0 values calculated for the nine primary study loci generated using random subsamples. The mean for all loci in each category is displayed as a grey triangle. Different letters over dashed bars indicate significantly different distributions of values for groups (all P < 0.01).

In contrast to the polygyne form, the local monogyne form is genetically diverse (Figure 2) and appears to comprise an outbred population—no significant deviation from Hardy-Weinberg expected genotype frequencies was observed using either a sample of 27 individuals scored at 35 loci (exact test, X^2=83.3, DF= 68, P=0.10) or a larger sample of 73 individuals scored at nine loci (exact test, X^2=25.2, DF=18, P=0.12) (see also refs. [27,33]). Furthermore, no evidence of genetic differentiation between monogyne sites—three local and one roughly 200km distant—was found (jackknife mean F_{ST} <0.01), suggesting panmixia in this form across northern Florida.

The substantial genetic diversity of polygyne workers is striking given the paucity of diversity in their mothers. Because these workers are produced sexually, their augmented diversity must derive from the mates of polygyne queens. To determine the identity of these mates, we compared polygyne worker MLGs with both the monogyne gene pool (all alleles found in this form) and the parthenogen gene pool (all alleles found in polygyne parthenogens and, hence, their sons). All MLGs for 693 workers sampled from 40 polygyne nests featured at least one, and usually several, loci containing non-maternal alleles that were absent from the parthenogen gene pool; furthermore, most or (typically) all non-maternal alleles in workers occurred in the monogyne gene pool. This suggests that polygyne workers almost invariably were sired by monogyne males rather than males of their own social form. We next genotyped the spermathecal contents of 56 polygyne queens from 17 nests. Observed sperm haplotypes were consistent with all of these queens being singly mated to haploid males (Appendix H). With one exception, sperm multilocus haplotypes contained two or more alleles found in the monogyne gene pool but not in the parthenogen gene pool (Figure

3, Appendix H). These results indicate that virtually all queens from our polygyne sites mate with monogyne males to produce workers. Evidently, no monogyne daughter progenies were sired by polygyne males, as all such daughters contained at least one locus with alleles absent from the parthenogen gene pool.

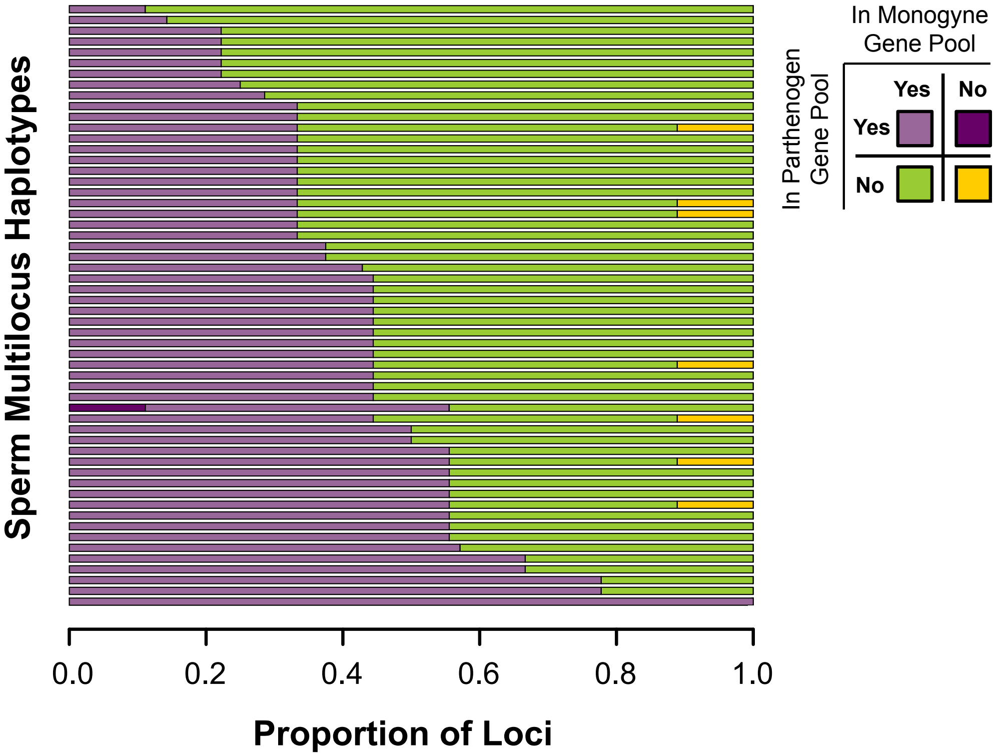


Figure 3 / Allelic composition of multilocus haplotypes of sperm in spermathecae of polygyne reproductive queens. Different colors in each bar represent the proportions of primary microsatellite loci comprising a given sperm multilocus haplotype that contain alleles characterized as present or absent in each social form.

To test whether this unexpected pattern of matings may result from reproductive impairment of polygyne males, we dissected adult males of both forms and quantified their reproductive development. Polygyne males exhibited significantly reduced development of their reproductive tissues compared to monogyne males (Mann-

Whitney test, median U=21, P=0.004) (Figure 4), consistent with a prevalence of reproductive defects in polygyne males. Male sterility is known to result from diploidy in *S. invicta*[40] and other Hymenoptera[41,42] and, indeed, we inferred that roughly one third of polygyne males were diploid based on heterozygosity at one or more loci (Appendix I). However, the minimal reproductive development of such diploid males was typical of all dissected polygyne males, including putative haploids (Mann-Whitney test, median U=18, P=0.3 for comparison between confirmed diploids and putative haploids). Related to this point, we may have considerably underestimated the frequency of polygyne male diploidy because: 1) all detected diploid males lacked paternal alleles (contained only maternal alleles; Appendix I), indicating they were produced asexually, 2) it is impossible to distinguish asexually produced diploid sons homozygous at surveyed loci from haploid sons, and therefore 3) such undetected homozygous diploid males are expected to be common because most polygyne reproductive queens were multilocus homozygotes. All monogyne males contained a single maternal allele at each locus, despite high heterozygosity at our marker loci, indicative of their haploidy.

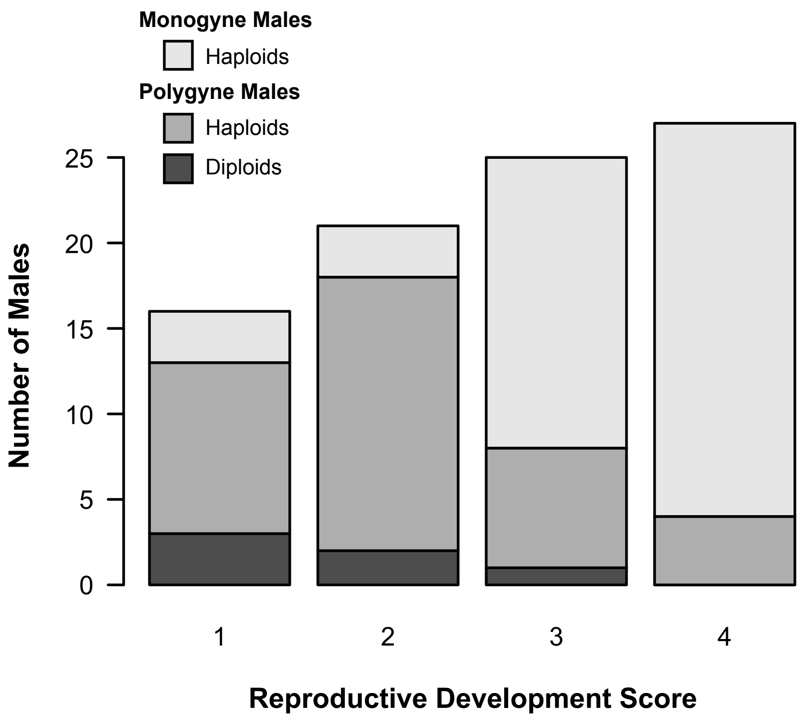
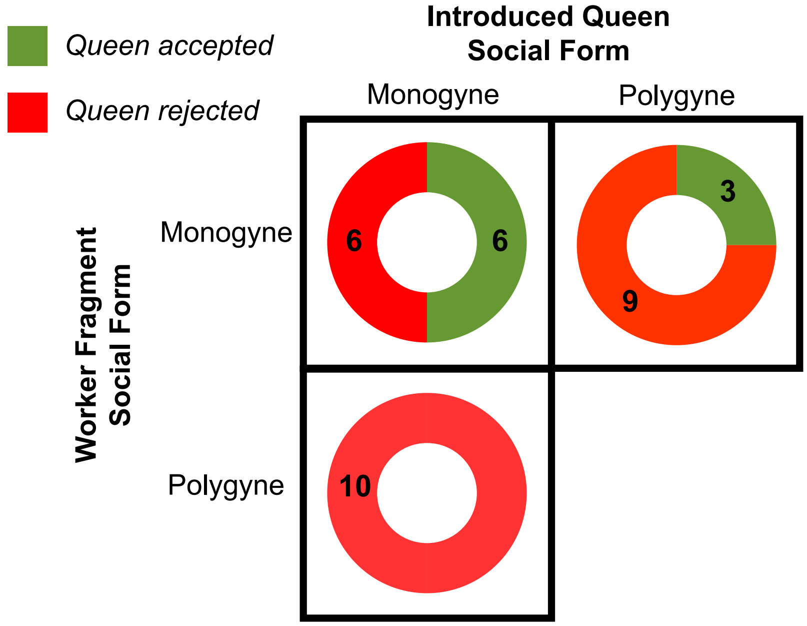


Figure 4 / Reproductive development of mature adult monogyne and polygyne *S. geminata* males. Higher scores indicate greater development of reproductive tissues. Diploid males were heterozygotes at one or more microsatellite loci, while haploid males each possessed only a single allele at each locus, and thus were inferred to be hemizygous. Monogyne males were all inferred hemizygotes (haploids).

Queen Introductions Between Social Forms



Queen Introductions Between Sites in Polygyne Form

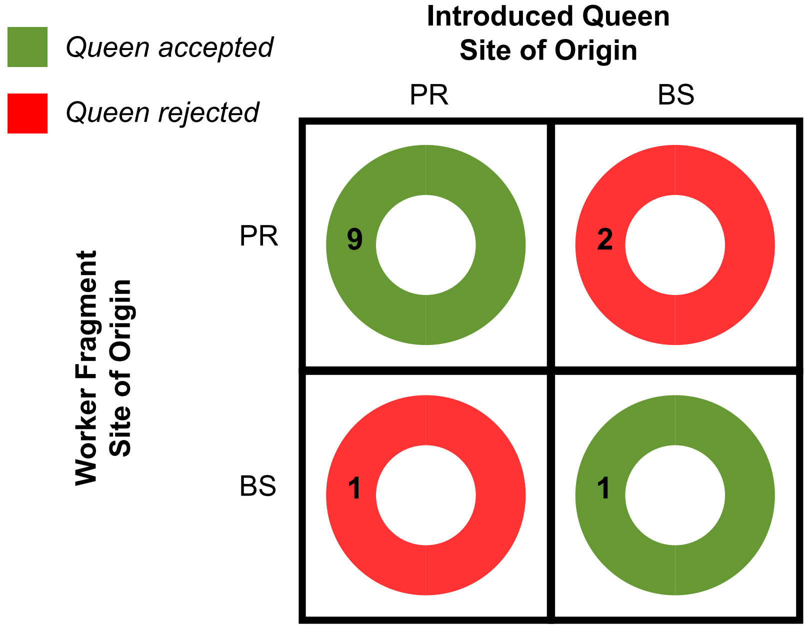


Figure 5 / Results of queen introduction behavioral assays. Donut charts show proportions of queens accepted or rejected by colony fragments composed of queenless adult workers, with actual numbers of trial outcomes denoted on each slice.

Conspecific ant social forms often are characterized by differences in worker tolerance of non-nestmates[43]. For example, in monogyne *S. invicta* aggression is displayed towards newly mated queens from different colonies seeking adoption[44]. In polygyne *S. invicta*, however, aggression against both worker and queen non-nestmates of the same form is greatly reduced, likely due to joint possession of cuticular semiochemical profiles associated with the polygyny-inducing supergene[44,45]. To investigate such discrimination in *S. geminata*, we introduced queens into dequeened colony fragments comprising workers from nests of the alternate form or site. Polygyne colony fragments accepted queens from their own site but rejected polygyne queens from the other site (as well as monogyne queens) (Figure 5), suggesting distinct queen semiochemical profiles at each polygyne site that may reflect divergent underlying genetic variation. In support of this, polygyne queens from our two study sites 19km apart, while displaying identical reproductive modes, appear quite genetically distinct at our markers. Four of the ten parthenogen MLGs occurred exclusively at one site while the remaining six occurred only at the other (Appendix G). Moreover, limited allele sharing was evident between polygyne sites; for instance, queens from the two sites had completely nonoverlapping sets of alleles at 20 of the 35 loci studied (Figure 6a). Importantly, considerably greater overlap was evident between each polygyne subpopulation and the monogyne form; virtually all alleles in polygyne queens were observed also in the monogyne form (Figure 6a), with exceptions typically involving highly polymorphic loci, for which some rarer alleles in the monogyne form likely remained unsampled (due in part to the reduced sample size for the expanded set of 26 loci).

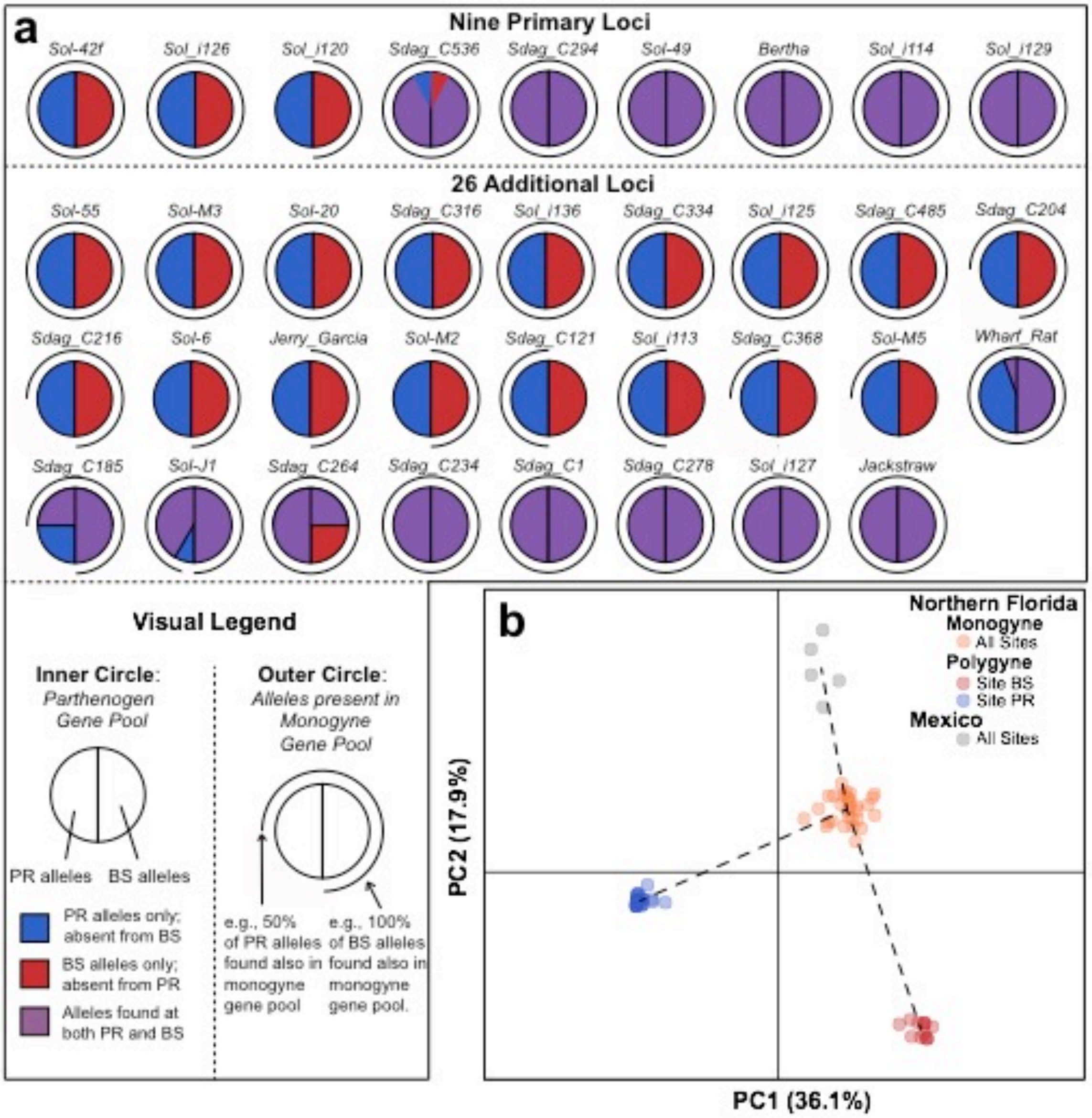


Figure 6 / Nuclear DNA population genetic structure in *S. geminata*. (a) Nine primary microsatellite loci were studied in 2277 individuals from 73 monogyne and 42 polygyne nests, while 26 additional loci were studied in a subset of 30 monogyne nests and 43 polygyne queens. Within the inner circle for each locus, the left side represents alleles found in site PR polygyne queens, and the right side represents alleles found in site BS queens. Blue and red shading represent alleles unique to each polygyne site, while purple shading represents alleles found at both sites. An outer circle (or portion thereof) surrounding each inner circle represents alleles in polygyne queens also observed in the local monogyne form, with wedges of the inner circle and portions of the outer circle scaled according to allele proportions at each polygyne site. Locus names are given above circles. **(b)** Scatterplots for four clusters of individuals recognized by DAPC comprising projections on the first two principal components; the analysis was based on genotypes at 32 microsatellite loci. Cluster centroids calculated using all principal components are connected by a minimum-spanning tree (dashed lines).

We examined nuclear population genetic structure further by conducting discriminant analysis of principal components (DAPC) using data from 32 microsatellite loci. For this analysis, we included polygyne queens from sites PR and BS, monogyne females, and *S. geminata* individuals from Mexico[46] as an outgroup. The analysis identified four clusters with membership corresponding perfectly to the above categories (Figure 6b). The monogyne cluster represents a hub connected to all other clusters by a minimum spanning tree, with the two polygyne clusters adjacent to the monogyne cluster rather than each other in multidimensional space, indicating that ants from the two polygyne sites are more similar genetically to those of the local monogyne form than to each other.

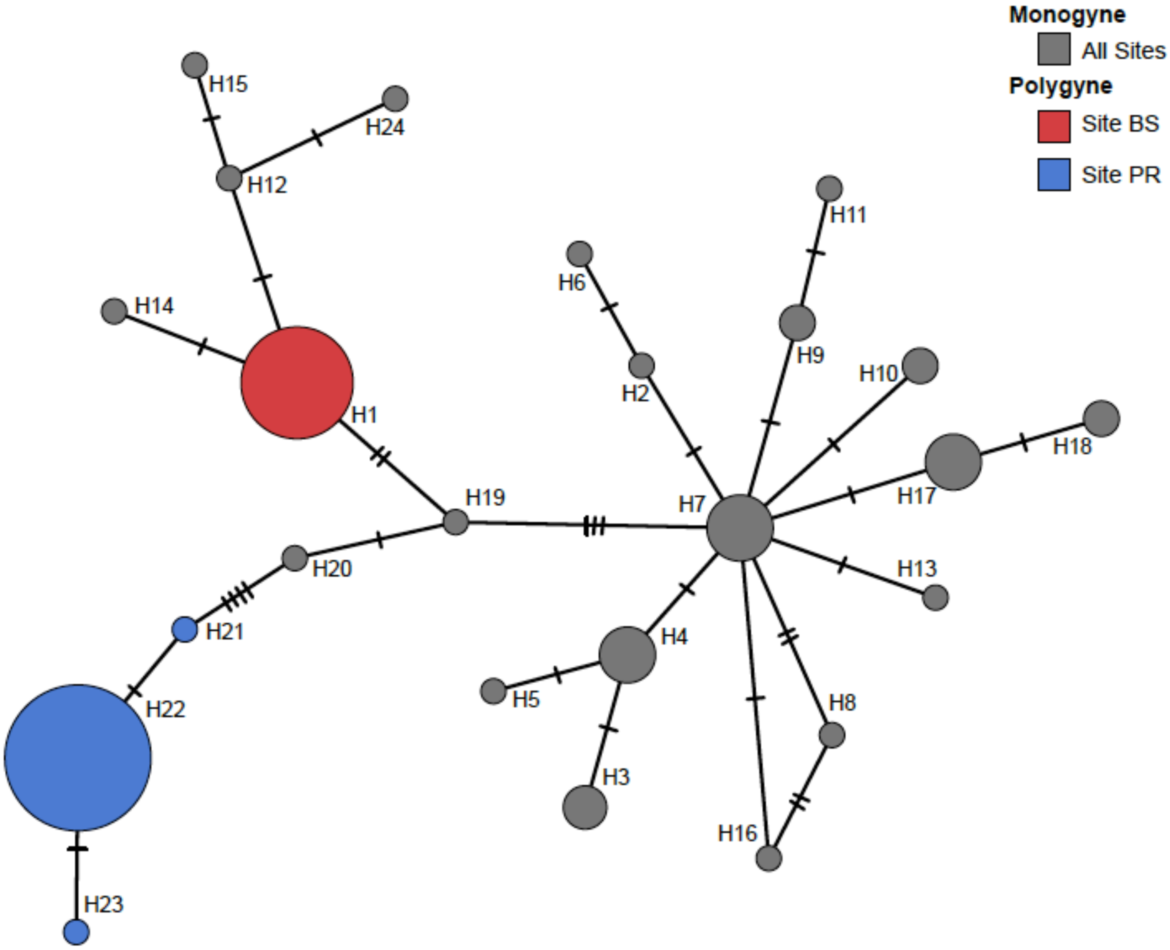


Figure 7 / Mitochondrial DNA haplotype diversity and population genetic structure in monogyne and polygyne *S. geminata*. Minimum-spanning network of 784bp segment of the mtDNA *COI* gene. Circles represent unique haplotypes, with areas proportional to the frequency of each in the total sample of 95 individuals. Tick marks denote single nucleotide substitutions. Blue haplotypes were found exclusively in polygyne nests from the PR site, red exclusively in polygyne nests from the BS site, and grey exclusively in monogyne nests. Haplotypes H14 and H15 occurred in Tallahassee, Florida, and the remainder in our Alachua Co., Florida study populations.

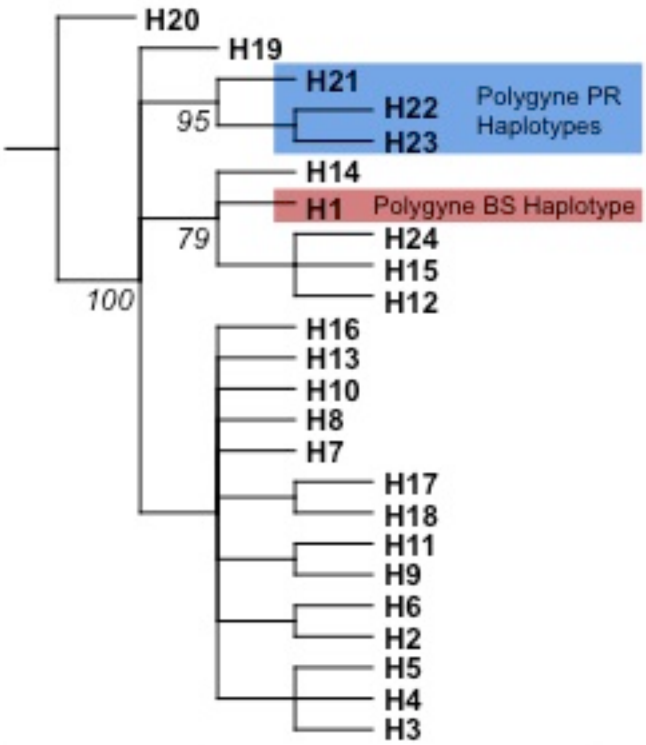


Figure 8 / Mitochondrial DNA haplotype phylogeny of monogyne and polygyne *S. geminata*. Maximum parsimony phylogeny of 784bp segment of the mtDNA *COI* gene (length=30, consensus of three such phylogenies). Bootstrap support values greater than 70 (out of 100 replicates) are shown below relevant nodes. Polygyne haplotypes are highlighted with colored boxes; the remaining haplotypes were found exclusively in monogyne individuals (H14 and H15 in individuals from Tallahassee, Florida, the remainder in individuals from our Alachua Co., Florida study population).

We examined mtDNA population structure by sequencing the *COI* gene for the same subset of individuals genotyped at the additional microsatellite loci and including supplementary sequences from a previous study of this population[27]. Twenty-four haplotypes were observed in total, with twenty recovered only in monogyne and the remainder only in polygyne individuals (Figure 7). One polygyne-exclusive haplotype (H1) was found only at site BS and was adjacent to monogyne-exclusive haplotypes in a minimum spanning network, separated by only one or two substitutions. The other three polygyne-exclusive haplotypes (H21, H22, and H23) occurred only in individuals from site PR; these haplotypes were linked by single substitutions but differed from the nearest monogyne-exclusive haplotype by four substitutions. Site-exclusive haplotypes from the two polygyne sites were diverged by a minimum of seven substitutions and separated by two monogyne-exclusive haplotypes in the network (Figure 7). Maximum parsimony phylogenies of the haplotypes revealed similar patterns of relationship (Figure 8)—minimum-length trees, all of which featured polyphyletic site-exclusive polygyne haplotype groups, had shorter tree lengths (30) than any trees in which monophyly of the polygyne haplotypes was enforced (minimum length=33). Together, the nuclear and mitochondrial genetic data suggest that the two polygyne subpopulations are genetically distinct and independent of one another but both likely evolved from local monogyne form.

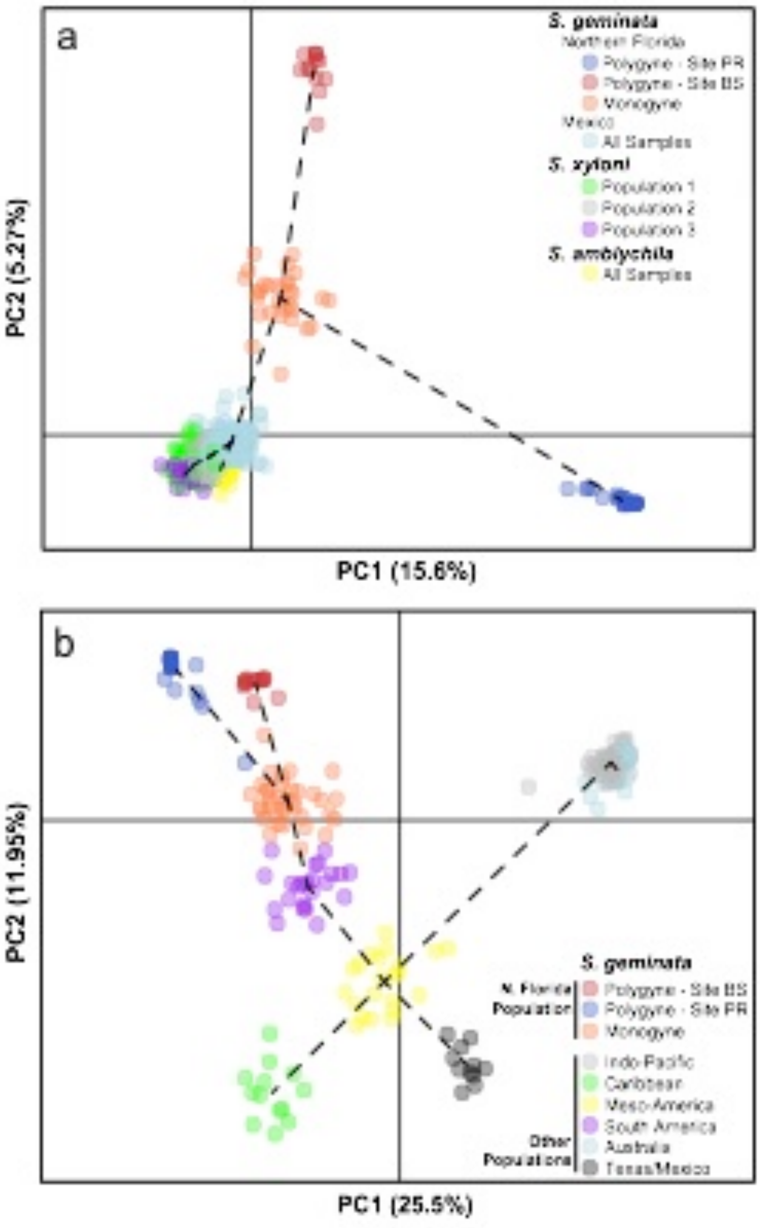
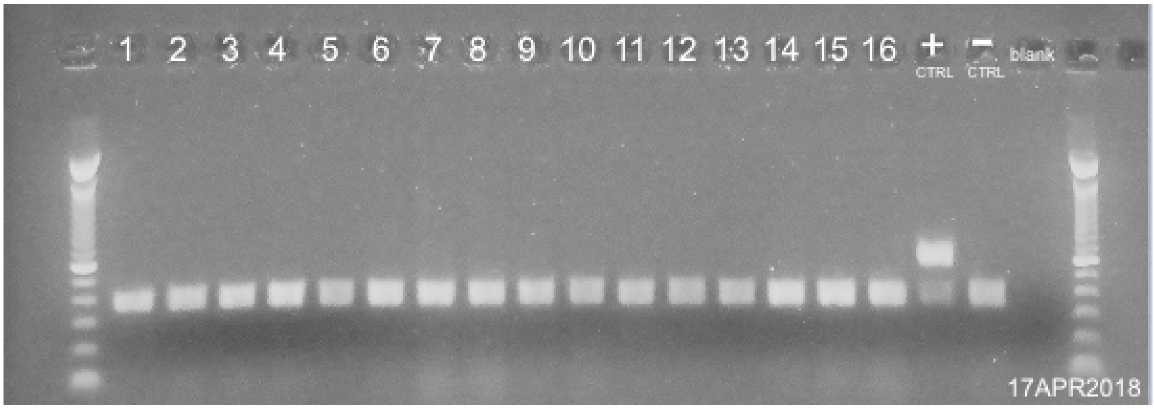


Figure 9 / Scatterplots for clusters of individuals of S. geminata and related fire ant species recognized by DAPC comprising projections on the first two principal components. Cluster centroids are connected by minimum-spanning trees (dashed lines). (a) Northern Florida S. geminata and other members of the North American fire ant clade (data from 32 microsatellite loci). Both Florida polygyne clusters are adjacent to the sympatric monogyne cluster in multidimensional space rather than to other S. geminata or heterospecific clusters. (b) Northern Florida S. geminata and other nominal conspecific populations from around the world (data from 23 microsatellite loci). Both Florida polygyne clusters are adjacent to the sympatric monogyne cluster in multidimensional space rather than to other S. geminata clusters.

Major causes of transitions from sexual to asexual reproduction in insects include hybridization between sexually reproducing species and parthenogenesis induction by endosymbionts (e.g., *Wolbachia*)[47,48]. A hybrid origin in polygyne *S. geminata* seems unlikely given the genetic similarity between the sympatric social forms in northern Florida. Nonetheless, hybridization is known to occur between *S. geminata* and the sympatric congener *S. xyloni*[49], and could also occur between unrecognized cryptic species regarded as nominal *S. geminata*, given the substantial geographic genetic structure reported in this species[50]. However, we found no evidence for such hybridization from DAPC analysis using added samples of *S. geminata* and other North American fire ants and appropriate microsatellite panels[46,50] (Figure 9). We also confirm that neither form of *S. geminata* at our study sites is infected with *Wolbachia*, as reported previously[27,51] (Figure 10), although we cannot rule out infection by other endosymbionts.



Well	Sample Code	Social Form	Site	Material
1	GBS17-1-g-1	Monogyne	BS	Daughter queen gaster
2	GKO17-19-g-1	Monogyne	KO	Daughter queen gaster
3	GKO17-21-g-1	Monogyne	KO	Daughter queen gaster
4	GKO17-23-g-1	Monogyne	KO	Daughter queen gaster
5	P-2-4-gem-a-110	Polygyne	BS	Daughter queen body
6	GBS15-15-5-gaster	Polygyne	BS	Daughter queen gaster
7	GBS15-13-4-gaster	Polygyne	BS	Daughter queen gaster
8	GBS15-13-5-gaster	Polygyne	BS	Daughter queen gaster
9	GBS15-5-3-gaster	Polygyne	BS	Daughter queen gaster
10	GBS15-19-19-gaster	Polygyne	BS	Daughter queen gaster
11	GPR15_2_Q1_FP1	Polygyne	PR	Daughter queen body
12	GPR15_10_Q1_FP1	Polygyne	PR	Daughter queen body
13	GPR15_12_Q7_FP1	Polygyne	PR	Daughter queen body
14	GPR17_30_g_1	Polygyne	PR	Daughter queen gaster
15	GPR16-1-Q1-fp35	Polygyne	PR	Daughter queen body
16	GPR16-2-Q1-fp11	Polygyne	PR	Daughter queen body
+ ctrl	ED499b	Positive control	—	Adult worker body
- ctrl	P16-17	Negative control	—	Adult worker body
blank	—	—	—	Water Blank

F

Figure 10 / Results of PCR assay for *Wolbachia* infection in *Solenopsis geminata*. Gel image depicts PCR amplification products for a 590-632 bp portion of the Wolbachia surface protein gene (wsp; fragment size varies depending on Wolbachia strain assayed) and for a 400 bp portion of the EF1α nuclear gene for individual samples from both monogyne and polygyne colonies. The EF1α reference amplicon appears for all samples except the blank, whereas the wsp fragment appears only in the positive control. Materials for the positive and negative controls are individuals of other fire ant species. DNA ladder (100 bp fragments) in each outer lane has several bands identified (including 600 bp major reference band).

CHAPTER FOUR

DISCUSSION

Ant species with variable reproductive strategies present a unique opportunity to study the factors influencing biological complexity[52]. Here, we report two apparently independent instances of the evolution of polygyne colony structure from a monogyne population in *S. geminata*. In both cases the origin of polygyny was linked with a transition to clonal queen production, an association all the more remarkable in light of its contrast with social polymorphism in other ants. In *S. invicta*, for example, extensive genetic data demonstrate that both female castes are produced sexually in both social forms[29-33]. The independent, coincident occurrences of two major transitions in *S. geminata* suggest evolutionary connections between clonality and cooperative reproduction. We hypothesize that such connections are caused by: 1) fitness costs due to both reduction of heterozygosity and the accumulation of deleterious alleles associated with reduced recombination, 2) genetic bottlenecks resulting from the foundation of clonal lineages from a sexual population, and 3) preservation of favorable allele combinations in queen genomes due to the lack of recombination associated with sexual reproduction.

Asexuality leads to reductions in heterozygosity under certain mechanisms of parthenogenesis[47] (but see ref. [53]) and to the accumulation of deleterious mutations due to a lack of recombination[54,55]. These effects can negatively impact organisms' reproductive potential. Indeed, we observed at least one such heterozygosity-related fitness cost—the high frequency of sterile, diploid males produced by polygyne queens. Diploid male production may be unavoidable for these queens, as diploid sons evidently are produced via the same reproductive mechanism (from unfertilized diploid eggs) as daughter queens, presumably due to a reduction in heterozygosity at the sex-determining locus (in most Hymenoptera, heterozygosity at this locus triggers female development while hemi- or homozygosity triggers male development[41,42]). Hence, diploid male production may be an inherent ancillary effect of clonal queen production in *S. geminata*. Similar obligate production of diploid males occurs in *S. invicta* when queens mate with males bearing a matching allele at the sex-determining locus. Such queens are unable to found colonies independently[30], likely due to the substantial costs of diploid male production[29,30,56]. Parthenogenetic *S. geminata* probably experience this same impediment, and also may endure other reproductive deficits related to both their extensive homozygosity and the expected accumulation of deleterious mutations.

Such fitness costs likely are important in the evolution of colony queen number in ants[14,18]. This is due in part to the nature of reproduction in societies with a sterile worker caste. For such societies, colony reproduction is successful only if sexual offspring are produced, which occurs in fire ants only once colonies have reached a substantial worker population[57,58]. Reaching this threshold requires immense reproductive output, which can be achieved either by a single highly fecund queen or by multiple queens of lower fecundity[18,56]. Reproductive deficiencies resulting from the genetic costs of asexual queen production may preclude independent reproduction by clonal queens in *S. geminata*, thus rendering them obligately polygyne.

While polygyny is a suitable strategy for low-fecundity queens[18,56,59], this option may be unavailable in ancestrally monogyne populations. In such populations workers or competing queens execute supernumerary reproductive queens[60,61], presumably to secure the inclusive fitness benefits of raising exclusively their own siblings or offspring as future reproductives. *Solenopsis invicta* workers discriminate among potential new queens on the basis of variable queen cuticular semiochemical profiles, which seem to be associated with alternate genetic variants[44,45,62]. Assuming a similar genetic basis for semiochemical profiles in *S. geminata*, and given the extensive genetic variation present in the monogyne form, each monogyne queen may have a distinctive profile. Hence supernumerary queens seeking adoption into established nests are readily distinguished from resident queens by workers and executed. We show that polygyne queens possess only a small subset of the genetic variation present in the local monogyne form at our markers, presumably due to a genetic bottleneck associated with the founding of each clonal subpopulation (as in ref. [7]). Parallel genetic bottlenecks likely occurred at the loci underlying semiochemical diversity, raising the possibility that polygyne queens at each site share virtually identical cuticular chemical profiles (similar to refs. [63-66]) and therefore are indistinguishable from other resident queens by resident workers. Our queen introduction assays appear to support this hypothesis; polygyne workers from each site accepted all introduced sitemate queens but rejected all non-sitemates. Finally, induction of genetic bottlenecks associated with the onset of asexuality may stabilize polygyny in an ultimate sense by minimizing inclusive fitness conflicts between reproducers, given that queens of the same or slightly derivative clones are highly related to one another.

Further insight into the origins of polygyny in *S. geminata* comes from comparison with the genetic determinants of social polymorphism in other ants. In *S. invicta*, polygyny is obligately associated with a large supergene over which recombination with the homologous region is suppressed, thus preventing interform gene flow over this portion of the genome even as it persists over the rest[24,67]. A comparable but non-homologous supergene has been shown to be associated with social polymorphism in the distantly related ant *Formica selysi*[68], suggesting that such restructuring of genome architectures may often be important for the evolution of polygyny[69], presumably by locking together through restricted recombination numerous alleles beneficial for this social syndrome. Polygyny appears to have evolved independently in *S. geminata* and *S. invicta*; the supergene in the latter evidently arose in the stem group of the socially polymorphic clade of South American fire ants[25,28], to which *S. geminata* is relatively distantly related[26]. Because queens are produced clonally in polygyne *S. geminata*, polygyny is associated with the entire genome of each parthenogenetic lineage. Thus, complex allele combinations, including those important for coordinated expression of the various individual- and colony-level elements of polygyny, effectively are locked together over many generations, likely further contributing to the evolutionary stability of polygyne colony structure.

While shifts to parthenogenesis may facilitate transitions to polygyny, it is also conceivable that the reverse is true—that “normal” *S. geminata* queens facultatively produce new queens via parthenogenesis in the context of polygyny (e.g., phenotypic plasticity in reproductive mode). However, polygyne queens produced daughter queens almost exclusively via parthenogenesis in our colony fragments, essentially a monogyne colony context, suggesting that such plasticity is not currently responsible for clonal queen production in the polygyne form. Nonetheless, we cannot rule out the possibility that selection on facultative parthenogenesis in ancestral associations of multiple queens led to genetic assimilation of this trait.

The persistence of polygyne *S. geminata* despite the remarkably low genetic diversity and presumed negative fitness consequences in queens is likely related to the high diversity present in the sexually produced worker caste, which may impart such benefits as enhanced colony function and disease resistance[70-72]. The elevated genetic diversity of workers results from queens mating almost exclusively with males from the monogyne form, suggesting dependence of polygyne colonies upon monogyne colonies for their persistence. Similarly, polygyne *S. invicta* queens are dependent upon matings with monogyne males in the invasive range[73-75], presumably due to extensive polygyne male diploidy[30] and reduced fertility of haploid males bearing the polygyny-inducing supergene[76]. An important difference between these convergent phenomena lies in the fate of the transferred monogyne alleles; *S. invicta* queens use monogyne sperm to produce new queens as well as workers, ensuring that monogyne genes introgress into the polygyne form, whereas polygyne *S. geminata* queens use sperm almost exclusively to produce sterile workers. We can only speculate on the mechanism limiting the number of sexually produced daughters that develop as queens, but it could stem from loss of phenotypic plasticity for worker development in parthenogenetic lineages (similar to refs. [49,77-79]). Importantly, a low proportion (0.04) of polygyne *S. geminata* daughter queens evidently were produced sexually via interform mating, but no reproductive queens were observed with such genotypes, suggesting that they are incapable of establishing themselves as reproductive queens in polygyne nests (as in ref. [80]). Clearly, large scale genome-wide introgression of monogyne alleles into the polygyne Mendelian gene pool is effectively precluded in *S. geminata*.

Advances in biological complexity are achieved through the evolution of cooperative reproduction by previously independent reproducers. Examples include the transition from asexual to sexual reproduction and from solitary to eusocial life[1]. Little is known, however, about interactions between transitions at different levels. In this study we investigated independent occurrences of an analogous major transition—from single-queen to multiple-queen colonies—in a fire ant. In both cases this increase in complexity was associated with a parallel transition from sexual to asexual reproduction, an ostensible simplification in complexity. We propose that the transition to parthenogenetic queen production indirectly led to a transition in colony social organization in both groups, implicating a perceived decrease in complexity at one level of biological organization in prompting an increase in complexity at another. Our findings thus illustrate that changes at one level of biological organization may have unexpected impacts at other levels.

REFERENCES

1 Szathmáry, E. & Smith, J. M. The major evolutionary transitions. *Nature* **374**, 227-232 (1995).

2 Mockford, E. L. Parthenogenesis in psocids (Insecta: Psocoptera). *American Zoologist* **11**, 327-339 (1971).

3 Tibayrenc, M. & Ayala, F. J. The clonal theory of parasitic protozoa: 12 years on. *Trends in Parasitology* **18**, 405-410 (2002).

4 Walser, J. C., Gugerli, F., Holderegger, R., Kuonen, D. & Scheidegger, C. Recombination and clonal propagation in different populations of the lichen Lobaria pulmonaria. *Heredity* **93**, 322 (2004).

5 Alberto, F. *et al.* Within□population spatial genetic structure, neighbourhood size and clonal subrange in the seagrass Cymodocea nodosa. *Molecular Ecology* **14**, 2669-2681 (2005).

6 Foucaud, J. *et al.* Sex and clonality in the little fire ant. *Molecular Biology and Evolution* **24**, 2465-2473 (2007).

7 Rabeling, C. *et al.* Cryptic sexual populations account for genetic diversity and ecological success in a widely distributed, asexual fungus-growing ant. *Proceedings of the National Academy of Sciences* **108**, 12366-12371 (2011).

8 Rehan, S. M., Schwarz, M. P. & Richards, M. H. Fitness consequences of ecological constraints and implications for the evolution of sociality in an incipiently social bee. *Biological Journal of the Linnean Society* **103**, 57-67 (2011).

9 Brand, P. *et al.* The nuclear and mitochondrial genomes of the facultatively eusocial orchid bee Euglossa dilemma. *G3: Genes, Genomes, Genetics* **7**, 2891-2898 (2017).

10 Michener, C. D. in *Social Insects* (ed Wolf Engels) Ch. 5, 123-146 (Springer Verlag, 1990).

11 Plateaux-Quénu, C., Plateaux, L. & Packer, L. Population-typical behaviours are retained when eusocial and non-eusocial forms of Evylaeus albipes (F.)(Hymenoptera, Halictidae) are reared simultaneously in the laboratory. *Insectes Sociaux* **47**, 263-270 (2000).

12 Doering, G. N., Scharf, I., Moeller, H. V. & Pruitt, J. N. Social tipping points in animal societies in response to heat stress. *Nature Ecology & Evolution*, 1 (2018).

13 Keller, L. & Vargo, E. L. in *Queen Number and Sociality in Insects* (ed Laurent Keller) Ch. 2, 16-44 (Oxford University Press, 1993).

14 Ross, K. G. & Keller, L. Ecology and evolution of social organization: insights from fire ants and other highly eusocial insects. *Annual Review of Ecology and Systematics* **26**, 631-656 (1995).

15 Hölldobler, B. & Wilson, E. O. The number of queens: an important trait in ant evolution. *Naturwissenschaften* **64**, 8-15 (1977).

16 Nonacs, P. Queen number in colonies of social Hymenoptera as a kin□selected adaptation. *Evolution* **42**, 566-580 (1988).

17 Nonacs, P. The effects of polygyny and colony life history on optimal sex investment. *Queen number and sociality in insects.*, 110-131 (1993).

18 Keller, L. Social life: the paradox of multiple-queen colonies. *Trends in Ecology & Evolution* **10**, 355-360 (1995).

19 Mullon, C., Keller, L. & Lehmann, L. Social polymorphism is favoured by the co-evolution of dispersal with social behaviour. *Nature Ecology & Evolution* **2**, 132 (2018).

20 Macom, T. E. & Porter, S. D. Comparison of polygyne and monogyne red imported fire ant (Hymenoptera: Formicidae) population densities. *Annals of the Entomological Society of America* **89**, 535-543 (1996).

21 Hölldobler, B. & Wilson, E. O. *The Ants*. (Harvard University Press, 1990).

22 Ross, K. G. Multilocus evolution in fire ants: effects of selection, gene flow and recombination. *Genetics* **145**, 961-974 (1997).

23 Ross, K. G. & Keller, L. Genetic control of social organization in an ant. *Proceedings of the National Academy of Sciences* **95**, 14232-14237 (1998).

24 Wang, J. *et al.* A Y-like social chromosome causes alternative colony organization in fire ants. *Nature* **493**, 664 (2013).

25 Krieger, M. J. B. & Ross, K. G. Identification of a major gene regulating complex social behavior. *Science* **295**, 328-332 (2002).

26 Pitts, J. P., Camacho, G. P., Gotzek, D., McHugh, J. V. & Ross, K. G. Revision of the fire ants of the Solenopsis saevissima species-group (Hymenoptera: Formicidae). *Proceedings of the Entomological Society of Washington* **120**, 308-411 (2018).

27 Ross, K. G., Krieger, M. J. B. & Shoemaker, D. D. Alternative genetic foundations for a key social polymorphism in fire ants. *Genetics* **165**, 1853-1867 (2003).

28 Krieger, M. J. B. & Ross, K. G. Molecular evolutionary analyses of the odorant-binding protein gene Gp-9 in fire ants and other Solenopsis species. *Molecular Biology and Evolution* **22**, 2090-2103 (2005).

29 Ross, K. G., Vargo, E. L. & Fletcher, D. J. Colony genetic structure and queen mating frequency in fire ants of the subgenus Solenopsis (Hymenoptera: Formicidae). *Biological Journal of the Linnean Society* **34**, 105-117 (1988).

30 Ross, K. G. & Fletcher, D. J. C. Genetic origin of male diploidy in the fire ant, Solenopsis invicta (Hymenoptera: Formicidae), and its evolutionary significance. *Evolution* **39**, 888-903 (1985).

31 Ross, K. G. & Fletcher, D. J. C. Comparative study of genetic and social structure in two forms of the fire ant Solenopsis invicta (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology* **17**, 349-356 (1985).

32 Ross, K. G. Differential reproduction in multiple-queen colonies of the fire ant Solenopsis invicta (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology* **23**, 341-355 (1988).

33 Ross, K. G., Krieger, M. J. B., Shoemaker, D. D., Vargo, E. L. & Keller, L. Hierarchical analysis of genetic structure in native fire ant populations: results from three classes of molecular markers. *Genetics* **147**, 643-655 (1997).

34 Pearcy, M., Aron, S., Doums, C. & Keller, L. Conditional use of sex and parthenogenesis for worker and queen production in ants. *Science* **306**, 1780-1783 (2004).

35 Fournier, D. *et al.* Clonal reproduction by males and females in the little fire ant. *Nature* **435**, 1230 (2005).

36 Ohkawara, K., Nakayama, M., Satoh, A., Trindl, A. & Heinze, J. Clonal reproduction and genetic caste differences in a queen-polymorphic ant, Vollenhovia emeryi. *Biology Letters* **2**, 359-363 (2006).

37 Pearcy, M., Goodisman, M. A. D. & Keller, L. Sib mating without inbreeding in the longhorn crazy ant. *Proceedings of the Royal Society of London B: Biological Sciences* **278**, 2677–2681 (2011).

38 Leniaud, L., Darras, H., Boulay, R. & Aron, S. Social hybridogenesis in the clonal ant Cataglyphis hispanica. *Current Biology* **22**, 1188-1193 (2012).

39 Halkett, F., Simon, J.-C. & Balloux, F. Tackling the population genetics of clonal and partially clonal organisms. *Trends in Ecology & Evolution* **20**, 194-201 (2005).

40 Krieger, M. J. B., Ross, K. G., Chang, C. W. Y. & Keller, L. Frequency and origin of triploidy in the fire ant Solenopsis invicta. *Heredity* **82**, 142 (1999).

41 Crozier, R. H. Heterozygosity and sex determination in haplo-diploidy. *The American Naturalist* **105**, 399-412 (1971).

42 Stouthamer, R., Luck, R. F. & Werren, J. H. Genetics of sex determination and the improvement of biological control using parasitoids. *Environmental Entomology* **21**, 427-435 (1992).

43 Gotzek, D. & Ross, K. G. Genetic regulation of colony social organization in fire ants: an integrative overview. *The Quarterly Review of Biology* **82**, 201-226 (2007).

44 Keller, L. & Ross, K. G. Selfish genes: a green beard in the red fire ant. *Nature* **394**, 573-575 (1998).

45 Trible, W. & Ross, K. G. Chemical communication of queen supergene status in an ant. *Journal of Evolutionary Biology* **29**, 502-513 (2016).

46 Chialvo, P., Gotzek, D. A., Shoemaker, D. & Ross, K. G. Genetic analyses reveal cryptic diversity in the native North American fire ants (Hymenoptera: Formicidae: Solenopsis). *Systematic Entomology* **43**, 109-122 (2018).

47 Rabeling, C. & Kronauer, D. J. C. Thelytokous parthenogenesis in eusocial Hymenoptera. *Annual Review of Entomology* **58**, 273-292 (2013).

48 Ma, W. J. & Schwander, T. Patterns and mechanisms in instances of endosymbiont□induced parthenogenesis. *Journal of Evolutionary Biology* (2017).

49 Cahan, S. H. & Vinson, S. B. Reproductive division of labor between hybrid and nonhybrid offspring in a fire ant hybrid zone. *Evolution* **57**, 1562-1570 (2003).

50 Gotzek, D., Axen, H. J., Suarez, A. V., Helms Cahan, S. & Shoemaker, D. Global invasion history of the tropical fire ant: a stowaway on the first global trade routes. *Molecular Ecology* **24**, 374-388 (2015).

51 Shoemaker, D. D., Ross, K. G., Keller, L., Vargo, E. & Werren, J. H. Wolbachia infections in native and introduced populations of fire ants (Solenopsis spp.). *Insect Molecular Biology* **9**, 661-673 (2000).

52 Keller, L. Uncovering the Biodiversity of Genetic and Reproductive Systems: Time for a More Open Approach: American Society of Naturalists EO Wilson Award Winner Address. *The American Naturalist* **169**, 1-8 (2007).

53 Warren, W. C. *et al.* Clonal polymorphism and high heterozygosity in the celibate genome of the Amazon molly. *Nature Ecology & Evolution* **2**, 669 (2018).

54 Smith, J. M. *The Evolution of Sex*. (Cambridge University Press, 1978).

55 Felsenstein, J. The evolutionary advantage of recombination. *Genetics* **78**, 737-756 (1974).

56 DeHeer, C. J. & Tschinkel, W. R. The success of alternative reproductive tactics in monogyne populations of the ant Solenopsis invicta: significance for transitions in social organization. *Behavioral Ecology* **9**, 130-135 (1998).

57 Trible, W., Shoemaker, D. D. & Gotzek, D. Sociometry of Solenopsis geminata (Hymenoptera: Formicidae) reveals variation in colony-level phenotypes in fire ants. *Myrmecological News*, 47-53 (2018).

58 Tschinkel, W. R. Sociometry and sociogenesis of colonies of the fire ant Solenopsis invicta during one annual cycle. *Ecological Monographs* **63**, 425-457 (1993).

59 McInnes, D. A. & Tschinkel, W. R. Queen dimorphism and reproductive strategies in the fire ant Solenopsis geminata (Hymenoptera: Formicidae). *Behavioral Ecology and Sociobiology* **36**, 367-375 (1995).

60 Fletcher, D. J. C. & Blum, M. S. Regulation of queen number by workers in colonies of social insects. *Science* **219**, 312-314 (1983).

61 Bernasconi, G. & Keller, L. Reproductive conflicts in cooperative associations of fire ant queens (Solenopsis invicta). *Proceedings of the Royal Society of London B: Biological Sciences* **263**, 509-513 (1996).

62 Eliyahu, D., Ross, K. G., Haight, K. L., Keller, L. & Liebig, J. Venom alkaloid and cuticular hydrocarbon profiles are associated with social organization, queen fertility status, and queen genotype in the fire ant Solenopsis invicta. *Journal of Chemical Ecology* **37**, 1242-1254 (2011).

63 Giraud, T., Pedersen, J. S. & Keller, L. Evolution of supercolonies: the Argentine ants of southern Europe. *Proceedings of the National Academy of Sciences* **99**, 6075-6079 (2002).

64 Holway, D. A., Suarez, A. V. & Case, T. J. Loss of intraspecific aggression in the success of a widespread invasive social insect. *Science* **282**, 949-952 (1998).

65 Tsutsui, N. D. & Case, T. J. Population genetics and colony structure of the Argentine ant (Linepithema humile) in its native and introduced ranges. *Evolution* **55**, 976-985 (2001).

66 Teseo, S. *et al.* Genetic distance and age affect the cuticular chemical profiles of the clonal ant Cerapachys biroi. *Journal of Chemical Ecology* **40**, 429-438 (2014).

67 Pracana, R., Priyam, A., Levantis, I., Nichols, R. A. & Wurm, Y. The fire ant social chromosome supergene variant Sb shows low diversity but high divergence from SB. *Molecular Ecology* **26**, 2864-2879 (2017).

68 Purcell, J., Brelsford, A., Wurm, Y., Perrin, N. & Chapuisat, M. Convergent genetic architecture underlies social organization in ants. *Current Biology* **24**, 2728-2732 (2014).

69 Linksvayer, T. A., Busch, J. W. & Smith, C. R. Social supergenes of superorganisms: do supergenes play important roles in social evolution? *BioEssays* **35**, 683-689 (2013).

70 Cole, B. J. & Wiernasz, D. C. The selective advantage of low relatedness. *Science* **285**, 891-893 (1999).

71 Hughes, W. O. H. & Boomsma, J. J. Genetic diversity and disease resistance in leaf-cutting ant societies. *Evolution* **58**, 1251-1260 (2004).

72 Mattila, H. R. & Seeley, T. D. Genetic diversity in honey bee colonies enhances productivity and fitness. *Science* **317**, 362-364 (2007).

73 Ross, K. G. & Shoemaker, D. D. An unusual pattern of gene flow between the two social forms of the fire ant Solenopsis invicta. *Evolution* **47**, 1595-1605 (1993).

74 Ross, K. G. & Shoemaker, D. D. Nuclear and mitochondrial genetic structure in two social forms of the fire ant Solenopsis invicta: insights into transitions to an alternate social organization. *Heredity* **78**, 590 (1997).

75 Shoemaker, D. D. & Ross, K. G. Effects of social organization on gene flow in the fire ant Solenopsis invicta. *Nature* **383**, 613 (1996).

76 Lawson, L. P., Vander Meer, R. K. & Shoemaker, D. Male reproductive fitness and queen polyandry are linked to variation in the supergene Gp-9 in the fire ant Solenopsis invicta. *Proceedings of the Royal Society of London B: Biological Sciences*, rspb20120315 (2012).

77 Cahan, S. H. & Keller, L. Complex hybrid origin of genetic caste determination in harvester ants. *Nature* **424**, 306 (2003).

78 Romiguier, J., Fournier, A., Yek, S. H. & Keller, L. Convergent evolution of social hybridogenesis in Messor harvester ants. *Molecular Ecology* **26**, 1108-1117 (2017).

79 Cahan, S. H. *et al.* Loss of phenotypic plasticity generates genotype-caste association in harvester ants. *Current Biology* **14**, 2277-2282 (2004).

80 Schwander, T., Keller, L. & Cahan, S. H. Two alternate mechanisms contribute to the persistence of interdependent lineages in Pogonomyrmex harvester ants. *Molecular Ecology* **16**, 3533-3543 (2007).

81 Ascunce, M. S. *et al.* Global invasion history of the fire ant Solenopsis invicta. *Science* **331**, 1066-1068 (2011).

82 Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M. & Shipley, P. MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Resources* **4**, 535-538 (2004).

83 Jombart, T. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* **24**, 1403-1405 (2008).

84 Leigh, J. W. & Bryant, D. popart: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* **6**, 1110-1116 (2015).

85 Goloboff, P. A., Farris, J. S. & Nixon, K. C. TNT, a free program for phylogenetic analysis. *Cladistics* **24**, 774-786 (2008).

86 Zhou, W., Rousset, F. & O'Neill, S. Phylogeny and PCR–based classification of Wolbachia strains using wsp gene sequences. *Proceedings of the Royal Society of London B: Biological Sciences* **265**, 509-515 (1998).

87 Dobson, S. L. *et al.* Wolbachia infections are distributed throughout insect somatic and germ line tissues. *Insect Biochemistry and Molecular Biology* **29**, 153-160 (1999).

88 Shoemaker, D. D. *et al.* Distribution and prevalence of Wolbachia infections in native populations of the fire ant Solenopsis invicta (Hymenoptera: Formicidae). *Environmental Entomology* **32**, 1329-1336 (2003).

89 Raymond, M. & Rousset, F. GENEPOP (version 1. 2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* **86**, 248-249 (1995).

90 Rousset, F. Genepop’007: a complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources* **8**, 103-106 (2008).

APPENDIX A

SAMPLE SIZES FOR GENOTYPING STUDY

Sample sizes shown for all genotyped S. geminata individuals* in this study. For categories in which more than one individual was sampled per nest or isolation fragment, the numbers of fragments and nests are given in brackets and/or parentheses, respectively. Dashes indicate no genotypes scored.

	Number of Loci Scored	
	Nine†	**Thirty-five^**
Monogyne Form		
Total**		
Nests	73	30
Reproductive queens	16	8
Daughter queens	86 (22)	6
Workers	843 (73)	16
Males	96 (14)	—
KO Site		
Nests	51	21
Reproductive queens	12	8
Daughter queens	82 (21)	6
Workers	582 (51)	7
Males	96 (14)	—
BS Site		
Nests	11	9
Reproductive queens	3	—
Daughter queens	4 (1)	—
Workers	140 (11)	9
Males	—	—
PR Site		
Nests	11	—
Reproductive queens	1	—
Daughter queens	—	—
Workers	121 (11)	—
Males	—	—
Polygyne Form		
PR Site		
Total††		
Nests	35	15
Reproductive queens	106 (19)	16 (13)
Daughter queens	167 (17)	14(11)
Workers	609 (35)	15 (15)
Males	91 (15)	—
Sperm	48 (15)	—
Whole Colony^^		
Nests	32	13
Reproductive queens	72 (14)	12 (11)
Daughter queens	93 (13)	10 (9)
Workers	351 (32)	13 (13)
Males	71 (10)	—
Sperm	25 (9)	—
Colony Fragment***		
Fragments	40 (20)	4 (3)
Reproductive queens	34 (14)	4 (3)
Daughter queens	74 [9] (6)	4 (3)
Workers	258 [24]	2 (2)

	(12)	
Males	20 [7] (6)	—
Sperm	23 (11)	—
BS Site		
Total††		
Nests	7	7
Reproductive queens	20 (6)	6 (6)
Daughter queens	36 (6)	7 (6)
Workers	84 (5)	6 (6)
Males	33 (5)	—
Sperm	8 (2)	—
Whole Colony^^		
Nests	7	5
Reproductive queens	18 (4)	4 (4)
Daughter queens	35 (5)	6 (5)
Workers	68 (6)	5 (5)
Males	32 (4)	—
Sperm	8 (2)	—
Colony Fragment***		
Fragments	4 (3)	2 (2)
Reproductive queens	2 (2)	2
Daughter queens	14	1
Workers	36 [3] (2)	1
Males	1	—
Sperm	—	—

* Including spermathecal contents of reproductive queens ("Sperm").

† Primary microsatellite loci.

^ Primary loci plus 26 additional microsatellite loci.

** All field-collected material; sites pooled.

†† If fragment was made from whole field-collected colony, then these are combined in this table.

^^ Field-collected nests with multiple reproductive queens.

*** Colony fragments headed by single isolated queens for progeny studies.

APPENDIX B

DIVERSITY STATISTICS AND ALLELE FREQUENCIES FOR THE NINE PRIMARY MICROSATELLITE LOCI STUDIED IN *S. GEMINATA*.

Locus	Number of Alleles Observed	Expected Heterozygosity (H_{exp})	Allele	Allele Frequencies		
					95% Confidence Limits	
				Mean	Lower	Upper
Bertha	11	0.825	196	0.007	0.007	0.007
			204	0.018	0.007	0.034
			206	0.007	0.007	0.007
			208	0.070	0.048	0.089
			210	0.215	0.181	0.247
			212	0.266	0.233	0.301
			214	0.061	0.041	0.082
			216	0.187	0.158	0.215
			218	0.095	0.075	0.116
			220	0.070	0.055	0.089
			230	0.012	0.007	0.014
Sdag_C294	14	0.779	100	0.015	0.013	0.016
			102	0.015	0.013	0.016
			104	0.074	0.047	0.097
			106	0.068	0.029	0.108
			108	0.389	0.329	0.453
			110	0.221	0.156	0.284
			112	0.053	0.027	0.086
			116	0.031	0.014	0.047
			118	0.063	0.029	0.100
			120	0.039	0.015	0.061
			122	0.018	0.013	0.030
			124	0.017	0.013	0.030
			126	0.019	0.013	0.033
			132	0.015	0.013	0.016
Sdag_C536	12	0.749	95	0.024	0.014	0.029
			97	0.007	0.007	0.007
			99	0.025	0.007	0.042
			103	0.086	0.063	0.106
			105	0.142	0.113	0.171
			107	0.448	0.407	0.493
			109	0.110	0.090	0.130
			111	0.088	0.068	0.107
			113	0.026	0.007	0.042
			115	0.023	0.014	0.029
			117	0.013	0.007	0.021
			119	0.010	0.007	0.014
Sol-42f	31	0.937	144	0.007	0.007	0.007
			146	0.009	0.007	0.014
			148	0.007	0.007	0.007
			150	0.007	0.007	0.007
			154	0.012	0.007	0.014
			156	0.007	0.007	0.007
			158	0.026	0.014	0.036
			160	0.046	0.035	0.057
			162	0.030	0.014	0.043
			164	0.033	0.021	0.045
			166	0.076	0.056	0.100
			168	0.079	0.057	0.100
			170	0.105	0.085	0.127
			172	0.097	0.069	0.125
			174	0.057	0.035	0.083
			176	0.050	0.035	0.066

			178	0.089	0.064	0.113
			180	0.040	0.028	0.050
			182	0.082	0.057	0.106
			184	0.038	0.014	0.058
			186	0.015	0.007	0.028
			188	0.016	0.007	0.028
			192	0.009	0.007	0.014
			194	0.014	0.014	0.015
			196	0.007	0.007	0.007
			202	0.007	0.007	0.007
			204	0.011	0.007	0.021
			206	0.007	0.007	0.007
			208	0.007	0.007	0.007
			212	0.007	0.007	0.007
			214	0.021	0.014	0.022
Sol-49	7	0.709	148	0.023	0.014	0.035
			152	0.079	0.062	0.097
			154	0.444	0.408	0.479
			156	0.247	0.212	0.281
			158	0.158	0.125	0.188
			160	0.028	0.027	0.028
			162	0.021	0.014	0.028
Sol_i114	3	0.120	301	0.936	0.917	0.952
			303	0.064	0.048	0.083
			305	0.007	0.007	0.007
Sol_i120	15	0.892	310	0.018	0.014	0.021
			316	0.009	0.007	0.014
			318	0.035	0.021	0.049
			320	0.033	0.021	0.049
			322	0.050	0.029	0.069
			324	0.071	0.050	0.089
			326	0.142	0.114	0.169
			328	0.209	0.174	0.243
			330	0.051	0.041	0.063
			332	0.085	0.063	0.110
			334	0.113	0.086	0.139
			336	0.049	0.035	0.063
			338	0.047	0.034	0.063
			340	0.084	0.068	0.103
			342	0.009	0.007	0.014
Sol_i126	5	0.247	187	0.858	0.829	0.889
			190	0.127	0.097	0.158
			193	0.007	0.007	0.007
			196	0.010	0.007	0.014
			212	0.007	0.007	0.007
Sol_i129	3	0.514	150	0.488	0.451	0.528
			154	0.014	0.007	0.021
			158	0.498	0.458	0.535

APPENDIX C

DIVERSITY STATISTICS AND ALLELE FREQUENCIES FOR 26 ADDITIONAL MICROSATELLITE LOCI STUDIED IN *S. GEMINATA*.

Locus	Number of Alleles Observed	Expected Heterozygosity (H_{exp})	Allele	Mean Frequency
Jackstraw	13	0.847	103	0.050
			105	0.050
			109	0.017
			111	0.283
			113	0.050
			115	0.017
			119	0.033
			123	0.017
			125	0.183
			127	0.017
			129	0.067
			131	0.133
			133	0.083
Jerry_Garcia	4	0.569	208	0.074
			216	0.019
			228	0.537
			230	0.370
Sdag_C1	12	0.874	269	0.038
			271	0.173
			273	0.038
			279	0.077
			281	0.038
			283	0.154
			285	0.154
			287	0.115
			289	0.154
			297	0.019
			299	0.019
			301	0.019
Sdag_C121	13	0.877	161	0.052
			167	0.034
			169	0.034
			171	0.069
			173	0.190
			175	0.190
			177	0.034
			179	0.138
			181	0.121
			183	0.034
			185	0.069
			189	0.017
			197	0.017
Sdag_C185	2	0.296	155	0.819
			157	0.181
Sdag_C204	8	0.842	171	0.161
			173	0.107
			175	0.107
			177	0.196
			179	0.232
			181	0.107
			183	0.071
			187	0.018
Sdag_C216	9	0.832	213	0.034
			215	0.172

			217	0.034
			219	0.259
			221	0.207
			223	0.103
			225	0.086
			227	0.086
			229	0.017
Sdag_C234	4	0.596	121	0.554
			124	0.161
			127	0.268
			130	0.018
Sdag_C264	9	0.837	125	0.196
			129	0.217
			131	0.217
			133	0.043
			135	0.109
			137	0.087
			139	0.087
			141	0.022
			143	0.022
Sdag_C278	5	0.674	108	0.190
			112	0.017
			114	0.397
			116	0.362
			118	0.034
Sdag_C316	8	0.825	232	0.037
			234	0.074
			236	0.148
			238	0.074
			240	0.185
			242	0.278
			244	0.167
			246	0.037
Sdag_C334	2	0.158	187	0.914
			189	0.086
Sdag_C368	14	0.882	191	0.018
			193	0.018
			195	0.125
			199	0.018
			201	0.107
			203	0.143
			205	0.054
			207	0.214
			209	0.089
			211	0.036
			213	0.089
			215	0.018
			217	0.018
			223	0.054
Sdag_C485	11	0.838	122	0.017
			126	0.233
			128	0.200
			130	0.183
			132	0.150
			134	0.067
			136	0.067
			138	0.017
			142	0.017

			144	0.033
			146	0.017
Sol_i113	14	0.903	165	0.019
			169	0.093
			171	0.074
			173	0.148
			175	0.093
			177	0.093
			179	0.074
			183	0.074
			185	0.093
			187	0.148
			189	0.019
			191	0.019
			193	0.037
			195	0.019
Sol_i125	3	0.263	176	0.850
			179	0.117
			182	0.033
Sol_i127	3	0.271	220	0.846
			226	0.058
			228	0.096
Sol_i136	4	0.688	65	0.348
			67	0.348
			69	0.261
			71	0.043
Sol-6	10	0.817	121	0.154
			129	0.058
			131	0.077
			133	0.096
			135	0.346
			137	0.115
			139	0.077
			141	0.038
			143	0.019
			145	0.019
Sol-20	8	0.768	108	0.019
			110	0.037
			112	0.370
			114	0.259
			116	0.093
			118	0.056
			120	0.093
			122	0.074
Sol-55	4	0.283	173	0.018
			175	0.839
			177	0.107
			179	0.036
Sol-J1	6	0.643	248	0.019
			252	0.019
			258	0.519
			260	0.241
			262	0.167
			264	0.037
Sol-M2	14	0.892	285	0.020
			286	0.100
			288	0.040
			292	0.080

			296	0.020
			298	0.100
			300	0.020
			302	0.180
			304	0.080
			306	0.160
			308	0.100
			310	0.060
			312	0.020
			318	0.020
Sol-M3	7	0.813	212	0.111
			214	0.111
			216	0.185
			218	0.296
			220	0.111
			222	0.167
			224	0.019
Sol-M5	17	0.907	354	0.017
			358	0.103
			360	0.086
			362	0.121
			364	0.086
			366	0.017
			368	0.034
			370	0.190
			372	0.052
			374	0.017
			376	0.052
			378	0.052
			380	0.034
			382	0.034
			386	0.034
			388	0.052
			394	0.017
Wharf_Rat	5	0.693	309	0.339
			311	0.357
			315	0.036
			319	0.250
			321	0.018

APPENDIX D

METADATA AND GENOTYPES FOR ADDITIONAL EXEMPLARY SAMPLES OF MONOGYNE NESTS.

			Primary Microsatellite Loci																	
Colony Code	**Sample Code**	**Sex/Caste**	***Bertha***		***Sdag_C294***		***Sdag_C536***		***Sol-42f***		***Sol-49***		***Sol_i114***		***Sol_i120***		***Sol_i126***		***Sol_i129***	
GKO15-1	GKO15-1-1	Reproductive queen	210	212	112	118	107	107	160	182	154	158	301	301	320	328	187	190	150	150
GKO15-1	GKO15-1-2	Daughter queen	210	210	112	118	107	107	160	170	154	158	301	301	310	320	187	187	150	150
GKO15-1	GKO15-1-3	Daughter queen	210	212	112	112	107	107	160	170	154	154	301	301	310	328	187	190	150	150
GKO15-1	GKO15-1-4	Daughter queen	210	212	112	112	107	107	160	170	154	154	301	301	310	328	187	187	150	150
GKO15-1	GKO15-1-M1	Male	210	210	118	118	107	107	160	160	154	154	301	301	328	328	187	187	150	150
GKO15-1	GKO15-1-M2	Male	212	212	—	—	107	107	—	—	—	—	301	301	328	328	190	190	150	150
GKO15-1	GKO15-1-M3	Male	212	212	118	118	107	107	160	160	158	158	301	301	320	320	187	187	150	150
GKO15-1	GKO15-1-M4	Male	210	210	112	112	107	107	160	160	154	154	301	301	320	320	187	187	150	150
GKO15-1	GKO15-01-a	Worker	210	210	—	—	107	107	160	170	154	158	301	301	310	320	187	190	150	150
GKO15-1	GKO15-01-b	Worker	210	210	—	—	107	107	170	182	154	158	301	301	310	320	187	190	150	150
GKO15-1	GKO15-01-c	Worker	210	212	—	—	107	107	160	170	154	154	301	301	310	320	187	190	150	150
GKO15-1	GKO15-01-d	Worker	210	210	—	—	107	107	160	170	154	154	301	301	310	328	187	187	150	150
GKO15-1	GKO15-01-e	Worker	210	210	—	—	107	107	160	170	154	158	301	301	310	328	187	187	150	150
GKO15-1	GKO15-01-f	Worker	210	212	—	—	107	107	170	182	154	158	301	301	310	328	187	190	150	150
GKO15-1	GKO15-01-g	Worker	210	212	—	—	107	107	170	182	154	158	301	301	310	320	187	187	150	150
GKO15-1	GKO15-01-h	Worker	210	212	—	—	107	107	170	182	154	154	—	—	310	328	187	190	150	150
GKO15-1	GKO15-01-i	Worker	210	210	—	—	107	107	160	170	154	154	301	301	310	328	187	190	150	150
GKO15-1	GKO15-01-j	Worker	210	212	—	—	107	107	170	182	154	154	301	301	310	328	187	190	150	150
GKO15-1	GKO15-01-k	Worker	210	212	—	—	107	107	160	170	154	154	301	301	310	320	187	190	150	150
GKO15-1	GKO15-01-l	Worker	210	212	—	—	107	107	160	170	154	158	—	—	310	320	187	187	150	150
GKO15-12	GKO15-12-1	Reproductive queen	208	210	106	118	99	107	178	192	152	156	301	303	328	342	187	190	158	158
GKO15-12	GKO15-12-2	Daughter queen	208	214	106	118	103	107	172	192	152	154	301	303	328	328	187	190	150	158
GKO15-12	GKO15-12-3	Daughter queen	208	214	106	106	99	103	172	192	152	154	301	303	328	342	187	190	150	158
GKO15-12	GKO15-12-4	Daughter queen	210	214	106	118	99	103	—	—	—	—	—	—	328	328	—	—	150	158
GKO15-12	GKO15-12-M1	Male	208	208	118	118	107	107	178	178	152	152	—	—	328	328	—	—	158	158
GKO15-12	GKO15-12-M2	Male	208	208	106	106	107	107	192	192	152	152	—	—	342	342	—	—	158	158
GKO15-12	GKO15-12-M3	Male	210	210	106	106	99	99	192	192	152	152	—	—	342	342	—	—	158	158
GKO15-12	GKO15-12-M4	Male	210	210	106	106	107	107	192	192	152	152	—	—	328	328	—	—	158	158
GKO15-12	GKO15-12-a	Worker	208	214	—	—	99	103	—	—	152	154	301	303	—	—	187	187	150	158
GKO15-12	GKO15-12-b	Worker	210	214	—	—	103	107	—	—	152	154	301	301	328	328	187	187	150	158
GKO15-12	GKO15-12-c	Worker	208	214	—	—	103	107	172	178	154	156	301	303	328	328	187	190	150	158
GKO15-12	GKO15-12-d	Worker	208	214	—	—	99	103	172	178	154	156	301	301	328	328	187	190	150	158
GKO15-12	GKO15-12-e	Worker	210	214	—	—	99	103	172	192	152	154	301	301	328	342	187	187	150	158

GKO15-12	GKO15-12-h	Worker	208	214	—	—	103	107	172	192	154	156	301	301	328	342	187	190	150	158
GKO15-12	GKO15-12-i	Worker	208	214	—	—	103	107	172	178	154	156	301	303	328	328	187	190	150	158
GKO15-12	GKO15-12-j	Worker	210	214	—	—	99	103	172	192	152	154	301	301	328	328	187	190	150	158
GKO15-12	GKO15-12-k	Worker	210	214	—	—	103	107	172	178	154	156	301	303	328	342	187	190	150	158
GKO15-12	GKO15-12-l	Worker	208	214	—	—	103	107	172	178	154	156	301	303	328	342	187	187	150	158
GKO15-19	GKO15-19-1	Reproductive queen	210	230	114	114	107	119	176	178	158	158	301	301	326	326	187	196	158	158
GKO15-19	GKO15-19-2	Daughter queen	210	210	106	114	109	119	176	178	154	158	301	301	326	338	187	196	158	158
GKO15-19	GKO15-19-3	Daughter queen	210	210	106	114	107	109	176	178	154	158	301	301	326	338	187	187	158	158
GKO15-19	GKO15-19-4	Daughter queen	210	230	106	114	107	109	176	178	154	158	301	301	326	338	187	187	158	158
GKO15-19	GKO15-19-M1	Male	230	230	114	114	107	107	178	178	158	158	—	—	326	326	—	—	158	158
GKO15-19	GKO15-19-M2	Male	210	210	114	114	119	119	178	178	158	158	—	—	326	326	—	—	158	158
GKO15-19	GKO15-19-M3	Male	210	210	114	114	107	107	176	176	158	158	—	—	326	326	—	—	158	158
GKO15-19	GKO15-19-M4	Male	230	230	114	114	119	119	176	176	158	158	—	—	326	326	—	—	158	158
GKO15-19	GKO15-19-m5	Male	230	230	114	114	119	119	176	176	158	158	301	301	326	326	187	187	158	158
GKO15-19	GKO15-19-m6	Male	230	230	114	114	119	119	178	178	158	158	301	301	326	326	196	196	158	158
GKO15-19	GKO15-19-m7	Male	230	230	114	114	107	107	176	176	158	158	301	301	326	326	196	196	158	158
GKO15-19	GKO15-19-m8	Male	210	210	—	—	107	107	178	178	158	158	301	301	—	—	187	187	158	158
GKO15-19	GKO15-19-a	Worker	210	230	—	—	107	109	178	178	154	158	301	301	326	338	187	187	158	158
GKO15-19	GKO15-19-b	Worker	210	230	—	—	109	119	178	178	154	158	301	301	326	338	187	196	158	158
GKO15-19	GKO15-19-c	Worker	210	230	—	—	107	109	176	178	154	158	301	301	326	338	187	187	158	158
GKO15-19	GKO15-19-d	Worker	210	210	—	—	107	109	178	178	154	158	301	301	326	338	187	196	158	158
GKO15-19	GKO15-19-e	Worker	210	230	—	—	109	119	178	178	154	158	301	301	326	338	187	196	158	158
GKO15-19	GKO15-19-f	Worker	210	230	—	—	107	109	178	178	154	158	301	301	326	338	187	196	158	158
GKO15-19	GKO15-19-g	Worker	210	230	—	—	109	119	176	178	154	158	301	301	326	338	187	187	158	158
GKO15-19	GKO15-19-h	Worker	210	210	—	—	109	119	176	178	154	158	301	301	326	338	187	196	158	158
GKO15-19	GKO15-19-i	Worker	210	230	—	—	109	119	178	178	154	158	301	301	326	338	187	187	158	158
GKO15-19	GKO15-19-j	Worker	210	210	—	—	107	109	176	178	154	158	301	301	326	338	187	187	158	158
GKO15-19	GKO15-19-k	Worker	210	230	—	—	107	109	178	178	154	158	301	301	326	338	187	196	158	158
GKO15-19	GKO15-19-l	Worker	210	230	—	—	107	109	176	178	154	158	301	301	326	338	187	187	158	158
GKO15-2	GKO15-2-1	Reproductive queen	212	214	106	108	107	107	168	182	154	156	301	301	318	322	187	187	150	158
GKO15-2	GKO15-2-2	Daughter queen	212	212	106	108	107	115	164	168	154	154	301	301	322	328	187	187	150	158
GKO15-2	GKO15-2-3	Daughter queen	212	214	108	108	107	115	164	182	154	156	301	301	318	328	187	187	150	150
GKO15-2	GKO15-2-4	Daughter queen	212	214	108	108	107	115	164	168	154	154	301	301	322	328	187	187	150	150
GKO15-2	GKO15-2-M1	Male	212	212	108	108	107	107	182	182	156	156	—	—	322	322	—	—	158	158
GKO15-2	GKO15-2-M2	Male	214	214	108	108	107	107	168	168	156	156	—	—	318	318	—	—	158	158
GKO15-2	GKO15-2-M3	Male	214	214	106	106	107	107	168	168	156	156	—	—	322	322	—	—	150	150
GKO15-2	GKO15-2-M4	Male	214	214	108	108	107	107	182	182	154	154	—	—	322	322	—	—	158	158
GKO15-2	GKO15-2-m5	Male	212	212	106	106	107	107	168	168	154	154	301	301	322	322	187	187	158	158

GKO15-2	GKO15-2-m6	Male	212	212	108	108	107	107	182	182	156	156	301	301	318	318	187	187	158	158
GKO15-2	GKO15-2-m7	Male	214	214	108	108	107	107	168	168	156	156	301	301	322	322	187	187	150	150
GKO15-2	GKO15-2-m8	Male	212	212	106	106	107	107	182	182	154	154	301	301	322	322	187	187	158	158
GKO15-2	GKO15-02-a	Worker	212	214	—	—	107	115	164	168	154	154	301	301	322	328	187	187	150	158
GKO15-2	GKO15-02-b	Worker	212	212	—	—	107	115	164	182	154	156	301	301	322	328	187	187	150	158
GKO15-2	GKO15-02-c	Worker	212	214	—	—	107	115	164	182	154	154	301	301	318	328	187	187	150	158
GKO15-2	GKO15-02-d	Worker	212	212	—	—	—	—	—	—	154	156	301	301	318	328	187	187	150	158
GKO15-2	GKO15-02-e	Worker	212	212	—	—	107	115	164	182	154	156	301	301	318	328	187	187	150	150
GKO15-2	GKO15-02-f	Worker	212	214	—	—	107	115	164	168	154	154	301	301	318	328	187	187	150	158
GKO15-2	GKO15-02-g	Worker	212	212	—	—	107	115	164	168	154	156	301	301	322	328	187	187	150	158
GKO15-2	GKO15-02-h	Worker	212	214	—	—	107	115	164	168	154	154	301	301	318	328	187	187	150	158
GKO15-2	GKO15-02-i	Worker	212	214	—	—	107	115	164	182	154	156	301	301	318	328	187	187	150	158
GKO15-2	GKO15-02-j	Worker	212	212	—	—	107	115	164	168	154	154	301	301	322	328	187	187	150	158
GKO15-2	GKO15-02-k	Worker	212	212	—	—	107	115	164	182	154	156	301	301	318	328	187	187	150	158
GKO15-2	GKO15-02-l	Worker	212	214	—	—	107	115	164	182	154	156	301	301	322	328	187	187	150	150

APPENDIX E

METADATA AND GENOTYPES FOR ADDITIONAL EXEMPLARY SAMPLES OF POLYGYNE NESTS FROM BOTH SITES.

				Primary Microsatellite Loci																		Additional Locus	
Nest Code	**Site**	**Sample Code**	**Sex / Caste**	***Bertha***		***Sdag_C294***		***Sdag_C536***		***Sol-42f***		***Sol-49***		***Sol_i114***		***Sol_i120***		***Sol_i126***		***Sol_i129***		***Sdag_C185***	
GBS15-15	BS	GBS15-15-1	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	155	155
GBS15-15	BS	GBS15-15-2	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-sp2	Sperm	212	212	112	112	111	111	172	172	154	154	301	301	326	326	187	187	154	154	—	—
GBS15-15	BS	GBS15-15-3	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-sp3	Sperm	216	216	108	108	109	109	166	166	156	156	301	301	324	324	187	187	154	154	—	—
GBS15-15	BS	GBS15-15-4	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	155	155
GBS15-15	BS	GBS15-15-5	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-6	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-M1	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-M2	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-M3	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-M4	Male	210	210	—	—	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-m5	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-m6	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-m7	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-m8	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-15	BS	GBS15-15-a	Worker	210	216	—	—	105	109	162	168	154	154	301	301	318	322	190	193	154	154	155	155
GBS15-15	BS	GBS15-15-b	Worker	210	216	—	—	105	109	162	168	154	154	301	301	322	324	190	193	154	154	—	—
GBS15-15	BS	GBS15-15-c	Worker	210	216	—	—	—	—	—	—	154	154	—	—	—	—	187	193	—	—	—	—
GBS15-15	BS	GBS15-15-d	Worker	210	216	—	—	—	—	—	—	154	154	—	—	322	322	187	193	—	—	—	—
GBS15-15	BS	GBS15-15-e	Worker	210	216	—	—	109	109	162	164	154	154	301	301	322	324	190	193	154	154	—	—
GBS15-15	BS	GBS15-15-f	Worker	210	216	—	—	105	109	162	168	154	154	301	301	318	322	187	193	154	154	—	—
GBS15-15	BS	GBS15-15-g	Worker	210	212	—	—	107	109	162	172	154	154	301	301	322	326	187	193	154	154	—	—
GBS15-15	BS	GBS15-15-h	Worker	210	216	—	—	109	111	162	174	154	154	301	305	314	322	187	193	154	154	—	—
GBS15-15	BS	GBS15-15-i	Worker	210	216	—	—	109	111	162	168	154	154	301	305	318	322	187	193	154	154	—	—
GBS15-15	BS	GBS15-15-j	Worker	210	216	—	—	105	109	162	168	154	154	301	301	322	324	190	193	154	154	—	—
GBS15-15	BS	GBS15-15-k	Worker	210	216	—	—	105	109	162	168	154	154	301	301	318	322	190	193	154	154	—	—
GBS15-15	BS	GBS15-15-l	Worker	—	—	—	—	—	—	—	—	154	156	301	301	—	—	187	193	154	154	—	—
GBS15-19	BS	GBS15-19-1	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	155	155
GBS15-19	BS	GBS15-19-2	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-sp2	Sperm	216	216	108	108	109	109	164	164	154	154	301	301	324	324	187	187	154	154	—	—
GBS15-19	BS	GBS15-19-3	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-sp3	Sperm	216	216	110	110	111	111	174	174	154	154	305	305	314	314	187	187	154	154	—	—
GBS15-19	BS	GBS15-19-4	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-sp4	Sperm	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-5	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-6	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—

GBS15-19	BS	GBS15-19-8	Reproductive queen	210	210	108	108	109	109	—	—	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-9	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-10	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-11	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-sp11	Sperm	218	218	108	108	107	107	166	166	154	154	301	301	312	312	187	187	150	150	—	—
GBS15-19	BS	GBS15-19-12	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-sp14	Sperm	212	212	112	112	107	107	172	172	154	154	301	301	326	326	187	187	154	154	—	—
GBS15-19	BS	GBS15-19-15	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-16	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-17	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-18	Daughter queen	210	210	108	108	109	109	162	162	154	154	—	—	322	322	—	—	154	154	—	—
GBS15-19	BS	GBS15-19-19	Daughter queen	210	210	108	108	109	109	162	162	154	154	—	—	322	322	—	—	154	154	—	—
GBS15-19	BS	GBS15-19-20	Daughter queen	210	212	108	112	109	111	162	172	154	154	301	301	322	326	187	193	154	154	—	—
GBS15-19	BS	GBS15-19-M1	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-M2	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	196	154	154	—	—
GBS15-19	BS	GBS15-19-M3	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	196	—	—	—	—
GBS15-19	BS	GBS15-19-m5	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	196	154	154	—	—
GBS15-19	BS	GBS15-19-m6	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	196	154	154	—	—
GBS15-19	BS	GBS15-19-m7	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-19	BS	GBS15-19-m8	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	196	154	154	—	—
GBS15-19	BS	GBS15-19-a	Worker	210	216	—	—	109	111	162	174	154	154	301	301	322	326	187	193	154	154	155	155
GBS15-19	BS	GBS15-19-b	Worker	210	212	—	—	99	109	162	176	154	154	301	301	318	322	187	193	154	154	—	—
GBS15-19	BS	GBS15-19-c	Worker	210	216	—	—	105	109	162	168	154	154	301	301	318	322	187	193	154	154	—	—
GBS15-19	BS	GBS15-19-d	Worker	210	216	—	—	109	111	162	168	154	154	301	305	318	322	187	193	154	154	—	—
GBS15-19	BS	GBS15-19-e	Worker	210	216	—	—	109	109	162	166	154	156	301	301	322	324	187	193	154	154	—	—
GBS15-19	BS	GBS15-19-f	Worker	210	216	—	—	105	109	162	168	154	154	301	301	318	322	187	193	154	154	—	—
GBS15-19	BS	GBS15-19-g	Worker	210	216	—	—	105	109	162	168	154	154	301	301	318	322	190	193	154	154	—	—
GBS15-19	BS	GBS15-19-h	Worker	210	216	—	—	109	111	162	174	154	154	301	305	314	322	187	193	154	154	—	—
GBS15-19	BS	GBS15-19-i	Worker	210	216	—	—	109	109	162	168	154	154	301	301	318	322	187	193	154	154	—	—
GBS15-19	BS	GBS15-19-j	Worker	210	216	—	—	109	111	162	168	154	154	301	301	322	326	187	193	154	154	—	—
GBS15-19	BS	GBS15-19-k	Worker	210	216	—	—	105	109	162	166	154	156	301	301	318	322	190	193	154	154	—	—
GBS15-19	BS	GBS15-19-l	Worker	210	216	—	—	109	111	162	168	154	154	301	301	322	326	187	193	154	154	—	—
GBS15-5	BS	GBS15-5-1	Reproductive queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	155	155
GBS15-5	BS	GBS15-5-2	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	196	154	154	—	—
GBS15-5	BS	GBS15-5-3	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	155	155
GBS15-5	BS	GBS15-5-4	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-5	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-6	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-7	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-8	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-9	Daughter queen	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-M1	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—

GBS15-5	BS	GBS15-5-M2	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-M3	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-m6	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-m7	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-m8	Male	210	210	108	108	109	109	162	162	154	154	301	301	322	322	193	193	154	154	—	—
GBS15-5	BS	GBS15-5-a	Worker	210	216	—	—	109	111	162	174	154	154	301	301	322	326	190	193	154	154	155	155
GBS15-5	BS	GBS15-5-b	Worker	210	212	—	—	99	109	—	—	154	154	301	301	322	332	187	193	154	154	—	—
GBS15-5	BS	GBS15-5-c	Worker	210	216	—	—	105	109	162	168	154	154	301	301	318	322	190	193	150	154	—	—
GBS15-5	BS	GBS15-5-d	Worker	210	212	—	—	109	109	—	—	154	154	301	301	322	332	187	193	154	154	—	—
GBS15-5	BS	GBS15-5-e	Worker	210	212	—	—	109	109	162	166	154	154	301	301	322	324	184	193	154	158	—	—
GBS15-5	BS	GBS15-5-f	Worker	210	216	—	—	109	111	162	168	154	154	301	301	322	326	187	193	154	154	—	—
GBS15-5	BS	GBS15-5-g	Worker	210	216	—	—	109	111	162	172	154	154	301	301	322	326	187	193	154	154	—	—
GBS15-5	BS	GBS15-5-h	Worker	210	216	—	—	107	109	162	172	154	154	301	301	322	326	187	193	154	154	—	—
GBS15-5	BS	GBS15-5-i	Worker	210	216	—	—	—	—	—	—	154	154	301	301	322	322	187	193	154	158	—	—
GBS15-5	BS	GBS15-5-j	Worker	—	—	—	—	109	111	—	—	154	154	—	—	322	324	—	—	154	154	—	—
GBS15-5	BS	GBS15-5-k	Worker	—	—	—	—	—	—	—	—	154	154	—	—	—	—	—	—	154	154	—	—
GBS15-5	BS	GBS15-5-l	Worker	210	216	—	—	105	109	162	168	154	154	301	301	318	322	190	193	150	154	—	—
GBS15-13	BS	GBS15-13-1	Reproductive queen	210	210	108	108	105	105	162	162	154	154	301	301	320	320	193	193	154	154	155	155
GBS15-13	BS	GBS15-13-2	Reproductive queen	210	210	108	108	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-3	Daughter queen	210	216	108	110	105	111	162	164	154	154	301	305	314	320	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-4	Daughter queen	210	210	108	108	105	109	164	164	154	154	301	301	320	320	193	196	154	154	—	—
GBS15-13	BS	GBS15-13-5	Daughter queen	210	216	108	110	105	111	162	164	154	154	301	305	314	320	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-6	Daughter queen	210	212	108	112	105	111	162	168	154	154	301	301	320	326	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-7	Daughter queen	210	210	108	108	105	105	162	162	154	154	301	301	320	320	193	193	154	154	155	155
GBS15-13	BS	GBS15-13-8	Daughter queen	210	216	108	110	105	111	162	164	154	154	301	305	314	320	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-9	Daughter queen	210	210	108	108	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-10	Daughter queen	210	210	108	108	105	109	164	164	154	154	301	301	320	320	193	196	154	154	—	—
GBS15-13	BS	GBS15-13-F-1	Daughter queen	210	210	—	—	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-F-2	Daughter queen	210	210	—	—	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-F-3	Daughter queen	210	210	—	—	105	109	164	164	154	154	301	301	320	320	193	196	154	154	—	—
GBS15-13	BS	GBS15-13-F-4	Daughter queen	210	210	—	—	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-F-5	Daughter queen	210	210	—	—	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-M-1	Male	210	210	—	—	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-M-2	Male	210	210	—	—	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-M-3	Male	210	210	—	—	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-M-4	Male	210	210	—	—	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-M-5	Male	210	210	—	—	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-M1	Male	210	210	108	108	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-M2	Male	210	210	108	108	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-M3	Male	210	210	108	108	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-M4	Male	210	210	108	108	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—
GBS15-13	BS	GBS15-13-m5	Male	210	210	108	108	105	105	162	162	154	154	301	301	320	320	193	193	154	154	—	—

GBS15-13	BS	GBS15-13-m6	Male	210	210	108	108	105	105	162	162	154	154	—	—	320	320	—	—	154	154	—	—
GBS15-13	BS	GBS15-13-a	Worker	210	216	—	—	105	111	162	164	154	154	301	305	314	320	187	193	154	154	155	155
GBS15-13	BS	GBS15-13-b	Worker	210	212	—	—	105	107	162	172	154	154	301	301	320	326	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-c	Worker	210	212	—	—	105	107	162	172	154	154	301	301	320	326	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-d	Worker	210	216	—	—	105	111	162	164	154	154	301	305	314	320	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-e	Worker	210	216	—	—	105	111	162	168	154	154	301	301	320	322	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-f	Worker	210	218	—	—	105	107	—	—	154	154	301	301	312	320	187	193	150	154	—	—
GBS15-13	BS	GBS15-13-g	Worker	210	216	—	—	105	111	162	168	154	154	301	305	318	320	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-h	Worker	210	216	—	—	105	111	162	168	154	154	301	305	320	322	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-i	Worker	210	218	—	—	—	—	—	—	154	156	301	301	320	328	193	193	150	154	—	—
GBS15-13	BS	GBS15-13-j	Worker	210	216	—	—	105	111	162	168	154	154	301	301	320	322	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-k	Worker	210	216	—	—	105	111	162	164	154	154	301	305	314	320	187	193	154	154	—	—
GBS15-13	BS	GBS15-13-l	Worker	210	216	—	—	105	111	162	168	154	154	301	305	318	320	187	193	154	154	—	—
GBS15-6	BS	GBS15-6-F-1	Daughter queen	210	210	—	—	109	109	162	162	154	154	301	301	322	322	193	196	154	154	155	155
GBS15-6	BS	GBS15-6-F-2	Daughter queen	210	210	—	—	109	109	162	162	154	154	301	301	322	322	193	196	154	154	—	—
GBS15-6	BS	GBS15-6-F-3	Daughter queen	210	212	—	—	109	111	162	166	154	154	301	301	322	322	187	193	154	154	—	—
GBS15-6	BS	GBS15-6-F-4	Daughter queen	210	210	—	—	109	109	162	162	154	154	301	301	322	322	196	196	154	154	155	155
GBS15-6	BS	GBS15-6-F-5	Daughter queen	210	212	—	—	109	111	162	166	154	154	301	301	322	322	187	196	154	154	—	—
GBS15-6	BS	GBS15-6-a	Worker	210	212	—	—	107	109	162	164	154	154	301	301	322	322	187	196	154	154	155	155
GBS15-6	BS	GBS15-6-b	Worker	210	212	—	—	107	109	162	164	154	154	301	301	322	322	187	193	154	154	—	—
GBS15-6	BS	GBS15-6-c	Worker	210	212	—	—	107	109	162	164	154	154	301	301	322	322	187	193	154	154	—	—
GBS15-6	BS	GBS15-6-d	Worker	210	212	—	—	109	111	162	166	154	154	301	301	322	322	187	193	154	154	—	—
GBS15-6	BS	GBS15-6-e	Worker	210	212	—	—	109	111	162	166	154	154	301	301	322	322	187	196	154	154	—	—
GBS15-6	BS	GBS15-6-f	Worker	210	212	—	—	109	111	162	166	154	154	301	301	322	322	187	196	154	154	—	—
GBS15-6	BS	GBS15-6-g	Worker	210	212	—	—	109	111	162	166	154	154	301	301	322	322	187	196	154	154	—	—
GBS15-6	BS	GBS15-6-h	Worker	210	212	—	—	109	111	162	166	154	154	301	301	322	322	187	196	154	154	—	—
GBS15-6	BS	GBS15-6-i	Worker	210	212	—	—	107	109	162	164	154	154	301	301	322	322	187	196	154	154	—	—
GBS15-6	BS	GBS15-6-j	Worker	210	212	—	—	109	111	162	166	154	154	301	301	322	322	187	196	154	154	—	—
GBS15-6	BS	GBS15-6-k	Worker	210	212	—	—	109	111	162	166	154	154	301	301	322	322	187	193	154	154	—	—
GBS15-6	BS	GBS15-6-l	Worker	210	212	—	—	109	111	162	166	154	154	301	301	322	322	187	196	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-W-01	Worker	210	212	—	—	109	109	160	162	154	158	301	301	322	324	187	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-W-02	Worker	210	220	—	—	109	111	160	172	154	160	301	301	322	326	187	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-W-03	Worker	210	216	—	—	107	109	160	166	154	154	301	301	322	324	187	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-W-04	Worker	210	216	—	—	107	109	160	166	154	154	301	301	322	324	187	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-W-05	Worker	210	218	—	—	107	109	160	168	154	156	301	301	322	322	187	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-W-06	Worker	210	212	—	—	109	113	160	182	154	154	301	303	322	326	187	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-W-07	Worker	210	216	—	—	99	109	160	170	154	154	301	303	322	326	187	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-W-08	Worker	210	218	—	—	107	109	160	166	154	154	—	—	322	322	187	193	150	154	—	—
GPR15-1	PR	GPR15-1-2	Reproductive queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-1	PR	GPR15-1-sp2	Sperm	218	218	108	108	103	103	168	168	156	156	301	301	332	332	187	187	150	150	—	—
GPR15-1	PR	GPR15-1-3	Reproductive queen	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-1	PR	GPR15-1-4	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157

GPR15-1	PR	GPR15-1-5	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-1	PR	GPR15-1-6	Daughter queen	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-1	PR	GPR15-1-7	Daughter queen	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-1	PR	GPR15-1-8	Daughter queen	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-1	PR	GPR15-1-9	Daughter queen	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-1	PR	GPR15-1-10	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-1	PR	GPR15-1-M1	Male	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR15-1	PR	GPR15-1-M2	Male	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-1	PR	GPR15-1-M3	Male	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-1	PR	GPR15-1-M4	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR15-1	PR	GPR15-1-m5	Male	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR15-1	PR	GPR15-1-m6	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-1	PR	GPR15-1-m7	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR15-1	PR	GPR15-1-m8	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR15-1	PR	GPR15-1-a	Worker	210	216	—	—	107	109	150	178	154	156	301	301	312	340	187	187	150	154	155	157
GPR15-1	PR	GPR15-1-b	Worker	210	210	—	—	99	109	150	168	154	154	301	301	312	334	187	187	150	154	—	—
GPR15-1	PR	GPR15-1-c	Worker	210	212	—	—	—	—	150	176	154	154	301	301	312	328	187	187	—	—	—	—
GPR15-1	PR	GPR15-1-d	Worker	210	216	—	—	97	109	150	150	154	156	301	301	312	332	187	187	154	158	—	—
GPR15-1	PR	GPR15-1-e	Worker	208	210	—	—	107	109	150	172	154	154	301	301	312	328	187	187	150	154	—	—
GPR15-1	PR	GPR15-1-f	Worker	210	218	—	—	99	109	150	166	154	156	301	301	312	338	187	187	150	154	—	—
GPR15-1	PR	GPR15-1-g	Worker	208	210	—	—	107	109	150	172	154	154	301	301	312	328	187	187	150	154	—	—
GPR15-1	PR	GPR15-1-h	Worker	210	216	—	—	99	109	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-1	PR	GPR15-1-i	Worker	210	212	—	—	105	109	150	176	154	156	301	301	312	338	184	187	154	158	—	—
GPR15-1	PR	GPR15-1-j	Worker	210	216	—	—	107	109	150	178	152	154	301	301	312	336	187	187	154	158	—	—
GPR15-1	PR	GPR15-1-k	Worker	210	212	—	—	103	109	150	168	154	154	301	301	312	326	187	187	154	154	—	—
GPR15-1	PR	GPR15-1-l	Worker	210	210	—	—	107	109	150	168	154	158	301	303	312	326	187	190	—	—	—	—
GPR15-2	PR	GPR15-2-1	Reproductive queen	210	210	108	108	109	109	158	158	154	154	301	301	312	312	187	187	154	154	155	155
GPR15-2	PR	GPR15-2-10	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-2	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-3	Daughter queen	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-2	PR	GPR15-2-4	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-5	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-6	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-7	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-8	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-9	Daughter queen	210	210	108	108	105	109	150	150	154	156	301	301	312	326	187	187	154	158	—	—
GPR15-2	PR	GPR15-2-F-1	Daughter queen	210	210	—	—	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-F-2	Daughter queen	210	210	—	—	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-F-3	Daughter queen	210	210	—	—	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-F-4	Daughter queen	210	210	—	—	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-F-5	Daughter queen	210	210	—	—	109	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-M1	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	157	157

GPR15-2	PR	GPR15-2-M2	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR15-2	PR	GPR15-2-M3	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR15-2	PR	GPR15-2-M4	Male	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR15-2	PR	GPR15-2-m5	Male	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR15-2	PR	GPR15-2-m6	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR15-2	PR	GPR15-2-m7	Male	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	155	155
GPR15-2	PR	GPR15-2-m8	Male	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-2	PR	GPR15-2-a	Worker	210	210	—	—	107	109	150	172	154	154	301	301	312	328	187	187	154	158	155	155
GPR15-2	PR	GPR15-2-b	Worker	210	214	—	—	107	109	150	176	152	154	301	301	312	330	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-c	Worker	210	212	—	—	97	109	148	150	154	156	301	301	312	326	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-d	Worker	210	210	—	—	99	109	150	178	154	158	301	301	312	338	187	187	154	158	—	—
GPR15-2	PR	GPR15-2-e	Worker	210	210	—	—	99	111	150	168	154	158	301	301	312	326	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-f	Worker	210	210	—	—	103	109	150	166	154	154	301	301	312	334	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-g	Worker	210	210	—	—	105	109	150	166	152	154	301	301	312	338	187	187	154	158	—	—
GPR15-2	PR	GPR15-2-h	Worker	210	210	—	—	—	—	150	160	154	156	301	301	312	324	187	190	150	154	—	—
GPR15-2	PR	GPR15-2-i	Worker	210	210	—	—	99	109	150	178	154	158	301	301	312	338	187	187	154	158	—	—
GPR15-2	PR	GPR15-2-j	Worker	210	216	—	—	103	109	150	150	154	154	301	301	312	326	187	187	154	158	—	—
GPR15-2	PR	GPR15-2-k	Worker	210	210	—	—	109	115	150	150	154	158	301	301	312	328	187	190	150	154	—	—
GPR15-2	PR	GPR15-2-l	Worker	210	210	—	—	107	109	150	180	154	156	301	301	312	326	187	190	150	154	—	—
GPR15-4	PR	GPR15-4-1	Reproductive queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR15-4	PR	GPR15-4-2	Reproductive queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR15-4	PR	GPR15-4-3	Daughter queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR15-4	PR	GPR15-4-4	Daughter queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR15-4	PR	GPR15-4-5	Daughter queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR15-4	PR	GPR15-4-6	Daughter queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR15-4	PR	GPR15-4-7	Daughter queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR15-4	PR	GPR15-4-8	Daughter queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR15-4	PR	GPR15-4-9	Daughter queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR15-4	PR	GPR15-4-10	Daughter queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR15-4	PR	GPR15-4-M1	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-4	PR	GPR15-4-M2	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-4	PR	GPR15-4-M3	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-4	PR	GPR15-4-a	Worker	210	210	—	—	99	109	150	160	154	156	301	301	312	324	187	190	150	154	—	—
GPR15-4	PR	GPR15-4-b	Worker	210	210	—	—	109	111	150	174	154	156	301	301	312	332	187	187	150	154	—	—
GPR15-4	PR	GPR15-4-c	Worker	210	216	—	—	99	109	150	160	154	156	301	301	312	328	187	187	154	158	—	—
GPR15-4	PR	GPR15-4-d	Worker	208	210	—	—	103	109	150	176	154	156	301	301	312	328	187	187	154	158	—	—
GPR15-4	PR	GPR15-4-e	Worker	210	214	—	—	105	109	158	158	152	154	301	301	312	338	187	190	150	154	—	—
GPR15-4	PR	GPR15-4-f	Worker	210	210	—	—	109	109	150	160	154	154	301	301	312	326	187	187	154	158	—	—
GPR15-4	PR	GPR15-4-g	Worker	210	210	—	—	105	109	150	172	154	156	301	301	312	332	187	190	150	154	—	—
GPR15-4	PR	GPR15-4-h	Worker	210	212	—	—	107	109	150	178	152	154	301	303	312	324	187	187	150	154	—	—
GPR15-4	PR	GPR15-4-i	Worker	210	216	—	—	99	109	150	160	154	156	301	301	312	328	187	187	154	158	—	—
GPR15-4	PR	GPR15-4-j	Worker	210	230	—	—	107	109	150	168	154	158	301	301	312	330	187	187	154	158	—	—

GPR15-4	PR	GPR15-4-k	Worker	210	212	—	—	107	109	150	178	154	156	301	301	312	328	187	187	150	154	—	—
GPR15-4	PR	GPR15-4-l	Worker	210	212	—	—	107	109	150	178	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-5	PR	GPR15-5-1	Reproductive queen	210	210	108	108	109	109	158	158	154	154	301	301	312	312	187	187	154	154	155	155
GPR15-5	PR	GPR15-5-sp1	Sperm	214	214	108	108	103	103	160	160	148	148	301	301	324	324	187	187	150	150	—	—
GPR15-5	PR	GPR15-5-2	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-5	PR	GPR15-5-sp2	Sperm	216	216	108	108	109	109	172	172	154	154	301	301	326	326	187	187	158	158	—	—
GPR15-5	PR	GPR15-5-3	Reproductive queen	210	210	108	108	109	109	158	158	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-5	PR	GPR15-5-4	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-5	PR	GPR15-5-5	Daughter queen	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-5	PR	GPR15-5-6	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-5	PR	GPR15-5-7	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-5	PR	GPR15-5-8	Daughter queen	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-5	PR	GPR15-5-9	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-5	PR	GPR15-5-10	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-5	PR	GPR15-5-M1	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR15-5	PR	GPR15-5-M2	Male	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR15-5	PR	GPR15-5-M3	Male	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	157	157
GPR15-5	PR	GPR15-5-M4	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-5	PR	GPR15-5-m5	Male	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-5	PR	GPR15-5-m6	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR15-5	PR	GPR15-5-m7	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR15-5	PR	GPR15-5-m8	Male	210	210	108	108	111	111	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR15-5	PR	GPR15-5-a	Worker	210	214	—	—	107	109	150	160	154	156	301	301	312	320	187	187	154	158	155	157
GPR15-5	PR	GPR15-5-b	Worker	210	212	—	—	107	111	150	168	154	156	301	301	312	328	187	190	150	154	—	—
GPR15-5	PR	GPR15-5-c	Worker	210	212	—	—	107	109	150	178	154	154	301	301	312	328	187	187	150	154	—	—
GPR15-5	PR	GPR15-5-d	Worker	210	210	—	—	107	109	150	176	152	154	301	301	312	330	187	187	150	154	—	—
GPR15-5	PR	GPR15-5-e	Worker	210	212	—	—	107	109	150	158	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-5	PR	GPR15-5-f	Worker	210	212	—	—	107	109	—	—	154	160	301	301	312	326	187	187	150	154	—	—
GPR15-5	PR	GPR15-5-g	Worker	210	216	—	—	109	117	150	170	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-5	PR	GPR15-5-h	Worker	210	216	—	—	109	117	150	170	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-5	PR	GPR15-5-i	Worker	210	210	—	—	107	109	150	176	152	154	301	301	312	330	187	187	150	154	—	—
GPR15-5	PR	GPR15-5-j	Worker	208	210	—	—	109	109	150	178	152	154	301	303	312	312	187	187	150	154	—	—
GPR15-5	PR	GPR15-5-k	Worker	210	214	—	—	—	—	150	188	148	154	301	301	312	328	187	187	154	158	—	—
GPR15-5	PR	GPR15-5-l	Worker	210	218	—	—	107	109	150	174	154	158	301	301	312	330	187	187	154	158	—	—
GPR15-7	PR	GPR15-7-1	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-7	PR	GPR15-7-10	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-7	PR	GPR15-7-2	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-7	PR	GPR15-7-3	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-7	PR	GPR15-7-4	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-7	PR	GPR15-7-5	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-7	PR	GPR15-7-6	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-7	PR	GPR15-7-8	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	155	157

GPR15-7	PR	GPR15-7-9	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-7	PR	GPR15-7-M1	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-7	PR	GPR15-7-M2	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR15-7	PR	GPR15-7-M3	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-7	PR	GPR15-7-M4	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR15-7	PR	GPR15-7-a	Worker	210	214	—	—	109	109	150	160	154	154	301	301	312	332	187	187	150	154	—	—
GPR15-7	PR	GPR15-7-b	Worker	210	212	—	—	107	109	150	200	154	156	301	301	312	328	187	190	150	154	—	—
GPR15-7	PR	GPR15-7-c	Worker	210	212	—	—	107	111	150	182	152	154	301	301	312	326	187	187	154	158	—	—
GPR15-7	PR	GPR15-7-d	Worker	208	210	—	—	109	117	150	176	154	154	301	301	312	338	187	187	150	154	—	—
GPR15-7	PR	GPR15-7-e	Worker	210	212	—	—	107	109	150	200	154	156	301	301	312	328	187	190	150	154	—	—
GPR15-7	PR	GPR15-7-f	Worker	—	—	—	—	105	109	—	—	154	156	301	301	—	—	187	187	—	—	—	—
GPR15-7	PR	GPR15-7-g	Worker	210	212	—	—	105	109	150	178	154	154	301	301	312	340	187	187	150	154	—	—
GPR15-7	PR	GPR15-7-h	Worker	210	214	—	—	99	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR15-7	PR	GPR15-7-i	Worker	210	216	—	—	105	111	150	168	154	156	301	301	312	328	187	187	150	154	—	—
GPR15-7	PR	GPR15-7-j	Worker	208	210	—	—	107	109	150	176	154	156	301	301	312	338	187	187	150	154	—	—
GPR15-7	PR	GPR15-7-k	Worker	210	216	—	—	105	111	150	168	154	156	301	301	312	328	187	187	150	154	—	—
GPR15-7	PR	GPR15-7-l	Worker	210	214	—	—	109	109	150	160	154	154	301	301	312	332	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q96	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q96-sp	Sperm	210	210	126	126	107	107	172	172	156	156	301	301	326	326	187	187	150	150	—	—
GPR16-1	PR	GPR16-1-Q97	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q98	Reproductive queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	—	—	154	154	—	—
GPR16-1	PR	GPR16-1-Q98-sp	Sperm	218	218	108	108	105	105	176	176	154	154	301	301	324	324	187	187	158	158	—	—
GPR16-1	PR	GPR16-1-Q99	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-1	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR16-1	PR	GPR16-1-2	Daughter queen	210	210	108	108	109	109	150	150	154	154	—	—	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-3	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-4	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-5	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-6	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-7	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-8	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-9	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-10	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-a	Worker	210	212	108	108	107	109	150	172	154	154	301	301	312	324	187	187	154	158	—	—
GPR16-1	PR	GPR16-1-b	Worker	210	216	108	108	105	109	150	178	154	154	301	301	312	334	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-c	Worker	210	212	108	110	107	109	150	164	154	154	301	303	312	324	187	187	154	158	—	—
GPR16-1	PR	GPR16-1-d	Worker	210	218	108	108	105	109	150	176	154	154	301	301	312	324	187	187	154	158	—	—
GPR16-1	PR	GPR16-1-e	Worker	210	210	108	110	109	117	150	168	154	156	301	305	312	332	187	190	150	154	155	155
GPR16-1	PR	GPR16-1-f	Worker	210	212	108	110	109	115	150	174	154	154	301	301	312	324	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-g	Worker	210	212	108	110	109	115	150	164	154	154	301	303	312	324	187	187	154	158	—	—
GPR16-1	PR	GPR16-1-h	Worker	210	218	108	108	103	107	150	174	152	152	301	301	312	324	187	187	154	158	—	—
GPR16-1	PR	GPR16-1-j	Worker	210	212	108	110	107	109	150	164	154	154	301	303	312	324	187	187	154	158	—	—

APPENDIX F

METADATA AND GENOTYPES FOR ADDITIONAL EXEMPLARY POLYGYNE NEST FRAGMENTS FROM BOTH SITES (INCLUDING ALL FRAGMENTS THAT PRODUCED DAUGHTER QUEENS)

					Primary Microsatellite Loci																		Additional Locus	
Nest Code	**Site**	**Fragment Code**	**Sample Code**	**Sex / Caste**	***Bertha***		***Sdag_C294***		***Sdag_C536***		***Sol-42f***		***Sol-49***		***Sol_i114***		***Sol_i120***		***Sol_i126***		***Sol_i129***		***Sdag_C185***	
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-Qn	Reproductive queen	210	210	108	108	109	109	160	160	154	154	301	301	322	322	193	193	154	154	155	155
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-01	Worker	210	212	—	—	109	113	160	166	154	154	—	—	—	—	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-02	Worker	210	212	—	—	109	113	160	166	154	154	—	—	—	—	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-03	Worker	210	212	—	—	109	113	160	166	154	154	—	—	322	322	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-04	Worker	210	212	—	—	109	113	160	166	154	154	—	—	—	—	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-05	Worker	210	212	—	—	109	113	160	166	154	154	—	—	—	—	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-06	Worker	210	212	—	—	109	113	160	166	154	154	—	—	322	322	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-07	Worker	210	212	—	—	109	113	160	166	154	154	—	—	322	322	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-08	Worker	210	212	—	—	109	113	160	166	154	154	—	—	322	322	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-09	Worker	210	212	—	—	109	113	160	166	154	154	301	301	322	322	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-10	Worker	210	212	—	—	109	113	160	166	154	154	301	301	322	322	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-11	Worker	210	212	—	—	109	113	160	166	154	154	301	301	322	322	187	193	154	158	—	—
P-2-3-gem	BS	P-2-3-gem-e	P-2-3-gem-e-W-12	Worker	210	212	—	—	109	113	160	166	154	154	301	301	322	322	187	193	154	158	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-Qn	Reproductive queen	210	210	108	108	109	109	160	160	154	154	301	301	322	322	193	193	154	154	155	155
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-110	Daughter queen	210	210	108	108	109	109	160	160	154	154	301	301	322	322	193	193	154	154	155	155
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-01	Daughter queen	—	—	—	—	109	109	160	160	154	154	—	—	—	—	193	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-02	Daughter queen	210	210	—	—	109	109	160	160	154	154	—	—	—	—	193	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-03	Daughter queen	210	210	—	—	109	109	160	160	154	154	—	—	—	—	193	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-04	Daughter queen	—	—	—	—	109	109	160	160	154	154	—	—	—	—	—	—	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-05	Daughter queen	—	—	—	—	109	109	160	160	154	154	—	—	—	—	193	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-06	Daughter queen	—	—	—	—	109	109	160	160	154	154	—	—	—	—	193	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-07	Daughter queen	—	—	—	—	109	109	160	160	154	154	—	—	—	—	—	—	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-08	Daughter queen	—	—	—	—	109	109	160	160	154	154	—	—	—	—	193	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-09	Daughter queen	210	210	—	—	109	109	160	160	154	154	—	—	—	—	193	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-10	Daughter queen	210	210	—	—	109	109	160	160	154	154	—	—	—	—	193	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-11	Daughter queen	210	210	—	—	109	109	—	—	—	—	—	—	—	—	193	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-12	Daughter queen	210	210	—	—	109	109	160	160	154	154	—	—	—	—	193	193	154	154	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-DQ-13	Daughter queen	210	210	—	—	—	—	160	160	154	154	—	—	—	—	193	193	—	—	—	—
P-2-4-gem	BS	P-2-4-gem-a	P-2-4-gem-a-111	Male	210	210	108	108	109	109	160	160	154	154	301	301	322	322	193	193	154	154	—	—
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-Qn	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-Sp	Sperm	212	212	—	—	107	107	178	178	156	156	301	301	340	340	187	187	150	150	—	—
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-FP1	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-a	Worker	210	212	108	108	107	109	150	178	154	156	—	—	312	340	—	—	150	154	155	157
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-b	Worker	210	212	108	108	107	109	150	178	154	156	301	301	312	340	187	187	150	154	—	—
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-c	Worker	210	212	—	—	107	109	150	178	154	156	301	301	312	340	187	187	150	154	—	—
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-d	Worker	210	212	—	—	107	109	150	178	154	156	301	301	312	340	187	187	150	154	—	—
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-e	Worker	210	212	—	—	107	109	150	178	154	156	301	301	312	340	187	187	150	154	—	—
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-f	Worker	210	212	108	108	107	109	150	178	154	156	301	301	312	340	187	187	150	154	—	—

		Q3																						
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-i	Worker	210	212	—	—	107	109	150	178	154	156	301	301	312	340	187	187	150	154	—	—
GPR15-10	PR	GPR15-10-Q3	GPR15-10-Q3-j	Worker	210	212	—	—	107	109	150	178	154	156	301	301	312	340	187	187	150	154	—	—
GPR15-10	PR	GPR15-10-Q1	GPR15-10-Q1-Qn	Reproductive queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-10	PR	GPR15-10-Q1	GPR15-10-Q1-FP1	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-10	PR	GPR15-10-Q1	GPR15-10-Q1-MP1	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-Qn	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-Sp	Sperm	214	214	108	108	113	113	164	164	154	154	303	303	334	334	187	187	150	150	—	—
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-FP1	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-FP2	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-MP1	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-MP2	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-a	Worker	210	214	108	108	109	113	150	164	154	154	301	303	312	334	187	187	150	154	155	155
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-b	Worker	210	214	—	—	109	113	150	164	154	154	301	303	312	334	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-c	Worker	210	214	—	—	109	113	150	164	154	154	301	303	312	334	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-d	Worker	210	214	108	108	109	113	150	164	154	154	301	303	312	334	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-e	Worker	210	214	108	108	109	113	150	164	154	154	301	303	312	334	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-f	Worker	210	214	108	108	109	113	150	164	154	154	301	303	312	334	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-g	Worker	210	214	108	108	109	113	150	164	154	154	301	303	312	334	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-h	Worker	210	214	108	108	109	113	150	164	154	154	301	303	312	334	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-i	Worker	210	214	108	108	109	113	150	164	154	154	301	303	312	334	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q7	GPR15-12-Q7-j	Worker	210	214	108	108	109	113	150	164	154	154	301	303	312	334	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-FP1	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-a	Worker	210	210	—	—	107	109	150	180	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-b	Worker	210	210	—	—	107	109	150	180	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-c	Worker	210	210	—	—	107	109	150	180	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-d	Worker	210	210	—	—	107	109	150	180	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-e	Worker	210	210	—	—	107	109	150	180	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-f	Worker	210	210	—	—	107	109	150	180	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-g	Worker	210	210	—	—	107	109	150	180	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-h	Worker	210	210	—	—	107	109	150	180	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-i	Worker	210	210	—	—	107	109	150	180	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-12	PR	GPR15-12-Q4	GPR15-12-Q4-j	Worker	210	210	—	—	107	109	150	180	154	154	301	301	312	326	187	187	150	154	—	—
GPR15-13	PR	GPR15-13-	GPR15-13-Q2-FP1	Daughter queen	210	210	108	108	109	111	150	150	154	154	301	301	312	312	187	187	154	154	—	—

		Q2																						
GPR15-13	PR	GPR15-13-Q2	GPR15-13-Q2-a	Worker	210	214	104	108	105	111	150	164	154	156	301	301	312	332	187	187	154	158	—	—
GPR15-13	PR	GPR15-13-Q2	GPR15-13-Q2-b	Worker	210	214	—	—	105	109	150	164	154	156	301	301	312	332	187	187	154	158	—	—
GPR15-13	PR	GPR15-13-Q2	GPR15-13-Q2-c	Worker	210	214	104	108	105	111	150	164	154	156	301	301	312	332	187	187	154	158	—	—
GPR15-13	PR	GPR15-13-Q2	GPR15-13-Q2-d	Worker	210	214	—	—	105	109	150	164	154	156	301	301	312	332	187	187	154	158	—	—
GPR15-13	PR	GPR15-13-Q2	GPR15-13-Q2-e	Worker	210	214	—	—	105	111	150	164	154	156	301	301	312	332	187	187	154	158	—	—
GPR15-13	PR	GPR15-13-Q2	GPR15-13-Q2-f	Worker	210	214	—	—	105	109	—	—	154	156	—	—	312	332	187	187	154	158	—	—
GPR15-13	PR	GPR15-13-Q2	GPR15-13-Q2-g	Worker	210	214	—	—	105	109	150	164	154	156	301	301	312	332	187	187	154	158	—	—
GPR15-13	PR	GPR15-13-Q2	GPR15-13-Q2-h	Worker	210	214	—	—	105	109	150	164	154	156	301	301	312	332	187	187	154	158	—	—
GPR15-13	PR	GPR15-13-Q2	GPR15-13-Q2-i	Worker	210	214	—	—	105	111	150	164	154	156	301	301	312	332	187	187	154	158	—	—
GPR15-13	PR	GPR15-13-Q2	GPR15-13-Q2-j	Worker	210	214	—	—	105	111	150	164	154	156	301	301	312	332	187	187	154	158	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-Qn	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-Sp	Sperm	216	216	108	108	111	111	176	176	154	154	301	301	322	322	187	187	150	150	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-FP1	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-FP2	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-MP1	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-a	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	—	—	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-b	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	—	—	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-c	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	—	—	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-d	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	—	—	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-e	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	—	—	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-f	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	—	—	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-g	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	—	—	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-h	Worker	210	216	—	—	109	111	150	176	154	154	—	—	312	322	—	—	—	—	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-i	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	—	—	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-j	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	—	—	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-m	Worker	210	216	108	108	109	111	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-n	Worker	210	216	108	108	109	111	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-o	Worker	210	216	108	108	109	111	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-p	Worker	210	216	108	108	109	111	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-q	Worker	210	216	108	108	109	111	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-r	Worker	210	216	108	108	109	111	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-s	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-t	Worker	210	216	108	108	109	111	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-u	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR15-2	PR	GPR15-2-Q1	GPR15-2-Q1-v	Worker	210	216	—	—	109	111	150	176	154	154	301	301	312	322	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-Qn	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-sp	Sperm	208	208	106	106	107	107	174	174	154	154	301	301	328	328	187	187	150	150	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp10	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp11	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp12	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp13	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—

GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp14	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp15	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp16	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp17	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp18	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp19	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp1	Daughter queen	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp20	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp21	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp22	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp23	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp24	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp25	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp26	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp27	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp28	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp29	Daughter queen	210	210	—	—	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp2	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp30	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp31	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp32	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp33	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp34	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp35	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp3	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp4	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp5	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp6	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp7	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp8	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-fp9	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-mp10	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-mp1	Male	210	210	108	108	109	109	150	150	—	—	301	301	312	312	187	187	154	154	155	157
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-mp2	Male	210	210	108	108	109	109	150	150	—	—	301	301	312	312	187	187	154	154	155	155
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-mp3	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-mp4	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-mp5	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-mp6	Male	210	210	108	108	109	109	150	150	154	154	—	—	312	312	187	187	154	154	155	157
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-mp7	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-mp8	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-mp9	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	157
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-a	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-b	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-c	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-d	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-e	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—

GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-f	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-g	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-h	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-i	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-j	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-k	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-l	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-x	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-1	PR	GPR16-1-Q1	GPR16-1-Q1-y	Worker	208	210	106	108	107	109	150	174	154	154	301	301	312	328	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-Qn	Reproductive queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-sp	Sperm	216	216	—	—	105	105	170	170	158	158	301	301	324	324	187	187	150	150	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp10	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp11	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp12	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp13	Daughter queen	210	210	—	—	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp14	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp15	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp16	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp17	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp18	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp19	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp1	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp20	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp21	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp22	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp23	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp24	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp25	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp26	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp27	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp28	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp29	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp2	Daughter queen	210	210	108	108	109	109	150	150	—	—	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp30	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp31	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp3	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp4	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp5	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp6	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp7	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp8	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-fp9	Daughter queen	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-mp1	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-mp2	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	157	157
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-mp3	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-mp4	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155

GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-mp5	Male	210	210	108	108	109	109	150	150	154	154	301	301	312	312	187	187	154	154	155	155
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-a	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-b	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-c	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-d	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-e	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-f	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-g	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-h	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-i	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-j	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-k	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-l	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-x	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—
GPR16-2	PR	GPR16-2-Q1	GPR16-2-Q1-y	Worker	210	216	108	118	105	109	150	170	154	158	301	301	312	324	187	187	150	154	—	—

APPENDIX G

UNIQUE MULTILOCUS GENOTYPES OBSERVED IN POLYGYNE QUEENS AT THE NINE PRIMARY LOCI.

The first two letters of each multilocus genotype (MLG) code denote the site at which it was exclusively observed. The number of individual queens for which each MLG was observed is shown, with the number of nests from which the queens were sampled in parentheses. A distinct color shading is given to each allele at a locus. Blue and red tones are reserved for alleles found exclusively in the MLGs of polygyne queens from either the PR or BS site, respectively, while purple is used for alleles found in the MLGs of reproductive queens from both sites. The first MLG listed for each site is the most frequent.

MLG code	**Reproductive Queens**	**Daughter Queens**	**All Queens**	**Primary Microsatellite Loci**																	
				Sol-42f		***Sol_i120***		***Sol_i126***		***Sdag_C536***		***Bertha***		***Sdag_C294***		***Sol-49***		***Sol_i114***		***Sol_i129***	
PR_1	72 (17)	66 (13)	138 (19)	150	150	312	312	187	187	109	109	210	210	108	108	154	154	301	301	154	154
PR_2	11 (6)	18 (9)	29 (11)	150	150	312	312	187	187	109	111	210	210	108	108	154	154	301	301	154	154
PR_3	9 (7)	11(6)	20 (9)	150	150	312	312	187	187	111	111	210	210	108	108	154	154	301	301	154	154
PR_4	11 (5)	5 (2)	16 (7)	158	158	312	312	187	187	109	109	210	210	108	108	154	154	301	301	154	154
BS_1	15 (3)	15 (3)	30 (3)	162	162	322	322	193	193	109	109	210	210	108	108	154	154	301	301	154	154
BS_2	2 (1)	6 (1)	8 (1)	162	162	320	320	193	193	105	105	210	210	108	108	154	154	301	301	154	154
BS_3	0	4 (2)	4 (2)	162	162	322	322	193	196	109	109	210	210	108	108	154	154	301	301	154	154
BS_4	0	1 (1)	1 (1)	162	162	322	322	196	196	109	109	210	210	108	108	154	154	301	301	154	154
BS_5	2 (2)	1 (1)	3 (2)	160	160	322	322	193	193	109	109	210	210	108	108	154	154	301	301	154	154
BS_6	0	3 (1)	3 (1)	164	164	320	320	193	196	105	109	210	210	108	108	154	154	301	301	154	154

APPENDIX H

GENOTYPES FOR POLYGYNE REPRODUCTIVE QUEENS AND THE SPERM IN THEIR SPERMATHECAE

Alleles in sperm that do not occur in the parthenogen gene pool are indicated by colored underlined font, with orange indicating alleles found in the monogyne form and blue indicating alleles not found in the monogyne form. Dashes indicate missing data.

	Collection				Primary Microsatellite Loci																	
Nest Code	**Site**	**Sample Code**	**Sample**	**Sample Origin**	***Bertha***		***Sdag_C294***		***Sdag_C536***		***Sol_i114***		***Sol_i120***		***Sol_i126***		***Sol_i129***		***Sol-42f***		***Sol-49***	
GBS15-15	BS	GBS15_15_2	Queen	Field-collected colony	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**322**	**322**	**193**	**193**	**154**	**154**	**162**	**162**	**154**	**154**
		GBS15_15_sp2	Sperm		**212**		**112**		**111**		**301**		**326**		**187**		**154**		**172**		**154**	
GBS15-15	BS	GBS15_15_3	Queen	Field-collected colony	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**322**	**322**	**193**	**193**	**154**	**154**	**162**	**162**	**154**	**154**
		GBS15_15_sp3	Sperm		**216**		**108**		**109**		**301**		**324**		**187**		**154**		**166**		**156**	
GBS15-19	BS	GBS15_19_2	Queen	Field-collected colony	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**322**	**322**	**193**	**193**	**154**	**154**	**162**	**162**	**154**	**154**
		GBS15_19_sp2	Sperm		**216**		**108**		**109**		**301**		**324**		**187**		**154**		**164**		**154**	
GBS15-19	BS	GBS15_19_3	Queen	Field-collected colony	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**322**	**322**	**193**	**193**	**154**	**154**	**162**	**162**	**154**	**154**
		GBS15_19_sp3	Sperm		**216**		**110**		**111**		**305**		**314**		**187**		**154**		**174**		**154**	
GBS15-19	BS	GBS15_19_4	Queen	Field-collected colony	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**322**	**322**	**193**	**193**	**154**	**154**	**162**	**162**	**154**	**154**
		GBS15_19_sp4	Sperm		**210**		**108**		**109**		**301**		**322**		**193**		**154**		**162**		**154**	
GBS15-19	BS	GBS15_19_7	Queen	Field-collected colony	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**322**	**322**	**193**	**193**	**154**	**154**	**162**	**162**	**154**	**154**
		GBS15_19_sp7	Sperm		**216**		**108**		**105**		**301**		**324**		**190**		**154**		**168**		**154**	
GBS15-19	BS	GBS15_19_11	Queen	Field-collected colony	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**322**	**322**	**193**	**193**	**154**	**154**	**162**	**162**	**154**	**154**
		GBS15_19_sp11	Sperm		**218**		**108**		**107**		**301**		**312**		**187**		**150**		**166**		**154**	
GBS15-19	BS	GBS15_19_sp14	Sperm	Field-collected colony	**212**		**112**		**107**		**301**		**326**		**187**		**154**		**172**		**154**	
GPR15-10	PR	GPR15_10_Q3_Qn	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR15_10_Q3_Sp	Sperm		**212**		**—**		**107**		**301**		**340**		**187**		**150**		**178**		**156**	
GPR15-12	PR	GPR15_12_Q7_Qn	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR15_12_Q7_Sp	Sperm		**214**		**108**		**113**		**303**		**334**		**187**		**150**		**164**		**154**	
GPR15-13	PR	GPR15_13_Q1_Qn	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR15_13_Q1_Sp	Sperm		**214**		**—**		**99**		**301**		**326**		**187**		**158**		**178**		**154**	
GPR15-2	PR	GPR15_2_Q1_Qn	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR15_2_Q1_Sp	Sperm		**216**		**108**		**111**		**301**		**322**		**187**		**150**		**176**		**154**	
GPR16-1	PR	GPR16_1_QIU5	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR16_1_QIU5_sp	Sperm		**214**		**108**		**109**		**301**		**340**		**187**		**150**		**160**		**154**	
GPR16-1	PR	GPR16_1_QIU6	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR16_1_QIU6_sp	Sperm		**216**		**108**		**107**		**301**		**—**		**187**		**158**		**—**		**154**	
GPR16-1	PR	GPR16_1_QIU8	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR16_1_QIU8_sp	Sperm		**212**		**106**		**109**		**301**		**326**		**187**		**150**		**182**		**160**	
GPR16-2	PR	GPR16_2_Q3_Qn	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR16_2_Q3_sp	Sperm		**226**		**108**		**109**		**301**		**324**		**187**		**150**		**178**		**154**	
GPR16-2	PR	GPR16_2_QIU1	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR16_2_QIU1_sp	Sperm		**210**		**108**		**99**		**301**		**326**		**190**		**150**		**168**		**154**	
GPR16-2	PR	GPR16_2_QIU2	Queen	Colony fragment	**210**	**210**	**108**	**108**	**111**	**111**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR16_2_QIU2_sp	Sperm		**212**		**122**		**107**		**301**		**338**		**187**		**150**		**188**		**154**	
GPR16-4	PR	GPR16_4_QIU5	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR16_4_QIU5_sp	Sperm		**210**		**114**		**103**		**301**		**326**		**187**		**158**		**160**		**152**	
GPR16-4	PR	GPR16_4_QIU6	Queen	Colony fragment	**210**	**210**	**108**	**108**	**109**	**109**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**
		GPR16_4_QIU6_sp	Sperm		**218**		**106**		**103**		**301**		**336**		**187**		**150**		**168**		**158**	
GPR16-4	PR	GPR16_4b_Q1_Qn	Queen	Colony fragment	**210**	**210**	**108**	**108**	**111**	**111**	**301**	**301**	**312**	**312**	**187**	**187**	**154**	**154**	**150**	**150**	**154**	**154**

		GPR16_4b_Q1_sp	Sperm		214		108		107		303		342		190		150		180		162	
GPR16-6	PR	GPR16_6_QIU1	Queen	Colony fragment	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_6_QIU1_sp	Sperm		214		110		107		305		332		187		150		—		—	
GPR16-6	PR	GPR16_6_QIU2	Queen	Colony fragment	210	210	108	108	109	111	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_6_QIU2_sp	Sperm		210		114		107		301		326		190		158		172		154	
GPR17-28	PR	GPR17-28-Q1	Queen	Colony fragment	210	210	108	108	—	—	301	301	—	—	187	187	—	—	150	150	154	154
		GPR17-28-Q1-sp	Sperm		210		106		107		303		326		187		150		—		—	
GPR17-28	PR	GPR17-28-Q2	Queen	Colony fragment	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR17-28-Q2-sp	Sperm		196		106		111		301		320		187		158		168		154	
GPR17-28	PR	GPR17-28-Q3	Queen	Colony fragment	210	210	108	108	111	111	301	301	312	312	187	187	154	154	—	—	—	—
		GPR17-28-Q3-sp	Sperm		210		106		107		301		334		187		150		190		152	
GPR17-28	PR	GPR17-28-Q4	Queen	Colony fragment	210	210	108	108	109	109	—	—	312	312	—	—	154	154	158	158	154	154
		GPR17-28-Q4-sp	Sperm		218		118		107		301		328		187		158		192		154	
GPR17-29	PR	GPR17-29-Q2	Queen	Colony fragment	210	210	108	108	109	109	301	301	312	312	187	187	154	154	158	158	154	154
		GPR17-29-Q2-sp	Sperm		212		108		105		—		328		187		150		170		156	
GPR17-31	PR	GPR17-31-Q2	Queen	Colony fragment	210	210	108	108	109	109	301	301	312	312	187	187	154	154	158	158	154	154
		GPR17-31-Q2-sp	Sperm		220		—		107		301		328		193		150		—		—	
GPR16-1	PR	GPR16-1-Q1-Qn	Queen	Colony fragment	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16-1-Q1-sp	Sperm		208		106		107		301		328		187		150		174		154	
GPR16-2	PR	GPR16-2-Q1-Qn	Queen	Colony fragment	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16-2-Q1-sp	Sperm		216		—		105		301		324		187		150		170		158	
GPR15-1	PR	GPR15_1_2	Queen	Field-collected colony	210	210	108	108	109	111	301	301	312	312	187	187	154	154	150	150	154	154
		GPR15_1_sp2	Sperm		218		108		103		301		332		187		150		168		156	
GPR15-5	PR	GPR15_5_1	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	158	158	154	154
		GPR15_5_sp1	Sperm		214		108		103		301		324		187		150		160		148	
GPR15-5	PR	GPR15_5_2	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR15_5_sp2	Sperm		216		108		109		301		326		187		158		172		154	
GPR16-1	PR	GPR16_1_Q96	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_1_Q96_sp	Sperm		210		126		107		301		326		187		150		172		156	
GPR16-1	PR	GPR16_1_Q98	Queen	Field-collected colony	210	210	108	108	109	109	—	—	312	312	—	—	154	154	150	150	154	154
		GPR16_1_Q98_sp	Sperm		218		108		105		301		324		187		158		176		154	
GPR16-2	PR	GPR16_2_Qn_1	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_2_Qn_1_sp	Sperm		230		108		107		301		328		190		150		168		156	
GPR16-2	PR	GPR16_2_Qn_2	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_2_Qn_2_sp	Sperm		214		120		107		301		330		187		150		176		152	
GPR16-2	PR	GPR16_2_Qn_3	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_2_Qn_3_sp	Sperm		210		110		107		301		328		190		158		166		156	
GPR16-2	PR	GPR16_2_Qn_4	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_2_Qn_4_sp	Sperm		210		114		99		301		322		187		158		178		154	
GPR16-3	PR	GPR16_3_Qn_1	Queen	Field-collected colony	—	—	—	—	111	111	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_3_Qn_1_sp	Sperm		210		118		99		301		334		187		158		146		154	
GPR16-3	PR	GPR16_3_Qn_2	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154

		GPR16_3_Qn_2_sp	Sperm		216		110		103		301		328		187		154		178		156	
GPR16-3	PR	GPR16_3_Qn_3	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_3_Qn_3_sp	Sperm		218		114		109		301		328		187		158		170		156	
GPR16-3	PR	GPR16_3_Qn_5	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_3_Qn_5_sp	Sperm		216		106		105		301		324		187		150		158		156	
GPR16-6	PR	GPR16_6_Qn_1	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_6_Qn_1_sp	Sperm		210		118		105		301		328		187		158		168		152	
GPR16-6	PR	GPR16_6_Qn_2	Queen	Field-collected colony	210	210	108	108	111	111	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_6_Qn_2_sp	Sperm		—		—		109		301		328		187		158		170		156	
GPR16-6	PR	GPR16_6_Qn_3	Queen	Field-collected colony	210	210	108	108	109	111	301	301	312	312	187	187	154	154	150	150	154	154
		GPR16_6_Qn_3_sp	Sperm		214		106		107		301		324		187		150		158		154	
GPR17-28	PR	GPR17_28_Qn_1	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	158	158	154	154
		GPR17_28_Qn_1_sp	Sperm		208		108		107		301		326		187		150		176		156	
GPR17-28	PR	GPR17_28_Qn_2	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR17_28_Qn_2_sp	Sperm		214		110		111		303		324		187		158		164		154	
GPR17-28	PR	GPR17_28_Qn_3	Queen	Field-collected colony	210	210	—	—	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR17_28_Qn_3_sp	Sperm		210		108		111		301		320		187		158		168		154	
GPR17-28	PR	GPR17_28_Qn_4	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR17_28_Qn_4_sp	Sperm		214		118		107		301		340		187		150		194		154	
GPR17-29	PR	GPR17_29_Qn_1	Queen	Field-collected colony	210	210	108	108	109	111	301	301	312	312	187	187	154	154	150	150	154	154
		GPR17_29_Qn_1_sp	Sperm		216		108		105		301		326		187		158		186		154	
GPR17-29	PR	GPR17_29_Qn_3_sp	Sperm	Field-collected colony	212		110		115		301		340		187		158		164		154	
GPR17-29	PR	GPR17_29_Qn_4	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	158	158	154	154
		GPR17_29_Qn_4_sp	Sperm		220		110		99		301		338		187		150		176		156	
GPR17-29	PR	GPR17_29_Qn_5	Queen	Field-collected colony	210	210	108	108	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		GPR17_29_Qn_5_sp	Sperm		210		112		103		301		326		187		158		186		154	
SDP14-1	PR	SDP14_1_Q_1	Queen	Field-collected colony	210	210	—	—	109	109	301	301	312	312	187	187	154	154	150	150	154	154
		SDP14_1_Q_1_sp	Sperm		210		—		107		301		340		187		158		158		158	

APPENDIX I

DIPLOID MALE PRODUCTION IN POLYGYNE *S. GEMINATA*

Proportions of males heterozygous (diploid) and of queens heterozygous, as well as proportions of diploid males with maternal alleles only, are shown for two sets of microsatellite loci. Sample sizes (numbers of males) are in parentheses.

	Site	Source of Males	Males Heterozygous	Queens Heterozygous^	Diploid Males with Maternal Alleles Only
Nine Loci*	PR	field-collected nests and study progenies	0.066 (91)	0.143 (203)	1.0 (6)
	BS	field-collected nests and study progenies	0.152 (33)	0.146 (48)	1.0 (5)
Ten Loci†	PR	field-collected nests and study progenies	0.369 (84)	0.867 (30)	1.0 (31)
	PR	study progenies with mothers heterozygous at *Sdag_C185*	0.400 (20)	1.0 (4)	1.0 (8)

^Includes reproductive and daughter queens. These data are shown to indicate the power to detect heterozygosity (diploidy) in males using the same markers.

*Primary loci used in this study.

†Nine primary loci plus locus *Sdag_C185*; the latter locus was included because most reproductive queens from site PR were heterozygotes for it.