THE GENETICS AND EPIGENETICS OF PLASTICITY IN SOCIAL INSECTS

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THE GENETICS AND EPIGENETICS OF PLASTICITY IN SOCIAL INSECTS

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LIST OF SYMBOLS AND ABBREVIATIONS

A_e	Effective number of alleles
A_n	Total number of alleles
ANOVA	Analysis of Variance test
B	Nonac's skew
CpG	Cytosine-Guanine dinucleotide
CpGo/e	Ratio of observed/expected CpG dinucleotides
DF	Degrees of freedom
D_r	Relative divergence in gene body methylation (e.g., between paralogs)
dS	Rate of synonymous substitutions per synonymous site
F'	Unbiased estimate of F_S accounting for sampling scheme
F_S	F-statistic measuring allele frequency differentiation between years
F_{ST}	F-statistic concerning differentiation among populations
GDA	Genetic Data Analysis Program
H_e	Effective heterozygosity
H_o	Observed heterozygosity
K_{e3}	Nielsen's effective paternity
mtDNA	Mitochondrial DNA
N_e	Effective population size
r_s	Spearman's rank correlation coefficient
r_{mm}	Inter-male relatedness
r_{ww}	Inter-worker relatedness
T_A	PCR primer annealing temperature

SUMMARY

The development of advanced societies marks a major transition in biological history. Highly social (eusocial) insects, in particular, comprise some of the most successful species on Earth due in large part to their high levels of cooperation and division of labor. The evolution of obligate sterility in worker individuals, shared brood care within colonies, and the development of differentiated castes are principal components to the success and survival of eusocial organisms. However, these behaviors are subject to evolutionary pressures from abiotic conditions such as environmental changes and the loss of natural habitats. When faced with such disruptions, social insects display a remarkable capacity for phenotypic plasticity and behavioral alterations.

This dissertation examines the evolutionary trajectory of this plasticity in eusocial insects from both a population genetics and a molecular perspective. First, at a population level, I investigate changes to the social form and life cycle of the southern yellowjacket *Vespula squamosa* and find polygynous colonies representing plasticity in changing environmental conditions. I also examine the population genetics and effective population size of the eastern yellowjacket *Vespula maculifrons* to investigate the mating behavior of population queens over time. Next, I probe the evolution of duplicated genes in the honeybee *Apis mellifera* and uncover strong correlations between gene body methylation and gene duplication in several contexts that could represent a significant molecular pathway to the plasticity of gene expression in highly social insects. Finally, to further understand the molecular underpinnings of plasticity, I examine the distribution of DNA

methylation within multiple insect genomes to explore its possible mechanistic role in eusocial systems.

These projects aim to provide insight into the evolution of eusocial organisms from both a population and a molecular level. The results of this research contribute to a greater understanding of the population dynamics of insect societies and the evolution of plasticity across eusocial insects.

CHAPTER 1. INTRODUCTION

The transition of insect populations from solitary societies to more advanced eusocial organization marks one of the most significant major transitions in evolutionary history (Maynard Smith & Szathmary, 1998; Wilson, 1971). Social insects, which include many species of bees, wasps, ants, and termites, are among the most successful extant organisms by many measures, including overall biomass (Fittkau and Klinge 1973; Wilson, 1987). The factors contributing to the evolution of eusociality include the development of several key behavioral phenotypes including obligate sterility, shared rearing of brood, and colony defense (Hölldobler and Wilson 1990; Ross and Matthews 1991). These behaviors are subject to influence from abiotic factors such as environmental disruptions, which can have effects on the success of the social unit as a whole (Hölldobler & Wilson, 1990; Queller & Strassmann, 1998; Ratnieks et al., 2006; Ross & Matthews, 1991). In some cases, social insects respond to these outside factors through phenotypic plasticity, wherein cooperative behaviors can change to facilitate the fitness interests of the colony (Andrew et al., 2013; Czaczkes & Heinze, 2015; Schurch et al., 2016; van Baaren & Candolin, 2018 Sih et al. 2011; Wong and Candolin 2015). This plasticity is of interest as changes to key behaviors such as mating could impact the intra-colony interactions and levels of relatedness that are essential for the survival of a eusocial system. However, much is still unknown regarding the genetic underpinnings of the evolution and maintenance of plasticity in eusocial insects.

The evolution of plasticity is crucial to the success of social insects, as the ability for specialization in colony behaviors depending on the caste and age of an individual

within the colony allows for an increase in the efficiency of the whole. For example, in a monogynous species, a reproductive division of labor in which queens monopolize the production of the next generation within the colony maintains levels of intra-colony relatedness and enables forager and nurse females to gain indirect fitness benefits by ensuring the development of the queen's offspring. In a colony with a single queen who only mates once, these individuals would share a relatedness of 75%, possibly leading to workers forgoing their own reproductive potential to care for their sisters. This benefit model relating to the evolution of obligate worker sterility relies on workers altruistically sacrificing their own direct fitness benefits by not reproducing new queens or workers, although workers have been shown to produce male offspring in the case of the queen being absent (Kovacs and Goodisman 2007; Ross 1986; Ross and Carpenter 1991). It has been a topic of debate as to whether such altruism could facilitate the evolution of eusocial insects multiple times phylogenetically (Hughes et al., 2008; but see Wilson et al., 2008; Nowak et al., 2010; Bourke et al., 2011). In some cases, worker sterility is facultative and is enforced within the colony through behaviors such as policing and manipulation of worker nutrition (Kapheim et al., 2011; Wenseleers, et al., 2004).

The evolution of a reproductive division of labor as a behavioral phenotype represents a significant departure from solitary species in cost and benefit in order to maintain high relatedness within colonies. Further compounding this, many social insect species exhibit polyandry, in which a single colony queen can reproduce with multiple male mates (Kraus and Moritz 2010; Strassmann 2001). In such cases, colony relatedness and genetic diversity are dependent on the number of male mates that produce colony offspring (Ross 1986; Goodisman et al. 2007; Goodisman et al. 2007; Kovacs et al. 2008;

Johnson et al. 2009; Kovacs and Goodisman 2012). Such decreases in relatedness between individuals within a colony can potentially reduce the benefit associated with cooperation and may lead to conflict (Crozier and Fjerdingstad 2001; Ratnieks et al. 2006; Strassmann 2001). However, having multiple male mates may also have indirect benefits for a queen and a colony by increasing genetic variation within a colony of closely related individuals, allowing for a higher resistance to selective effects (Baer 2016; Crozier and Fjerdingstad 2001; Dobelmann et al. 2017; Tarpy and Seeley 2006; Vollet-Neto et al. 2019). The ability of social insects to maintain sufficiently high levels of relatedness within colonies is important to the fitness of populations and allows for the plastic expression of phenotypes across castes without negatively impacting colony structure.

While the development of morphologically and behaviorally disparate phenotypes expressed between sexes and castes is a hallmark of the evolution of eusociality, plasticity in expression can lead to genetic conflicts that decrease fitness (Wheeler 1986; Normark 2003; Whitman 2009; Simpson, et al. 2011). Differentiated phenotypes expressed from a common genome, or polyphenisms, are underpinned by caste- and sex-dependent expression of genes. When castes are expressing a common gene in the same manner, selection should favor the evolution of a genotype that is most beneficial to the fitness requirements of each caste member. However, if castes have different selective requirements or plastic expression at a single locus, genetic intra-locus conflicts can emerge, which may result in a decreased fitness for both castes as neither is able to reach optimal expression (Rice and Chippindale 2001; Bonduriansky and Chenoweth 2009; Connallon and Clark 2011; Pennell and Morrow 2013; Pennell, et al. 2018). This could

create an environment in which phenotypic plasticity is detrimental to overall fitness at an individual and a colony level.

One existing model for how such conflicts could be resolved is through gene duplication (Ellegren and Parsch 2007; Gallach and Betran 2011). New genetic material being introduced into the genome could allow for differential expression at a single locus, and the potential for the adaptation and resolution of intra-locus conflict (Holland 1994; Li, et al. 2018). Previous work in insect duplication has shown that genes that have been duplicated are correlated at a higher level with caste-biased expression than are singleton genes (Chau and Goodisman 2017). However, evidence also suggests that the majority of duplicate gene copies become nonfunctionalized and are removed through negative selection (Lynch and Conery 2000). Therefore, while gene duplication represents a plausible mechanism for phenotypic plasticity in insects, it remains unclear how such a system evolved or is maintained in insect genomes.

A particular area of interest in furthering understanding of the evolution and regulation of phenotypic plasticity is epigenetics. Epigenetic information is a means of altering the genome without directly changing the encoding of the DNA. As such, the possible implications of epigenetic changes, and particularly DNA methylation, in phenotypic plasticity have been a target of study across taxa. DNA methylation is a heritable epigenetic modification that is found across a wide array of species, including animals, plants, bacteria, and fungi (Suzuki and Bird 2008; Feng, et al. 2010; Niederhuth, et al. 2016; Bewick, et al. 2019a). Previous work has shown causal associations between gene expression and DNA methylation of gene promoters in vertebrate systems (Jones 2012). However, in insect genomes, DNA methylation is found at much lower levels and

is represented in a “mosaic” distribution, rather than being found globally (Wang, et al., 2006; Zemach, et al., 2010). For this reason, a potential functional role of DNA methylation in insects is still unclear (Simmen, et al. 1999; Suzuki and Bird 2008; Feng, et al. 2010; Zemach, et al. 2010; Bewick et al. 2019a, b). Indeed, many insect studies have shown that there is no consistent causative association between gene body methylation and gene expression (Roudier et al., 2009; Bewick et al., 2016; Bewick and Schmitz, 2017). Despite this, some insect species with no significant DNA methylation at all still possess enzymes associated with *de novo* methylation of new sites and with maintenance of historical methylation marks (Bewick et al., 2017; Provataris, et al., 2018; Lewis et al., 2020). This conservation could indicate an as yet unclear role that DNA methylation plays in the insect genome. Alternative hypotheses for the continued presence of DNA methylation in insects posit that it may affect the integrity of transcription enzyme binding and the promotion of alternative gene splicing, although there is evidence that these roles are not consistently found across species (Bird 2002; Lorincz, et al. 2004; Zilberman and Henikoff 2007; Luco, et al. 2010; Maunakea, et al. 2010; Shukla, et al. 2011; Harris, et al. 2019).

This thesis aims to study the genetic and epigenetic underpinnings of plasticity in eusocial insect systems and investigates possible cases of plastic expression in insect species. From a molecular perspective, most of the research on this topic concerns functional associations between DNA methylation and gene expression, which has been disputed in many contexts such as phylogenetic clade, caste differentiation, and level of sociality. However, in some areas such as gene length and gene family size, reproducible correlations with DNA methylation have been discovered. Therefore, established

correlations in genic methylation of insects allow for the hypothesis that DNA methylation could be targeted to genes mechanistically and in broader genetic contexts, rather than based strictly on the functional role of the gene.

Chapter two of this dissertation is focused on the interesting dynamics of perennial colonies of the southern yellowjacket, *Vespula squamosa*. Although *V. squamosa* colonies typically display annual life cycles and are headed by a single colony queen, perennial colonies display striking plasticity in response to warming climates by exhibiting polygyny and persistence over multiple years. We genotyped individuals from perennial *V. squamosa* colonies at 10 polymorphic microsatellite loci and one mitochondrial DNA locus. Overall, we found that perennial and annual colonies did not show significant genetic differences, which indicates that the formation of perennial social forms represents a case of phenotypic plasticity, rather than a speciation event. Additionally, we found that relatedness of nestmates from perennial colonies was significantly lower than relatedness of nestmates from annual colonies, as would be expected in a system with multiple polyandrous queens. Mitochondrial DNA analyses showed that the perennial colonies were semi-closed systems, in which all individuals in the colony belonged to a single matriline despite the presence of multiple reproductive queens. Overall, our results indicated that perennial *V. squamosa* colonies showed substantial changes to their social biology compared to typical annual colonies and demonstrate variation in social behaviors in highly social species.

Chapter three investigates the population genetics of the highly social wasp, *Vespula maculifrons*, including examinations of changes to the social mating over time and the first estimations of its effective population size. We sought to interrogate the hypothesis that the N_e of eusocial species were relatively low due to the reproductive division of labor

that characterizes eusocial taxa. We found that effective population size ranged in the hundreds of individuals, which is similar to estimates in other, non-eusocial taxa. Additionally, we estimated levels of polyandry in *V. maculifrons* in different years in order to determine if queen mating system varied over time and found no change in the number of male mates per colony queen, nor to the paternity skew of the mates. Overall, the results of this chapter indicate a stability in the population dynamics of a eusocial insect over time, both in the mating system of queens and in an effective population size that is larger than expected which could indicate a higher resistance to selection than in a population with lower genetic diversity.

Chapter four is centered on the molecular evolution of a eusocial insect and a possible hypothesis regarding how phenotypic plasticity can be expressed in the genome. We studied duplicated genes of the honeybee *Apis mellifera*, to investigate whether DNA methylation was associated with gene duplication and genic evolution. Interestingly, we found that levels of gene body methylation were significantly lower in duplicate genes than in single copy genes, implicating a possible role of DNA methylation in post-duplication gene maintenance. Also, we discovered associations of gene body methylation with the location, length, and time since divergence of paralogous genes. We also found that divergence in DNA methylation was associated with divergence in gene expression in paralogs, although the relationship was not completely consistent with a direct link between DNA methylation and gene expression. Overall, our results provide further insight into genic methylation and how its association with duplicate genes might facilitate evolutionary processes and adaptation.

Finally, chapter five seeks to further elucidate the molecular underpinnings of plasticity by examining the global distribution of methylation across insect genomes. We examined levels of DNA methylation of genes across chromosomes in seven insect species with the goal of determining whether patterns of DNA methylation are similar across chromosomes in insect taxa or if global methylation distribution varies phylogenetically. We found that the mean levels of genic methylation differed significantly across chromosomes in all insect species, and that the magnitude of this difference showed a potential phylogenetic signal. We also found that the variance in genic methylation dispersion differed across chromosomes in all tested species, however there was no clear patterning phylogenetically for methylation variance. Additionally, we used CpGo/e as a proxy for DNA methylation data in order to incorporate a larger sample of insect species and extend the results of this chapter. The results from this study regarding potential specific targets and chromosomal distributions of DNA methylation in insects yield important insights into the evolution of the DNA methylation patterns that factor in to plasticity.

This dissertation aims to investigate plasticity in eusocial insects from multiple perspectives. Investigations into the population dynamics of two *Vespula* species examine colony-level evolution in response to evolutionary time and changes in climate. Additionally, molecular analysis of epigenetics in several insect species show associations between DNA methylation and genes in the context of duplication and location within the genome. Overall, these chapters examine phenotypic plasticity in eusocial species and add to the understanding of its genetic basis. The results of these projects will contribute to the field of social insect research by expanding the focus regarding the evolution of phenotypic

plasticity from direct causal gene expression changes to a more mechanistic examination of their genetic underpinnings.

CHAPTER 2. SOCIAL STRUCTURE OF PERENNIAL *VESPULA SQUAMOSA* WASP COLONIES[1]

2.1 Abstract

Many highly social species show variation in their social structure in response to different environmental conditions. For example, colonies of the yellowjacket wasp *Vespula squamosa* are typically headed by a single reproductive queen and survive for only a single season. However, in warmer climates, *V. squamosa* colonies sometimes persist for multiple years and can grow to extremely large size. We used genetic markers to understand patterns of reproduction and recruitment within these perennial colonies. We genotyped *V. squamosa* workers, pre-reproductive queens, and males from perennial colonies in the southeastern United States at 10 polymorphic microsatellite loci and one mitochondrial DNA locus. We found that *V. squamosa* from perennial nests were produced by multiple reproductives, in contrast to typical annual colonies. Relatedness of nestmates from perennial colonies was significantly lower than relatedness of nestmates from annual colonies. Our analyses of mitochondrial DNA indicated that most *V. squamosa* perennial colonies represented semi-closed systems whereby all individuals belonged to a single matriline despite the presence of multiple reproductive females. However, new queens recruited into perennial colonies apparently mated with non-nestmate males. Notably, perennial and annual colonies did not show significant genetic differences, supporting the hypothesis that perennial colony formation represents an instance of social plasticity.

[1] Dyson, C. J., Crossley, H. G., Ray, C. H., & Goodisman, M. A. (2022). Social structure of perennial *Vespula squamosa* wasp colonies. *Ecology and Evolution*, *12*(2), e8569. https://doi.org/10.1002/ece3.8569

Overall, our results indicate that perennial *V. squamosa* colonies show substantial changes to their social biology compared to typical annual colonies and demonstrate variation in social behaviors in highly social species.

2.2 Introduction

The evolution of advanced societies represented an important and successful major transition in biological history (Maynard Smith & Szathmary, 1998; E. O. Wilson, 1971). The most remarkable of these societies are displayed by social insects, which include ants, termites, social bees, and social wasps. Social insects play major roles in many ecological communities and are considered among the most successful of animal taxa (E. O. Wilson, 1987). The success of social insects arises from their use of cooperative and helping behaviors to complete important tasks such as rearing young, defending the colony, and foraging for food. These behaviors, which are critical to the growth and survival of social insect populations, could be affected by changes to outside factors such as environmental conditions. Social insects may respond to such disturbances through behavioral plasticity leading to changes in cooperative actions (Andrew et al., 2013; Czaczkes & Heinze, 2015; Schurch et al., 2016; van Baaren & Candolin, 2018). Such changes could ultimately lead to evolution of the social structures that define insect societies and are of primary importance to their persistence, survival, and success (Hölldobler & Wilson, 1990; Queller & Strassmann, 1998; Ratnieks et al., 2006; Ross & Matthews, 1991).

The goal of this study was to understand the potential differences between social systems and behaviors in annual and perennial colonies of a social insect. Specifically, we studied changes in the societies of the highly social wasp, *Vespula squamosa* (Figure 2-1

and Figure 2-2A). *V. squamosa*, locally known as the southern yellowjacket, is a common social wasp found throughout the Southeast of the United States and extending through Mexico and into Central America (Akre et al., 1980; Hunt et al., 2001; Landolt et al., 2009). Typically, single queens initiate new nests after a period of overwintering (natural history reviewed by Edwards, 1980; Greene, 1991; Spradbery, 1973). The new queen may construct her own incipient nest. Alternatively, newly-emerged queens sometimes invade the nests of congeners and take over the already established colony (Allen et al., 2020; MacDonald & Matthews, 1975, 1984). Regardless of the method of nest initiation, the single queen remains wholly responsible for the production of offspring within the nest as long as she is present.

Within-colony genetic diversity in *V. squamosa* is directly related to queen mate number, as queens of all *Vespula* taxa mate with multiple males (polyandry) and all annual *Vespula* colonies are headed by a single queen (monogyny) (Foster & Ratnieks, 2001; Goodisman et al., 2007a, 2007b; Goodisman, Matthews, & Crozier, 2001; Goodisman et al., 2002; Hoffman et al., 2008; Kovacs & Goodisman, 2012; Ross, 1986; Wenseleers et al., 2005). A mature *V. squamosa* nest consists of several layers of comb and may contain ~5,000 cells used to produce thousands of individual wasps (MacDonald & Matthews, 1984) (Figure 2-2B). *V. squamosa* colonies then produce new queens and males that proceed on mating flights to propagate with reproductives from other colonies. Newly-mated queens find locations to hibernate during the winter and the old colony, including all remaining workers, males, and queens, die off as winter approaches.

Figure 2-1. Queen and worker *V. squamosa* social wasps inside the nest. This study investigates the genetic structure of *V. squamosa* perennial colonies, which can grow to extreme size and have profound ecological impacts.

However, *V. squamosa* colonies sometimes can persist for more than one year (Deets & Fritz, 2002; Ross & Matthews, 1982; Ross & Visscher, 1983). When conditions are favorable, including sufficiently high temperatures and adequate food availability, nests can continue to grow rather than dying off in the winter. These nests can take on extremely large size very quickly, growing exponentially so that after only two years they may be ten times larger than a typical annual nest (Figure 2-2C-E). One perennial *V. squamosa* nest was estimated to contain ~475,000 cells (Pickett et al., 2001), which is 100X larger than a typical, annual nest. The residents of this massive colony were estimated to have consumed ~215 kg of arthropod prey or perhaps 5,000,000 prey items.

This study used genetic approaches to interrogate the social structure of perennial *V. squamosa* colonies. We determined the relationships of nestmates and the reproductive patterns of individuals within colonies. We also investigated the origin of individuals within colonies, and possible genetic differences between the annual and perennial social forms. We interpret our results in light of our understanding of the factors that contribute to variation in sociality. Overall, our research provides insight into the evolution and plasticity of social systems in highly social species.

Figure 2-2. The southern yellowjacket, *Vespula squamosa*. (A) *Vespula squamosa* workers inside of an active colony. (B) Comb from a mature, annual colony of *V. squamosa*. (C) Perennial colony "MO", with small satellite colony at base of tree indicated by arrow. (D) Perennial *V. squamosa* colony in a home. (E) Large perennial *V. squamosa* colony invading structure. Photocredits: Michael Goodisman (A, B) and Charles Ray (C, D, E).

2.3 Materials and Methods

2.3.1 Sample Collection

Perennial colonies of *V. squamosa* were collected in Alabama, USA between July 19, 2019 and July 3, 2020. Samples were collected from eight perennial colonies (Table 2-1). One colony had a small apparent satellite colony that was also sampled for analysis (Figure 2-2C). For collection, colonies were treated with carbon dioxide or pyrethrin and piperonyl butoxide depending on the location of the nest.

Individuals were manually extracted from the nest material. Workers were collected from all colonies. In addition, a small number of presumptive gynes (pre-reproductive queens) and males were sampled from a subset of colonies. Individuals were preserved in 95% ethanol and transported to the lab for genetic analysis.

2.3.2 Laboratory analysis

DNA was extracted from the rear leg of individual wasps using the Chelex® protocol (Walsh et al., 1991). The genotypes of all individuals were determined at ten polymorphic microsatellite loci including: LIST-2003, LIST-2004, LIST-2007, LIST-2008, LIST-2013, LIST-2015, LIST-2019, LIST-2020, Rufa05, and VMA-6 (Daly et al., 2002; Foster et al., 2001; Hasegawa & Takahashi, 2002; Hoffman et al., 2008). Additionally, we sequenced a portion of the mitochondrial DNA (mtDNA) of a subset of individuals from each nest using the primers CB1 and CB2 (Chiotis et al., 2000). The mtDNA primers amplified a 458 bp segment of the cytochrome b gene.

PCRs were used to amplify DNA at the microsatellite loci and the cytochrome b mtDNA locus. PCRs were generally conducted in a final volume of 15 μL composed of: 6.4 μL deionized water, 2.4 μL 25mM MgCl2, 1.5 μL 10x PCR buffer, 1.2 μL 2.5mM dNTPs, 1 μL Taq polymerase, 0.75 μL each of 10 μM reverse and fluorescence-tagged forward primers, and 1 μL of DNA. The PCR amplification profile used for each locus was: 2 min at 94°C, 35 cycles for 30 sec at 94°C, 30 sec at locus-specific annealing temperature (Table 2-2), 30 sec at 72°C, and then a final extension for 5.5 min at 72°C. PCR products were run on a 3% agarose gel at 100V to verify amplification for each individual.

After confirmation of amplification, microsatellite genotypes were analyzed using the fragment analysis module of an ABI 3100 sequencer. Scoring was completed using a combination of GeneMapper v4.0 (Applied Biosystems, Foster City, CA) and manual scoring of peaks. mtDNA amplicons were sequenced using the CB1 primer by Eton Biosciences.

2.3.3 Microsatellite Genetic Analysis

In total, 790 individuals from eight perennial colonies were newly genotyped in this study (Table 2-1). In addition, we incorporated data from a prior study of annual *V. squamosa* colonies into this investigation for comparative purposes (Hoffman et al., 2008). This previous study of annual colonies included data from 485 workers from 13 annual colonies, which were collected in and around Atlanta, GA, USA between July 2004 and July 2005, and were previously genotyped at seven of the ten loci used in this study (Table 2-1).

Table 2-1. Locations and number of workers, gynes, and males genotyped at nuclear microsatellite (and mitochondrial) markers from perennial and annual *V. squamosa* colonies.

Social form	Colony ID	Latitude	Longitude	Workers	Gynes	Males	Total
Perennial	HA	33.1175	-86.1227	80 (13)			80 (13)
	KL	32.1253	-85.9370	80 (14)	48 (10)		128 (24)
	MA	33.9681	-87.8050	82 (13)	2 (2)	24 (9)	108 (24)
	MC	33.2197	-86.3014	80 (14)	4 (4)	30 (12)	114 (30)
	MO	32.3855	-86.3099	75 (21)	33 (19)	18 (16)	126 (56)
	SP	32.4669	-87.2127	66 (14)			66 (14)
	ST	31.1180	-87.4632	80 (14)	5 (4)		85 (18)
	WA	31.0482	-87.7152	80 (14)	3 (2)		83 (16)
Total				**623 (117)**	**95 (41)**	**72 (37)**	**790 (195)**
Annual	2	33.8952	-84.6341	40 (2)			40 (2)
	4	33.9926	-84.2873	11 (2)			11 (2)
	5	33.7916	-84.3741	40 (2)			40 (2)
	6	33.7916	-84.3741	40 (2)			40 (2)
	11	34.5877	-84.0029	40 (2)			40 (2)
	12	34.5895	-84.0051	40 (2)			40 (2)
	21	33.7330	-84.3737	40 (2)			40 (2)
	22	33.7330	-84.3737	40 (2)			40 (2)
	26	34.0933	-84.1946	40 (2)			40 (2)
	31	33.9650	-84.5408	40 (2)			40 (2)
	32	33.9302	-83.3941	40 (1)			40 (2)
	42	34.0027	-84.3816	35 (2)			35 (2)
	48	33.7330	-84.3737	39 (2)			39 (2)
Total				**485 (25)**			**485 (25)**
Grand Total				**1108 (142)**	**95 (41)**	**72 (37)**	**1275 (220)**

Population genetic statistics for each microsatellite locus, including effective and observed number of alleles, as well as expected and observed heterozygosity, were estimated using SPAGEDi (Hardy & Vekemans, 2002). Perennial and annual locus statistics were compared using paired t-tests and Wilcoxon rank sum tests implemented in JMP Pro 15 (SAS Inc, 2019).

We investigated potential genetic differences between perennial colony 'MO' and its putative, small, satellite colony (Figure 2-2C). We used the program GENEPOP (Rousset, 2008) to determine if the distribution of worker, male, and gyne genotypes differed between the satellite and the parent nest.

Similarly, we used GENEPOP to conduct genotypic probability tests to determine whether the distribution of genotypes of workers sampled from perennial colonies differed from the distribution of gynes sampled from the same colony. Such a result would be expected if the reproductives (queens or males) within colonies contributed differentially to the production of gynes and workers. The resulting P-values from these analyses were then combined across perennial colonies using Stouffer's Z-transform test to determine the overall significance of genetic differences between castes for all colonies.

We then investigated the distribution of genotypes of workers, gynes, and males within perennial colonies to determine if individuals of each caste were produced by more than one reproductive queen. For example, the presence of three homozygous genotypes among the workers or gynes, or three different alleles among the males, would indicate that these individuals arose from multiple reproductives. We then used the program COLONY (Jones & Wang, 2010) to provide estimations of parentage and number of queens within each perennial colony.

We estimated the relatedness of nestmate workers, gynes, and males from measures of genetic variability, as determined by the relationship $r = 2F_{ST} / (1 + F_{IT})$ (Pamilo, 1989). Standard errors of the mean (SEM) were calculated from the locus-specific relatedness

estimates. Significant differences between estimates of relatedness were determined based on whether 95% confidence intervals (1.96 ± SEM) overlapped.

Next, we investigated the putative genetic differences between perennial and annual colonies. First, we compared the number of alleles segregating within perennial colonies to the number of alleles segregating within annual colonies. This provided information on the number and origin of reproductives within colonies of the different social forms.

Then, we investigated if the perennial and annual *V. squamosa* colonies displayed genetic isolation by distance. We first calculated paired F_{ST} values between all colonies using GENEPOP. Pairwise geographic distances were calculated between each of the colonies using GenAlEx 6.5 (Peakall & Smouse, 2012). We then used a Mantel test within GENEPOP and Spearman's rank correlation coefficient (r_s) to determine the significance of the correlation between geographic and genetic distance.

Finally, we examined genetic differences between perennial and annual colonies of *V. squamosa* using the program GDA (Lewis & Zaykin, 2000). *Vespula* species within their native ranges do not show significant variation in allele frequency and genetic diversity over time (Dyson et al., 2021). Thus, analysis of genetic differences between *V. squamosa* annual and perennial colonies in this study would provide a rudimentary test of genetic differentiation between social forms. We used a hierarchical analysis of genetic structure to measure genetic differences between both 'social form' (annual or perennial) and 'colony within social form'. Estimates of theta for these levels (θ_S and θ_C, respectively) provided information of genetic differentiation of social forms, while controlling for genetic differences between colonies.

2.3.4 Mitochondrial Genetic Analysis

We obtained the sequence of 223 individuals from 8 perennial and 13 annual colonies at the PCR-amplified cytochrome b fragment (Table 2-1). Sequences from both the perennial and annual colonies were newly-obtained in this study. Quality assessment of unassembled mtDNA sequences was performed manually. The program CHROMAS was used to visualize chromatogram output and identify low-quality or ambiguous base calls for trimming. Sequences for cytochrome b were aligned by MUSCLE (Edgar, 2004). MEGA X was used to trim low-quality bases from the reads, resulting in a final cytochrome b fragment of 338 bp (Kumar et al., 2018). Polymorphic bases among mtDNA sequences and haplotype diversity were estimated using DnaSP v6 (Rozas et al., 2017).

Table 2-2. Variability and amplification metrics for microsatellite and mtDNA loci used in analysis of perennial *V. squamosa* individuals.

Locus	A_n	A_e	H_e	H_o	T_A (°C)	Size (bp)
LIST2003	35	9.41	0.894	0.806	55	172-226
LIST2004	15	4.73	0.789	0.812	55	119-160
LIST2007	30	14.1	0.929	0.887	55	141-194
LIST2008	29	7.09	0.859	0.750	55	125-161
LIST2013	26	7.94	0.874	0.879	55	169-205
LIST2015	9	2.79	0.642	0.637	55	162-174
LIST2019	3	1.55	0.356	0.333	60	122-133
LIST2020	21	11.0	0.909	0.861	55	324-415
RUFA5	13	5.67	0.824	0.766	58	154-175
VMA6	29	9.16	0.891	0.867	58	269-303
mtDNA Cytochrome b*	5*	-	0.564*	-	45	458

*Total number of alleles, A_n; Effective number of alleles, A_e; Expected heterozygosity, H_e; Observed heterozygosity, H_o; PCR primer annealing temperature, T_A; Range of amplicon allele sizes, Size. * Number of haplotypes and haplotype diversity for mtDNA marker*

We generated a phylogeny of the mtDNA haplotypes. In order to create a rooted phylogeny of *V. squamosa* haplotype sequences, additional *Vespula* cytochrome b mtDNA sequences were downloaded from GenBank for inclusion in the analysis. These samples consisted of two additional *Vespula squamosa* sequences (Landolt et al., 2010; Lopez-Osorio et al., 2014), as well as sequences from two outgroup species, *V. maculifrons* (Landolt et al., 2010; Lopez-Osorio et al., 2014) and *V. germanica* (Eloff et al., 2020). Phylogenetic relationships were determined using MEGA X and maximum likelihood trees were constructed following the HKY nucleotide substitution model. Trees were tested using the bootstrap method with 500 replications.

Finally, we investigated if there were differences in mtDNA frequencies between the perennial and annual colonies. We used GENEPOP to determine the significance of differences in haplotype frequency between social forms. Due to the lack of intra-colony haplotype diversity within our samples, we collapsed our dataset so that information from each colony was represented by only a single individual. We then analyzed this reduced dataset consisting of 21 individuals (8 perennial and 13 annual) for differences between social forms using a probability test.

2.4 Results

2.4.1 Microsatellite Genetic Analysis

We identified one perennial colony, MO, that apparently had developed a small satellite colony near it (Figure 2-2C). A probability test of genotypic differentiation was used to determine if individuals sampled from the parent nest and satellite nest differed genetically. The genotypes of workers, gynes, and males sampled from the parent and satellite nest showed substantial but nonsignificant differences ($P = 0.051$, $P = 0.9685$, and $P = 0.0584$, respectively). Thus, we combined individuals from the MO parent and satellite nest together for all subsequent analyses.

Table 2-3. Tests of genotypic differentiation between gynes and workers in six perennial *V. squamosa* colonies and for all colonies combined.

Colony	N_G	N_W	χ^2	DF	P
KL	48	80	31.64	20	0.0473*
MA	2	82	40.19	18	0.0020**
MC	4	80	16.23	20	0.7022
MO	75	33	77.25	16	0.0001***
ST	5	80	18.34	20	0.5648
WA	3	80	20.66	20	0.4172
All					0.0001***

*Number of gynes, N_G; Number of workers, N_W; Chi-square statistic, χ^2; Degrees of freedom, DF; P-value, P with * P < 0.05, ** P < 0.01, *** P < 0.0001; Combined P-value from Z-transform analysis, All.*

We next investigated if the distribution of gyne and worker genotypes within perennial colonies differed significantly. Our analysis uncovered evidence that the distribution of gyne genotypes differed significantly from that of workers in 3 of the 6 colonies (Table 2-3). We then used Stouffer's Z-transform analysis to provide a secondary test of significance across all six colonies and found that the differences in genotypic distributions were highly supported (P < 0.001). Thus, there is some evidence of genetic differences between castes in perennial colonies.

The genotypes of workers, gynes, and males within perennial colonies were analyzed to determine if they were consistent with having been produced by more than a single queen. A single-queen model would demand that the genotype of every individual include one of the colony queen's two alleles. We found that individuals from perennial colonies could not have been produced by a single queen in all colonies and for all castes.

Moreover, iterative maximum-likelihood reconstructions of family relationships by the program COLONY produced a mean estimation of queen number of 21 for perennial colonies (colony HA: 14, KL: 29, MA: 9, MC: 21, MO: 25, SP: 18, ST: 32, WA: 20).

We next calculated the relatedness of nestmate workers, gynes, and males from perennial colonies (Figure 2-3). We found that the relatedness of perennial workers (0.130 ± 0.0095) was significantly lower than that of annual workers (0.368 ± 0.0164). The relatedness of gynes in perennial nests was 0.179 ± 0.0262, which did not differ significantly from the estimate for perennial workers. The relatedness of males from perennial colonies was 0.211 ± 0.0216, which was significantly below the value of 0.5 expected if males were produced by a single queen.

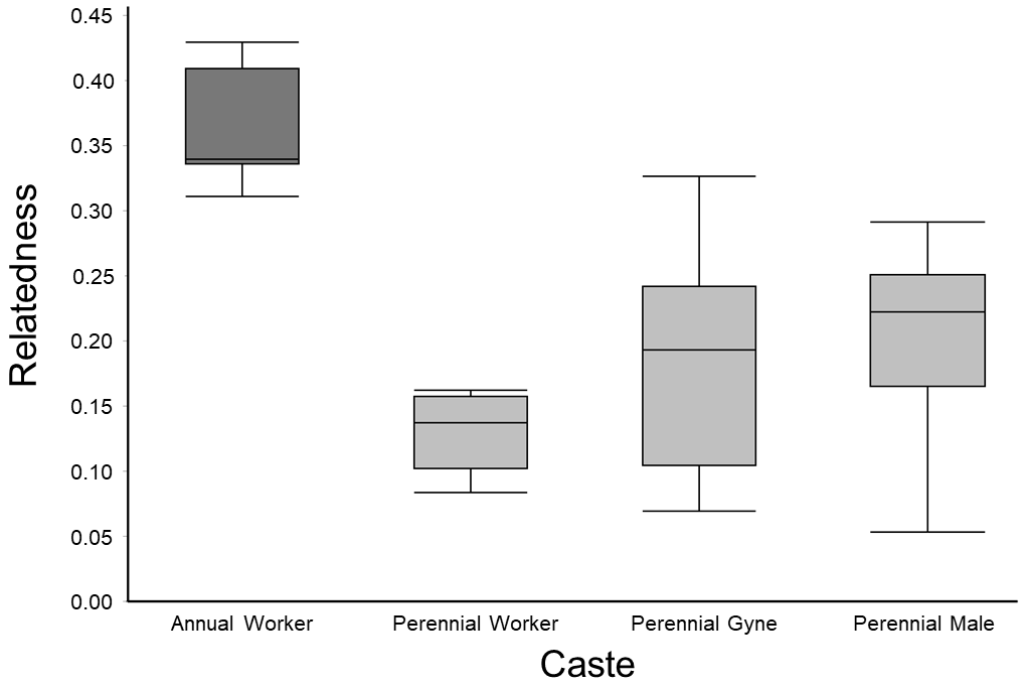

Figure 2-3. Relatedness between individuals of different castes in perennial and annual *V. squamosa* colonies. Boxes display first quartile, median, and third quartile values, whereas whiskers represent values within 1.5X the interquartile range. Workers sampled from annual colonies were significantly more highly related than those sampled from perennial colonies.

We next investigated genetic differences between the perennial and annual *V. squamosa* social forms. Our analyses revealed that the mean number of alleles present within perennial *V. squamosa* individuals was double to triple the mean number of alleles in annual colonies at 6 of the 7 loci, and differed significantly between social forms overall (paired t-test, t = -2.555, P = 0.0432; Figure 2-4). Specifically, the number of alleles in perennial colonies was significantly greater than those in annual colonies for all loci (Wilcoxon 2-sample test, P < 0.001) except for LIST2019 (P = 0.0541). The effective number of alleles, however, did not differ significantly between the social forms, suggesting that many of the observed alleles in perennial colonies were present at relatively low frequency (paired t-test, t = -2.284, P = 0.0625).

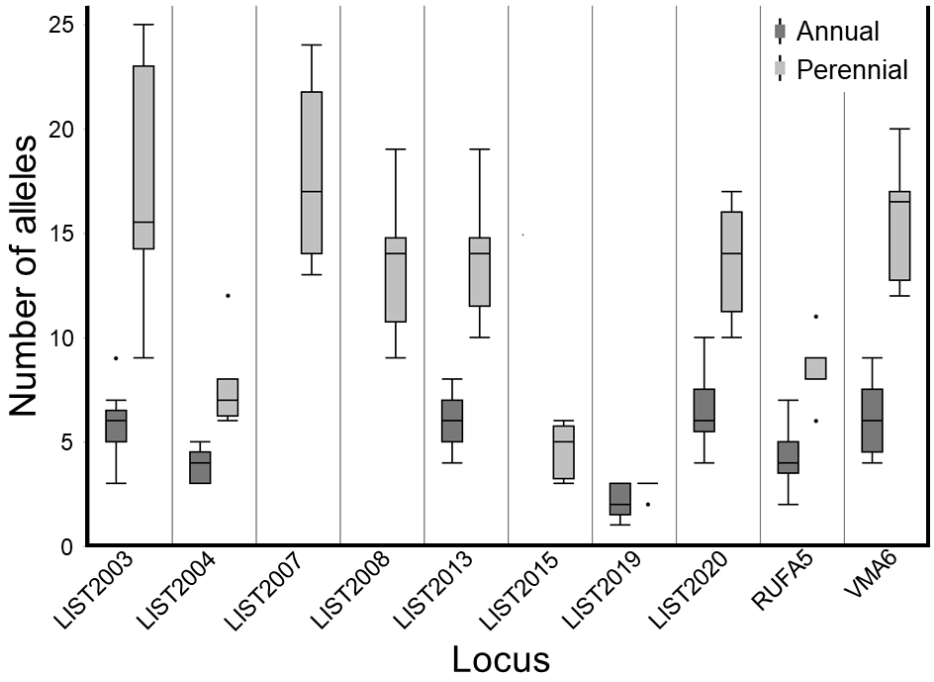

Figure 2-4. Number of alleles segregating at microsatellite loci in perennial and annual social forms of *V. squamosa*. Boxes display first quartile, median, and third quartile values, whereas whiskers represent values within 1.5X the interquartile range. Overall, perennial colonies showed a significantly higher number of alleles than annual colonies.

Genetic differences between colonies were estimated by calculating F_{ST} between all pairs of colonies (Figure 2-5). The F_{ST} values for the annual colonies were generally substantially higher than those for the perennial colonies (t-test, $t = -11.1438$, $p < 0.001$). Moreover, the pairwise F_{ST} values for inter-social form comparisons were more moderate and fell between the values for the annual and perennial colonies.

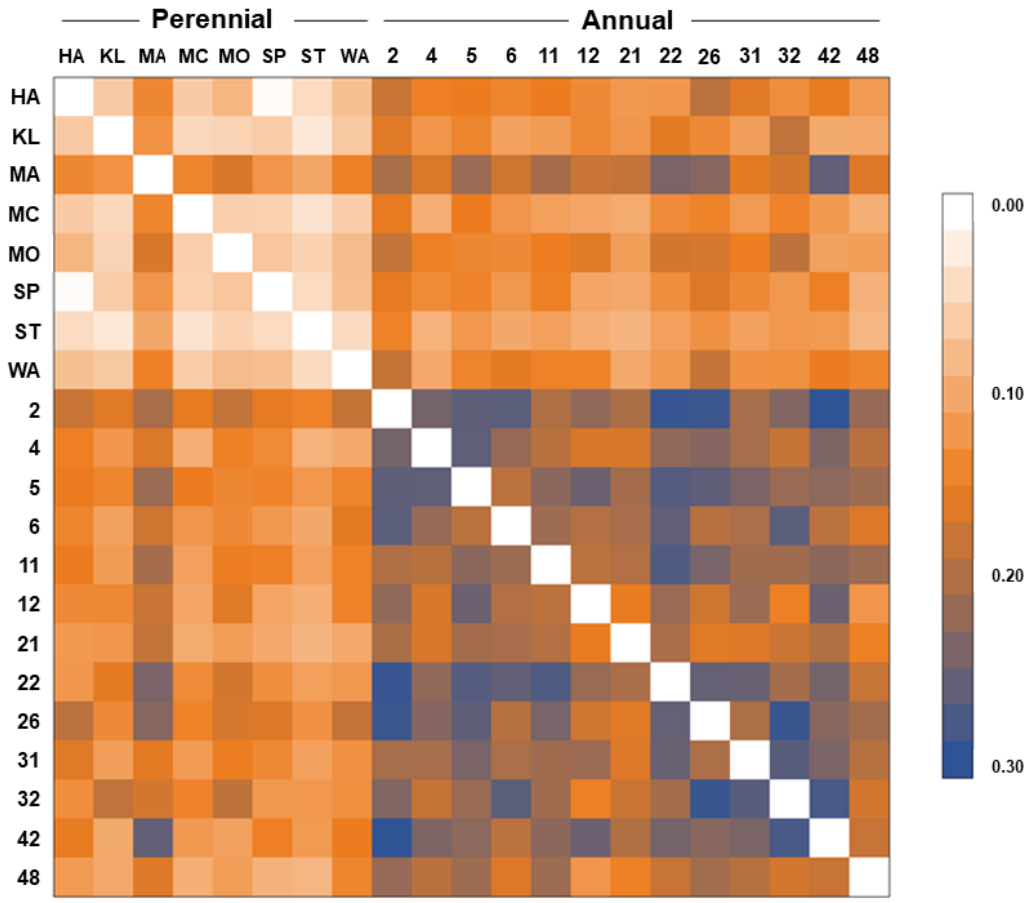

Figure 2-5. Pairwise F_{ST} values between all *V. squamosa* colonies. Annual colonies (denoted by numerical labels; N = 13) displayed a higher degree of differentiation between colonies than perennial colonies (denoted by two-letter name of collection site; N = 8).

We used Mantel tests to investigate genetic isolation by distance for the annual and perennial colonies. Annual colonies, perennial colonies, and all colonies combined showed no evidence of genetic isolation by distance across their respective collection ranges (P = 0.404, P = 0.541, P = 0.995, respectively). That is, colonies that were more distantly separated geographically from each other did not display significantly more genetically differentiation due to distance. Thus, overall, we find no evidence of population genetic isolation in *V. squamosa*.

Genetic differences between the perennial and annual social forms were further investigated using a hierarchical analysis of genetic structure. In particular, we were interested in understanding genetic differences between social forms while controlling for differences between colonies within social forms. The hierarchical analysis of variance of the nuclear microsatellite markers revealed no evidence of genetic differences between social forms (θ_S). In contrast there were substantial genetic differences among colonies within social form (Figure 2-6). Thus, overall, there was no evidence of genetic differentiation between annual and perennial social forms of *V. squamosa*.

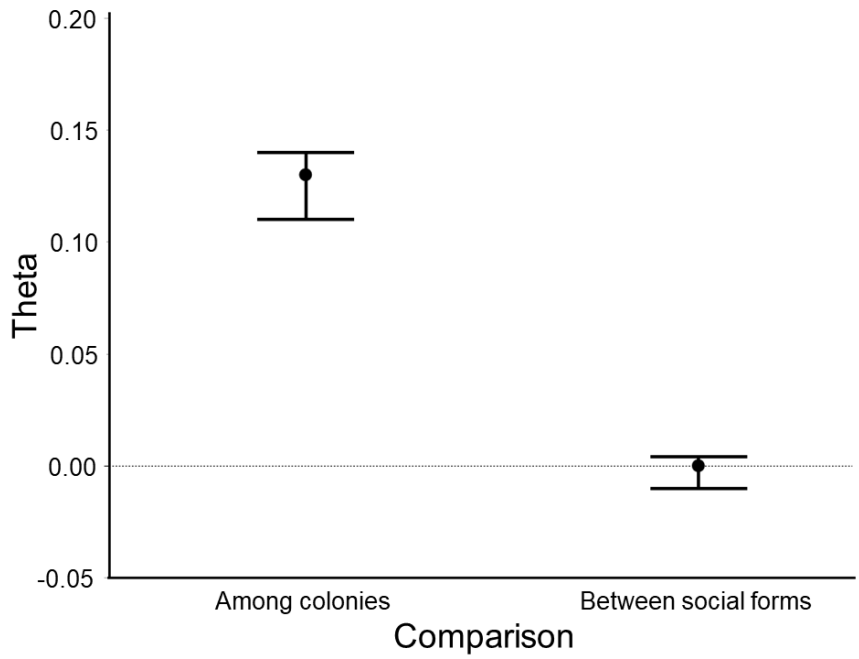

Figure 2-6. Means and 95% confidence intervals for comparisons of genetic differentiation among colonies within social forms (Θ_C) and between social forms (Θ_S).

2.4.2 Mitochondrial Genetic Analysis

We identified a total of five variable bases within our trimmed 338 bp sequence of the cytochrome b gene, indicating the presence of five unique haplotypes in our sequenced *V. squamosa* samples. Four of the haplotypes were found in the annual samples, while all five were present in the perennial samples (Figure 2-7A-B). Overall, we calculated a total haplotype diversity in our *V. squamosa* samples of 0.6116. Annual colonies (0.7267) had a similar haplotype diversity to perennial colonies (0.5641).

All annual colonies displayed a single mitochondrial haplotype, which is to be expected in a closed system with a single reproductive queen. Notably, however, we also found that all individuals from 7 out of 8 perennial colonies possessed only a single haplotype, suggesting that all individuals belonged to a single matriline. The only multiple-

haplotype perennial colony (MA) contained a single worker that differed in haplotype from the rest of the sampled individuals in that colony. This haplotype was confirmed by re-sequencing the individual in question.

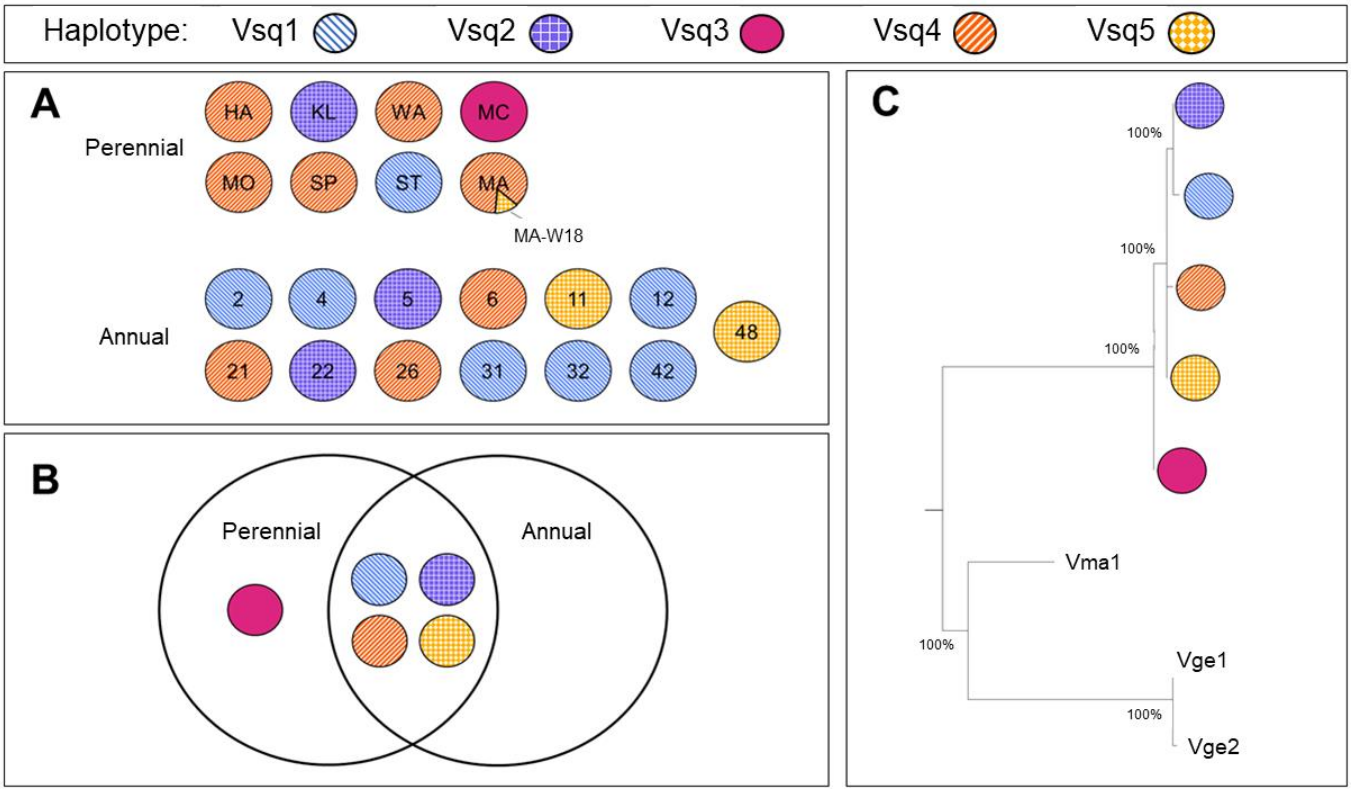

Figure 2-7. Mitochondrial haplotypes for cytochrome b in *V. squamosa*. (A) Haplotype frequencies within each colony. (B) Distribution of haplotypes in annual and perennial social forms. (C) Maximum-likelihood phylogeny of five mitochondrial haplotypes with *V. germanica* (Vge) and *V. maculifrons* (Vma) outgroups.

A maximum-likelihood phylogeny was generated to understand the relationships among the five different haplotypes (Figure 2-7C). We found that the five haplotypes identified in *V. squamosa* sorted into a monophyletic group when compared to the sequences from the outgroups *V. maculifrons* and *V. germanica*. We also investigated if the social forms showed significant differences in mtDNA haplotype frequency using an abbreviated dataset consisting of only a single individual per colony. We found no

significant differences in haplotype frequency between social forms using a test of genic differentiation (P = 0.2077).

2.5 Discussion

The goal of this study was to gain a greater understanding of variation in the social structure of a highly social insect. We specifically investigated the social systems of large, perennial *V. squamosa* colonies. Our goal was to understand how the social structure of perennial colonies differs from that of typical annual colonies and provide insight into future changes in social behavior.

2.5.1 Reproduction and recruitment within perennial colonies

We found that *V. squamosa* perennial colonies were always headed by multiple queens. The genotypes of sampled workers, gynes, and males were consistent with reproduction by multiple reproductive females. This was supported by maximum-likelihood estimations of queen number that indicated that colonies could be headed by 20 or more queens. However, we note that the exact assessments of queen number are likely to be complicated by queen polyandry, suggesting that actual estimates of queen number should be viewed cautiously. Regardless, our data clearly demonstrate that perennial colonies are headed by many reproductive queens. This differs substantially from annual *V. squamosa* colonies, which are always headed by a single queen that produces all workers, gynes, and males as long as she is present (Hoffman et al., 2008). This variation in colony queen number represents an important change in life history and social biology (Crozier & Pamilo, 1996; Keller, 1993).

Multiple-queen (polygyne) *V. squamosa* colonies could arise through one of two mechanisms. First, new queens could be recruited from within their natal nests. Alternatively, foreign queens from unrelated *V. squamosa* colonies could enter into already established colonies and begin producing new progeny. One way to distinguish these mechanisms is to study patterns of maternally-inherited genetic markers, such as the mtDNA (Goodisman & Ross, 1998). If new queens are recruited from their natal nest, then nestmates should always possess only a single mtDNA haplotype (i.e., that of the original mother queen). However, if foreign individuals enter nests to reproduce, then individuals from a single colony may contain multiple mtDNA haplotypes representing the matrilines of each new queen.

The overall result from our analysis of mtDNA was that workers, gynes, and males from perennial nests almost always possessed a singular mtDNA haplotype. There was a single worker from one perennial colony (MA) that possessed a mtDNA haplotype inconsistent with the rest of the colony. The importance of this individual is hard to interpret, as it could represent a rare event such as worker drift or even cross contamination. Therefore, overall, the data suggest that members of *V. squamosa* perennial colonies, including the multiple female reproductives, generally originate from their own parental nest.

The finding that perennial *V. squamosa* colonies recruit nestmate queens fits with general expectations from kin selection theory (Crozier & Pamilo, 1996). That is, cooperation among individuals is expected to occur between relatives (Bergmuller et al., 2007; Sachs et al., 2004). *V. squamosa* workers can produce males if the colony loses its queen. But they do not mate and cannot produce female offspring. Therefore, they

generally do not gain direct fitness benefits by producing their own offspring. Instead, they receive indirect benefits by helping to rear relatives produced by the queen. Cooperation and reproductive altruism can only evolve if nestmates are related (Kay et al., 2020). Thus, the finding that *V. squamosa* are mostly closed societies fits with these expectations as the introduction of foreign queens into the colony would cause a decrease in relatedness, and thus indirect fitness, overall.

Although new *V. squamosa* queens were recruited from within their natal nests, the male mates of new queens were apparently not. Genetic analyses indicated that perennial colonies contained a substantial increase in the number of nuclear alleles compared to annual colonies. This increase in rare alleles was associated with a decrease in nestmate relatedness. Thus, there was apparent gene flow into perennial colonies. In particular, new queens presumably mated with non-nestmate males. However, these mated queens apparently returned to their natal nest to reproduce. *Vespula* are capable of inbreeding in the lab (Kovacs et al., 2008). However, *Vespula* rarely inbreed in natural circumstances and have evolved several mechanisms to avoid inbreeding (Goodisman et al., 2002; Martinez et al., 2018; Martinez et al., 2021; Masciocchi et al., 2020; Masciocchi et al., 2018). Thus perennial *V. squamosa* colonies increase in genetic diversity over time through queen outbreeding, which leads to lower nestmate relatedness overall.

Interestingly, previous studies of perennial colonies in other *Vespula* species have uncovered evidence for at least occasional queen recruitment from outside the nest (Gambino, 1991; Goodisman, Matthews, Spradbery, et al., 2001; Hanna et al., 2014; Loope et al., 2018; Scarparo et al., 2021). Thus, different *Vespula* species show variation in whether they accept non-nestmate queens into the colony (Loope et al., 2018). Importantly,

the loss of colony boundaries associated with non-nestmate recruitment and recruitment of multiple queens has been identified in other social insects, most notably in many invasive ants (Helantera et al., 2009; Suarez & Goodisman, 2021). Such breakdowns are associated with changes in environmental conditions and, perhaps, with genetic changes to populations.

2.5.2 Reproductive competition within perennial colonies

The presence of multiple reproductive queens within *V. squamosa* perennial colonies raises the possibility that queens may engage in various types of reproductive competition with each other (Foster & Ratnieks, 2001; Ratnieks et al., 2006; Wenseleers et al., 2004). For example, prior studies have found that nest cells sometimes hold multiple eggs, indicating a breakdown of colony reproductive integrity (Kovacs & Goodisman, 2007; Spradbery, 1973). We investigated if the genotype distribution of gynes differed from that of workers in perennial colonies. Such differences could arise if different queens, or queens' male mates, contributed differentially to gyne and worker production (Boomsma et al., 2014; Heinze, 2010; Ratnieks et al., 2006) or if there were some other genetic effects on caste formation (Anderson et al., 2008; Lo et al., 2009; Schwander et al., 2010).

Interestingly, we found evidence of genetic differences between gynes and workers in three of the six colonies analyzed. This indicates that different queens or males likely produced the two castes in these colonies. Our sample size was relatively small for these analyses and so some caution is warranted in interpreting the results. However, secondary significance tests supported the results of the analyses that there were differences in the

genotypes between castes in perennial nests. Therefore, reproductives in polygyne *V. squamosa* nests apparently contribute differentially to the different castes, but more rigorous analysis would be needed to elucidate the details of the system.

Prior studies have found that genotype can affect caste phenotype in *Vespula* (Kovacs & Goodisman, 2012; Kovacs et al., 2010; Perrard et al., 2012). However, a previous investigation in *Vespula* found no evidence for different patriline contributions to gynes and workers (Goodisman et al., 2007a). Therefore, it is more likely that queens within perennial colonies contribute differentially to gyne and worker production. One might expect that queens within perennial colonies would compete to produce gynes rather than workers, as gyne production would presumably lead to larger increases in direct fitness, since workers can only produce males under restricted circumstances. Such reproductive competition should be an important factor affecting behavioral evolution (Ratnieks et al., 2006; Tarpy et al., 2004). It thus appears that reproductive competition may take place in *V. squamosa* perennial colonies among different queens (Stewart et al., 2017).

We identified a single perennial colony that generated a putative satellite colony nearby to the main nest. This appeared to be an instance of incipient polydomy, which is the occupation of multiple nests by a single colony. Polydomy is more often associated with terrestrial social insects such as ants or termites (Debout et al., 2007; Ellis et al., 2017; Robinson, 2014). However, polydomy could, in principle, occur in wasps as well. We found that there were large differences in the genotypes of males and workers sampled from the bud and parent colony, but these differences were not statistically significant and were limited in the samples that could be collected for analysis. Nevertheless, it is

intriguing to consider if these polygyne perennial colonies can create buds that ultimately become genetically differentiated from their parent colony.

2.5.3 Genetic differences between perennial and annual colonies

We sought to investigate whether the formation of perennial *V. squamosa* colonies represents true evolution (i.e., genetic change) or phenotypic plasticity. We attempted to explore this question by testing for genetic differences between the nuclear and mtDNA genotypes of annual and perennial colonies. We found no evidence of genetic differences between the social forms at either set of markers. In addition, there was no evidence of genetic isolation by distance within the social forms. Thus, overall, we find no evidence of genetic differentiation among the social forms. We note that this analysis, which includes the use of only a few genetic markers, is insufficient to provide a strong test of genetic differentiation between social forms. Nevertheless, our analysis can be viewed as providing preliminary insight into the question of whether annual and perennial *V. squamosa* colonies belong to the same gene pool. And our data are consistent with the idea that the perennial colonies represent an instance of phenotypic plasticity rather than evolution.

Previous investigations of other social species have sometimes uncovered evidence for a genetic basis to complex social behavior (Gutierrez-Valencia et al., 2021; Schwander et al., 2014). For example, variation in social form in two different ant genera has a genetic basis (Brelsford et al., 2020; Wang et al., 2013). Thus, phenotypic plasticity in social behavior may be associated with traits becoming genetically fixed. Or mutations may arise that lead to variation in complex social behavior (Rubenstein et al., 2019). Our research

on *V. squamosa* social forms has not uncovered evidence of genetic differences at this time. But more in-depth genomic analysis is needed to determine if such genetic differences exist or if they are likely to arise in the future.

2.5.4 Future of Vespula perennial colonies

Perennial colonies are found in several *Vespula* taxa (Gambino, 1991; Goodisman, Matthews, Spradbery, et al., 2001; Hanna et al., 2014; Jeanne, 1980; Lester & Beggs, 2019; Loope et al., 2018; Loope & Rankin, 2021; Plunkett et al., 1989; Reed & Landolt, 2005; Ross & Visscher, 1983; Spradbery, 1991; Thomas, 1960; Visscher & Vetter, 2003; E. E. Wilson et al., 2009). These large, persistent colonies in other *Vespula* are often associated with invaded habitats (Eyer & Vargo, 2021). Therefore, it is notable that *V. squamosa* forms perennial colonies in its native habitat, but only in warmer regions.

The perennial colonies of *V. squamosa* are thought to have a substantial ecological effect on the local environment. Their large size means that they take many more prey than a typical annual nest. Additionally, they become much more of a human nuisance because they contain orders of magnitude more workers than a typical nest (Beggs et al., 2011; Lester & Beggs, 2019; E. E. Wilson & Holway, 2010; E. E. Wilson et al., 2009). *Vespula* perennial colonies are expected to increase their range in response to climate change, which will further increase negative interactions with humans (Demain, 2020; Komonen et al., 2020; Lester et al., 2017). Moreover, it has been found that relatively few *Vespula* wasps are needed to initiate a new introduced population (Brenton-Rule et al., 2018; Chau et al., 2015; Dobelmann et al., 2019; Eloff et al., 2020; Hanna et al., 2014; Schmack et al., 2019). Thus, *Vespula* wasps have had great success in both introduced and native populations

around the world (Beggs et al., 2011; Lester & Beggs, 2019; Lowe et al., 2000; Manfredini et al., 2019). It is possible that continued global warming combined with increased movement of propagules will lead to *Vespula* perennial colonies worldwide.

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CHAPTER 3. TEMPORAL ANALYSIS OF EFFECTIVE POPULATION SIZE AND MATING SYSTEM IN A SOCIAL WASP[2]

3.1 Abstract

Highly social species are successful because they cooperate in obligately integrated societies. We examined temporal genetic variation in the eusocial wasp *Vespula maculifrons* in order to gain a greater understanding of evolution in highly social taxa. First, we wished to test if effective population sizes of eusocial species were relatively low due to the reproductive division of labor that characterizes eusocial taxa. We thus estimated the effective population size of *V. maculifrons* by examining temporal changes in population allele frequencies. We sampled the genetic composition of a *V. maculifrons* population at three separate time points spanning a 13-year period. We found that effective population size ranged in the hundreds of individuals, which is similar to estimates in other, non-eusocial taxa. Second, we estimated levels of polyandry in *V. maculifrons* in different years in order to determine if queen mating system varied over time. We found no significant change in the number or skew of males mated to queens. In addition, mating skew was not significant within *V. maculifrons* colonies. Therefore, our data suggest that queen mate number may be subject to stabilizing selection in this taxon. Overall, our study provides novel insight into the selective processes operating in eusocial species by analyzing temporal genetic changes within populations.

[2] Dyson, C. J., Piscano, O. L., Durham, R. M., Thompson, V. J., Johnson, C. H., & Goodisman, M. A. (2021). Temporal analysis of effective population size and mating system in a social wasp. *Journal of Heredity*, *112*(7), 626-634. https://doi.org/10.1093/jhered/esab057

3.2 Introduction

The evolution of advanced societies represented an important and successful major transition in biological history (Maynard Smith and Szathmary 1998; Wilson 1971). The most remarkable societies are displayed by eusocial species, such as the eusocial insects, which consist primarily of ants, termites, social bees, and social wasps. Members of these species form colonies that consist of individuals that work together to complete tasks such as food acquisition, colony defense, and rearing of young (Hölldobler and Wilson 1990; Ross and Matthews 1991). The success of eusocial species stems from this division of labor and the intricate cooperative actions displayed by society members.

The social behaviors exhibited by eusocial species are expected to be subject to a variety of selective processes. For example, abiotic shifts may alter selective pressures affecting a variety of social actions (Sih et al. 2011; Wong and Candolin 2015). In addition, the ability of social species to adapt to changing environments is constrained by levels of population genetic variation. Taxa with low genetic variability may be unable to adapt to new conditions (Charlesworth 2009; Ellegren and Galtier 2016; Luikart et al. 2010).

The overall goal of this study was to gain a greater understanding of the evolutionary processes affecting social species. This study had two aims related to understanding behavioral and genetic changes in eusocial taxa over time. First, we were interested in testing hypotheses about how sociality affects effective population size. The genetically effective population size (N_e) is a critical measure of the ability of a species to adapt to the environment (Ellegren and Galtier 2016). N_e is inversely related to the strength of genetic drift in a population. N_e is also directly related to the amount of genetic variation

a population maintains. Importantly, N_e can be influenced by a variety of demographic factors (Charlesworth 2009; Ellegren and Galtier 2016). For example, the complex societies displayed by eusocial species are expected to have strong effects on N_e (Crozier 1979; Crozier and Pamilo 1996; Wilson 1971).

A defining characteristic of eusocial species is a reproductive division of labor. That is, some individuals reproduce (i.e., the queens and males), whereas others do not (workers and soldiers). Eusocial societies are composed of relatively few individuals of the breeding classes and many more individuals of sterile classes (Wilson 1971). If very few individuals in a species are reproductively capable, then N_e is expected to be very low relative to the census population size (Crozier 1979; Romiguier et al. 2014; Romiguier and Weyna In press). This presents somewhat of a paradox. That is, one would predict that eusocial species would have low N_e and, therefore, be unable to adapt to changing conditions. Instead, eusocial species are among the most dominant of animal taxa (Wilson 1990). Consequently, it remains unclear how eusocial taxa can both have low N_e and be evolutionarily successful.

We wished to investigate N_e in eusocial species to obtain further insight into the reasons for the success of eusocial taxa. Thus, the first aim of this study was to determine N_e in a widespread, eusocial insect species. For the first time, we investigated variance in allele frequencies in a population of a eusocial insect over multiple years in order to estimate N_e. Our goal was to test whether N_e was low, as predicted by demographic and genetic theory.

The second aim of this study was to determine if the polyandrous mating system of a eusocial species showed evidence of change over time. The females of many taxa are polyandrous (mate with more than a single male) (Kraus and Moritz 2010; Strassmann 2001). Polyandry has particularly wide-ranging effects in eusocial species. For example, polyandry decreases the relatedness of interacting nestmates. Decreases in nestmate relatedness potentially reduce the benefits associated with cooperation and may lead to conflict among individuals (Crozier and Fjerdingstad 2001; Ratnieks et al. 2006; Strassmann 2001).

However, multiple mating by queens may also have advantages (Baer 2016; Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001; Loope et al. 2017). Polyandrous mating systems may return direct benefits to the female if, for example, she gains extra nutrients by mating multiply (Arnqvist and Nilsson 2000; Jennions and Petrie 2000; Kvarnemo and Simmons 2013; Pizzari and Wedell 2013). Alternatively, multiple mating may return indirect benefits whereby the high levels of genetic variation displayed by a female's offspring are advantageous (Baer 2016; Crozier and Fjerdingstad 2001; Dobelmann et al. 2017; Tarpy and Seeley 2006; Vollet-Neto et al. 2019). Thus, one might expect that mate number could be strongly affected by selection over time.

Despite the importance of polyandry, little is known about evolutionary changes to mate number in social species. Mate number variation may arise through several different proximate mechanisms, including changes to mate preferences, alterations in physiology, or modifications of reproductive actions. Due to the genetic "bottleneck" of queen-monopolized reproduction, such changes to the mating system may also have the potential to affect the amount of genetic variability within the population, and thereby influence the

effective population size. We investigated if queen mate number varied across years in a eusocial wasp in order to better understand the evolution of mating behaviors in eusocial species.

We undertook our investigation of temporal genetic change in the eusocial wasp, *Vespula maculifrons*. *V. maculifrons*, commonly known as the eastern yellowjacket, is a highly social wasp found throughout the eastern half of the United States that exhibits common social traits such as caste formation and reproductive division of labor (Greene 1991; MacDonald and Matthews 1981). Single queens initiate new nests after a period overwintering. The queen constructs the incipient nest and rears the first cohort of workers. Once the workers mature, they take over the task of colony maintenance. However, the single queen remains wholly responsible for the production of all offspring within the nest as long as she is present. The workers do not mate and therefore do not produce female offspring, although workers can produce male offspring in queenless nests (Kovacs and Goodisman 2007; Ross 1986; Ross and Carpenter 1991).

V. maculifrons queens mate with multiple males (Greene 1991; MacDonald and Matthews 1981). Within-colony genetic diversity is, therefore, a function of queen mate number (Goodisman et al. 2007; Goodisman et al. 2007; Johnson et al. 2009; Kovacs and Goodisman 2012; Kovacs et al. 2008; Ross 1986). Multiple mating by males has also been noted in laboratory settings (Ross 1983). *V. maculifrons* shows no evidence of inbreeding in natural settings (Hoffman et al. 2008). Queens and males find mates outside of their natal colony; Vespula queens are estimated to travel 1000-5000 meters to find their mates (Masciocchi et al. 2018).

Importantly, *V. maculifrons* also displays a simple, annual life cycle (MacDonald and Matthews 1981). That is, new colonies are established in the spring and then die off in the winter of the same year. This is relevant because overlapping generations can be problematic when estimating effective population size (Luikart et al. 2010). However, one year represents one birth to death cycle of a *V. maculifrons* colony and demarcates a single generation.

Overall, this study investigated temporal changes in behavior and genetic structure of a eusocial species. We estimated changes in population allele frequencies over several years in order to test if eusocial species displayed relatively low N_e. We also determined whether the queen mating system varied over a 13-generation period. We believe that these two aims are of importance to further understanding the evolution of eusocial species in light of environmental change and associated susceptibility to selective pressures. In addition, mating and social systems strongly influence the effective population size of species. However, relatively few studies have investigated colony and population genetic changes to eusocial species over time, particularly in light of how mating system fluctuations could affect genetic variability within species. Thus, our results provide novel insight into the importance of social behavior on evolutionary processes and adaptation.

3.3 Materials and Methods

3.3.1 Sample Collection

We collected *V. maculifrons* colonies in and around the city of Atlanta, Georgia, USA in three different years (Figure 3-1). Specifically, we collected 13 colonies from 2004, 11 colonies from 2006, and 20 colonies from 2017 (Table S3). Colonies were

anesthetized with ether in the field, manually extracted from the ground, and then returned to the lab for collection of samples. Approximately 16 workers and the colony queen (when available) were sampled from each colony and placed in 95% ethanol for future genetic analyses.

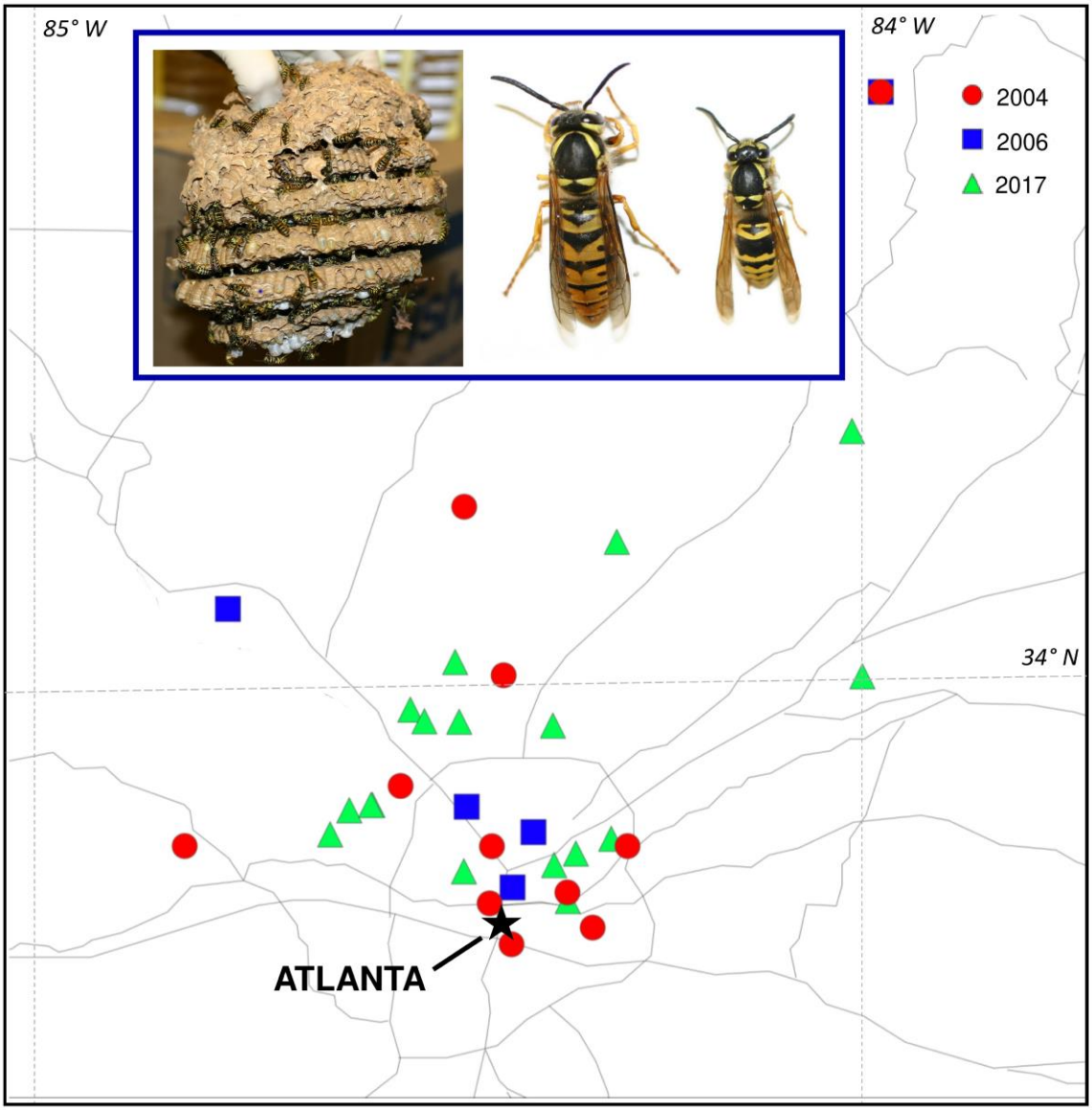

Figure 3-1. Locations of *V. maculifrons* nests collected around Atlanta, GA, USA at each of three time points. Inset: *V. maculifrons* small, mature nest, and *V. maculifrons* queen and worker.

DNA from individuals was extracted from rear leg tissue using an Omega Bio-Tek E.Z.N.A. tissue extraction kit (cat. D3396). The genotypes of all individuals were determined at sixteen polymorphic microsatellite loci: Rufa12, Rufa19, VMA-3, VMA-6, VMA-8, LIST2002, LIST2003, LIST2004, LIST2007, LIST2008, LIST2010, LIST2013, LIST2015, LIST2017, LIST2019, LIST2020 (Daly et al. 2002; Foster et al. 2001; Hasegawa and Takahashi 2002). These loci were chosen because they displayed no evidence of selection or linkage disequilibrium in previous studies in this species, and because their high diversity in *V. maculifrons* rendered possible male non-detection errors unlikely (Boomsma and Ratnieks 1996) (Table 3-1).

3.3.2 Microsatellite Genotyping

PCRs were used to amplify DNA at each of the sixteen microsatellite loci. PCRs were conducted using a 15 µL reaction composed of: 6.4 µL deionized water, 2.4 µL 25mM MgCl2, 1.5 µL 10x PCR buffer, 1.2 µL 2.5mM dNTPs, 1 µL Taq polymerase, 0.75 µL each of 10 µM reverse and fluorescence-tagged forward primers, and 1 µL of DNA. The PCR amplification cycle used for each locus was: 1 cycle for 2 min at 94°C, 35 cycles for 30 sec at 94°C, 30 sec at TA, 30 sec at 72°C, and a final extension for 5.5 min at 72°C. Annealing temperatures (T_A) are provided in Table 3-1.

Table 3-1. Allelic variability metrics at 16 microsatellite loci in *V. maculifrons*. Values represent means across three timepoints.

Locus	T_a	A_n	A_e	H_e	H_o	HWE
Rufa 12	55	8	3.59	0.721	0.712	0.514
Rufa 19	58	7	4.81	0.792	0.777	0.664
VMA 3	55	15	8.56	0.883	0.836	0.505
VMA 6	58	23	15.13	0.934	0.897	0.449
VMA 8	58	23	7.80	0.872	0.799	0.559
LIST 2002	55	13	5.75	0.826	0.714	0.594
LIST 2003	55	11	6.5	0.846	0.801	0.720
LIST 2004	55	13	6.53	0.847	0.857	0.311
LIST 2007	55	10	5.87	0.830	0.769	0.455
LIST 2008	55	9	4.71	0.788	0.738	0.467
LIST 2010	55	12	5.89	0.830	0.766	0.759
LIST 2013	55	14	7.72	0.870	0.777	0.486
LIST 2015	55	7	3.45	0.710	0.700	0.380
LIST 2017	55	10	4.11	0.757	0.731	0.358
LIST 2019	58	8	3.15	0.682	0.729	0.602
LIST 2020	55	13	7.95	0.874	0.861	0.569
Mean		12.25	6.34	0.816	0.779	0.596

T_a: Annealing temperature of PCR primers in °C; A_n: observed number of alleles; A_e: effective number of alleles; H_e: expected heterozygosity; H_o: observed heterozygosity; HWE: p-value for deviation from Hardy-Weinberg equilibrium.

PCR products were run on a 3% agarose gel at 100V to verify amplification for each individual. After confirmation of amplification, samples were analyzed using the fragment analysis module of an ABI 3100 sequencer. Scoring was completed using a combination of GeneMapper v4.0 and manual scoring of peaks. Worker genotypes obtained in this study are found in Table A-1.

We reconstructed the genotype of the colony queen and those of her male mates from the worker genotypes in each colony assuming Mendelian segregation of alleles and haplodiploid inheritance. As every worker inherits one of the queen's two alleles,

recapitulating the queen genotype and that of each haploid male mate can generally be accomplished using only the F1 generation genotypes. Reconstructed queen and male genotypes are found in Table A-2.

3.3.3 Population Genetic Analysis

Population genetic statistics for each locus, including expected and observed number of alleles, as well as expected and observed heterozygosity, were estimated using SPAGEDi (Hardy and Vekemans 2002). We then tested if the dataset consisting of inferred queen and male genotypes violated Hardy-Weinberg equilibrium using the program GenePop (Rousset 2008). Additionally, we tested for linkage disequilibrium among all pairs of microsatellite loci within years using GenePop.

We next investigated if our populations displayed genetic isolation by distance. Pairwise geographic distances were calculated between each of the colonies used in this study using GenAlEx 6.5 (Peakall and Smouse 2012). Next, genetic distances were calculated between each colony using GenePop. To calculate isolation by distance, we used a Mantel test within GenePop to determine significance of the correlation between geographic and genetic distance.

3.3.4 Effective Population Size

We used temporal genetic information to estimate N_e (Habel et al. 2014). Specifically, we estimated N_e based on the variance in population allele frequencies over time (Jorde and Ryman 2007; Krimbas and Tsakas 1971; Luikart et al. 2010). This approach benefits from an absence of overlapping generations (Jorde and Ryman 1995).

Thus this technique was appropriate for our analyses because different timepoints were taken without replacement and separated by at least one distinct generation.

We estimated N_e using a modified version of the temporal method of Jorde and Ryman (2007) as carried out by the program NeEstimator v2 (Do et al. 2014). In brief, allele frequencies at our 16 polymorphic loci were used to calculate measures of genetic differentiation (F_s) between time points. These measures of genetic differentiation were then used to estimate genetic drift and produce a pairwise harmonic mean estimate of $N_e = t / \{2[F_S-1/(2nx)-1/(2ny)]\}$ (Jorde and Ryman 2007), where t represents the number of generations between the two samples, F_s represents an average measure of genetic differentiation based on allele frequencies between the samples at the 16 polymorphic loci, and nx and ny represent the population sample sizes of the individuals in the comparison. Because *V. maculifrons* is haplodiploid, the focal calculation from Jorde and Ryman (2007) was modified by changing the constant representing the number of alleles per sampled individual from "2" to a weighted average within each estimation based on the ratio of males and females sampled (~1.5) to adjust for the haploid males. Confidence intervals were produced by jackknifing across the 16 loci 10,000 times. Jackknifing is used by NeEstimator v2 due to its tendency to produce precise intervals in repeated pairwise analyses.

We estimated N_e in *V. maculifrons* in four different sampling groups. First, we estimated N_e directly from the empirically obtained worker genotypes from this study. Second, we estimated N_e from the reconstructed genotypes of the colony queens and their male mates. Third, we estimated N_e from the inferred queen genotypes alone. And, finally, we determined N_e based solely on the inferred genotypes of the male mates. Using these

different samples to calculate Ne permitted for analysis based on the parental and filial generations, which allowed us to gauge the robustness of our estimates. Nevertheless, the inferred queen and male genotypes provided the most direct measure of genetically independent breeding individuals. However, we have no expectation that the estimates derived from the other samples would be substantially biased because we do not expect significant differences in allele frequencies between castes or sexes in our samples.

3.3.5 Mating System

We investigated aspects of the mating system of females based on the reconstructed male genotypes. First, we identified patrilines to determine the number of males mated to each queen. We then determined if the number of male mates per colony differed among years using ANOVA. We also used a Welch's test to investigate whether the variance in male mate number differed across years. Finally, we investigated if the distribution of queen mate number differed between pairs of years using a two-sample Kolmogorov-Smirnov Asymptotic test. All statistical analyses were performed in JMP Pro 15 (SAS Inc 2019).

We next calculated the effective paternity of queens within colonies. Effective paternity differs from raw paternity by factoring in unequal contributions of males to the reproduction of the next generation. The effective paternity per colony at each time point was calculated using the equation from Nielsen et al. (2003): $k_e3 = (n – 1)2/[\sum p_i2(n+1)(n-2)+3-n]$, where p is the proportion of offspring in a colony sired by male "i". Effective paternity across time points was compared using ANOVA.

Additionally, we calculated the paternity skew within colonies to determine if the levels of unequal mating differed across years. Higher values of paternity skew would be indicative of monopolization of reproductive success by a few males rather than a more even distribution across male mates. Skew was calculated using the binomial metric B in the program Skew Calculator (Nonacs 2003). Skew was considered to be significant if 95% confidence intervals did not overlap 0. ANOVA was used to determine whether mean skew differed across years.

Finally, we calculated the relatedness of nestmate workers in the program SPAGEDi (Hardy and Vekemans 2002) using the multi-locus estimator of Queller and Goodnight Queller and Goodnight (1989). Additionally, we calculated the relatedness of males mated to the same queen to determine if queens mated to genetically similar mates. Standard errors for these calculations were produced by jackknifing relatedness values over our 16 polymorphic loci. We compared our results against similar relatedness calculations in *V. maculifrons* from previous studies using a t-test in JMP Pro 15 (Hoffman et al. 2008).

3.4 Results

3.4.1 Population Genetic Analysis

We tested if the genotypes of individuals deviated from expectations under Hardy-Weinberg equilibrium. We found little evidence of deviations from HWE for most loci and timepoints. Overall, the data indicated no significant deviations from HWE in the combined male/queen dataset ($p = 0.5962$, Table 3-1). Additionally, tests for linkage disequilibrium were performed for each locus and for each timepoint. We found no

significant evidence of linkage disequilibrium within any locus or year (p = 0.7721, Table A-4).

We tested for genetic isolation by distance to determine whether the geographic distance between colonies correlated with their genetic distance. We found that there was no significant association between geographic and genetic distance of colonies within any of our three timepoints (p2004 = 0.3620; p2006 = 0.8460; p2017 = 0.1850). This indicates that colonies are not genetically correlated based on their location of collection. *Vespula* species have been shown in the past to not display significant genetic isolation by distance in their native habitats (Chau et al. 2015; Goodisman et al. 2001). Thus, our results in this study are consistent with the idea that *Vespula* wasps are able to move relatively freely throughout their geographic range.

3.4.2 Effective Population Size

We estimated N_e of *V. maculifrons* by analyzing the variance in population allele frequencies over time. Our estimate of N_e was calculated using genetic differences at 16 polymorphic loci across a 13-generation timeframe. We found harmonic mean values for N_e in our pairwise calculations of 31.1, 198.8, and 375.5 for our three comparisons using the genetically independent queen/male dataset (04-06, 04-17, 06-17, respectively; Table 3-2).

We found that the male-only and worker-only datasets produced estimates of N_e that were similar to those obtained from the queen/male dataset. However, the queen-only dataset tended to produce larger estimations of N_e (Table 3-2). The queen-only dataset also displayed the largest confidence intervals.

Table 3-2. Estimates of effective population size (N_e) in *V. maculifrons* with 95% confidence intervals in brackets. Four different sampling types were used for the analysis. W: worker genotypes; QM: queen and combined male genotypes; Q: queen genotypes; M: male mate genotypes.

Comparison	Sample	F_s	F'	N_e [C.I.]
2004 ↔ 2006	W	0.0637	0.0571	23.4 [16.5, 39.5]
	QM	0.0660	0.0428	31.1 [20.7, 62.8]
	Q	0.0878	0.0009	1498.1 [44.1, INF]
	M	0.0993	0.0671	19.9 [13.3, 39.3]
2004 ↔ 2017	W	0.0585	0.0532	162.8 [105.1, 362.5]
	QM	0.0625	0.0436	198.8 [121.6, 545.6]
	Q	0.0765	0.0104	833.3 [161.0, INF]
	M	0.0915	0.0647	134.0 [84.5, 322.3]
2006 ↔ 2017	W	0.0451	0.0398	184.4 [138.8, 275.1]
	QM	0.0382	0.0195	375.5 [228.8, 1050.0]
	Q	0.0754	0.0027	2716.1 [513.9, INF]
	M	0.0474	0.0222	330.8 [192.5, 1176.4]

F_s: F-statistic measuring allele frequency differentiation between years. F': unbiased estimate of F_s accounting for sampling scheme.

3.4.3 Mating System

We investigated if the number of males mated to queens heading *V. maculifrons* colonies differed among years. We found that there was no significant difference in the number of males mated to queens across the timepoints (F(2, 41) = 1.2250, P = 0.3043, Figure 3-2, Table A-3). We then used a nonparametric test to determine if the variance in queen mate number differed among years. We again found no differences in the variance in mate number across years (F = 1.0122, P = 0.3791).

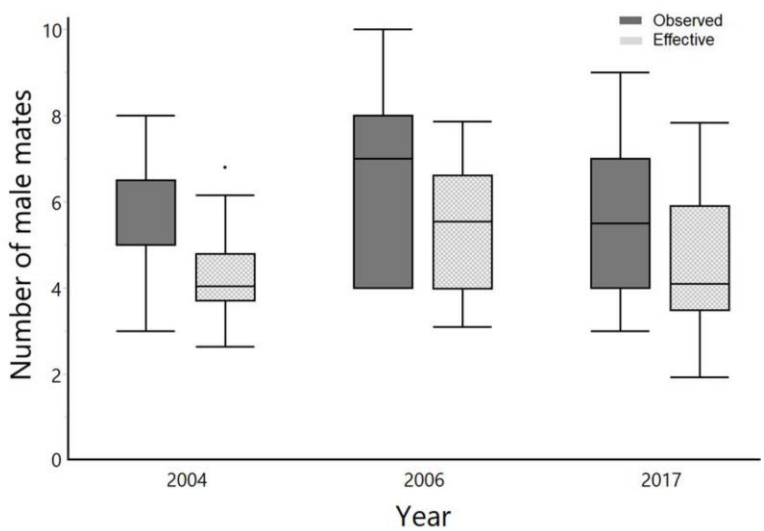

Figure 3-2. Number of male mates (dark gray) and effective number of male mates (light gray) per queen at three time points (N = 13, 11, and 20 colonies, respectively). Boxes display first quartile, median, and third quartile values, whereas whiskers represent values within 1.5X the interquartile range. Neither the raw number of mates nor the effective number of mates differed significantly across years.

Next, we evaluated the distribution of the number of male mates to determine whether the number of mates in each colony was distributed differently among years. For all three pairwise comparisons, we found no significant differences in the distributions of mate number (2004-2006: D = 0.3287, P = 0.5404; 2004-2017: D = 0.2731, P = 0.5995; 2006-2017: D = 0.2455, P = 0.7860).

To further examine the mating and reproductive behavior of *V. maculifrons*, we investigated differences in the effective paternity and paternity skew within the colonies at each time point. We found that there was no significant difference in mean effective paternity across time points (F(2,41) = 1.5632, P = 0.2217, Figure 3-2, Table A-3). In addition, there was no significant paternity skew found at any of the three time points, and no difference in skew across years (F(2,41) = 0.9211, P = 0.4062, Figure 3-3, Table A-3).

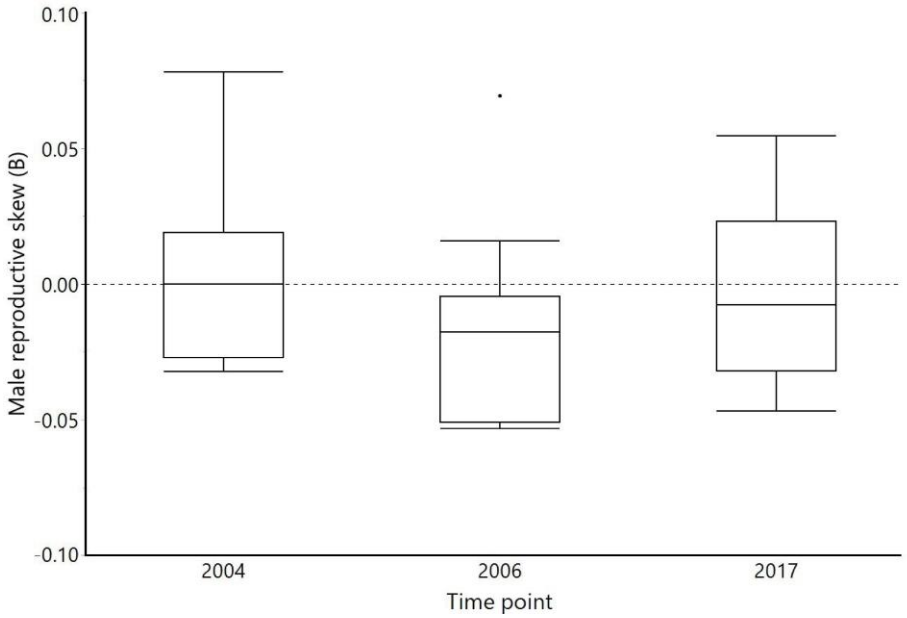

Figure 3-3. Reproductive skew (*B*) of males mated to single queens in *V. maculifrons*. Boxes display first quartile, median, and third quartile values, whereas whiskers represent values within 1.5X the interquartile range. Mating skew did not vary significantly from 0 (dashed line) in any of the three time points, and did not differ significantly across years.

Finally, we estimated relatedness among nestmate workers in *V. maculifrons*. We found mean nestmate worker relatedness was similar in our three timepoints of 2004, 2006, and 2017 (Table 3-3. Relatedness (± S.E.M) between nestmate workers (r_{ww}) and males mated to the same queen (r_{mm}) in *V. maculifrons*. Relatedness estimates did not differ significantly across years., Table A-3). We found that these values did not differ among years (F(2,48) = 2.0095, P = 0.1452). Additionally, relatedness was calculated for males

mating to the same queen within each colony. We found that the male mates of queens showed low, but significant, relatedness to each other (Table 3-3, Table A-3). These values, however, did not differ significantly among years ($F(2,48) = 0.1186$, $P = 0.8884$) and are likely indicative of a very small proportion of males originating from the same parent colony before mating with single queens.

Table 3-3. Relatedness (± S.E.M) between nestmate workers (r_{ww}) and males mated to the same queen (r_{mm}) in *V. maculifrons*. Relatedness estimates did not differ significantly across years.

Relatedness	2004	2006	2017
r_{ww}	0.365 ± 0.014	0.367 ± 0.025	0.396 ± 0.014
r_{mm}	0.061 ± 0.013	0.076 ± 0.013	0.072 ± 0.014

3.5 Discussion

This study investigated genetic and life history changes in a eusocial insect population. We estimated the effective population size (N_e) of a eusocial taxon in order to test if highly social species displayed lower N_e than other species. We also attempted to determine if the female mating system in a eusocial taxon was subject to variation across years, because social and mating systems can have strong effects on N_e. Therefore, we explore the results of these two research questions together to better understand the nature of genetic and behavioral change in social taxa.

3.5.1 Effective population size in eusocial species

We found that the effective population size for *V. maculifrons* ranged in the 100s of individuals (Table 3-2). The only exception was in the 2004-2006 comparisons where

estimates were on the order of just a few 10s of individuals. Additionally, our estimates based on queen genotypes seemed substantially larger than the other estimates. However, the variance in these estimates was also substantially larger, likely due to the small sample size of queens.

We found that the estimates of N_e in *V. maculifrons* were small. Indeed, when examining the results of the queen/male dataset, our results yield an N_e of less than 1,000 across each pairwise comparison. To the best of our knowledge, estimates of the census population size of *V. maculifrons* have not been published. However, this is a common insect and we believe that the effective population size is orders of magnitude smaller than the census population size of this species. This suggests that the ratio of N_e/N for the species is very small.

Populations with relatively low effective population sizes are expected to show high variance in allele frequencies (i.e., higher genetic drift) over time (Nei and Tajima 1981). Moreover, deleterious mutations have the potential to become fixed in populations with low N_e due to the diminished efficacy of selection, particularly in association with other environmental conditions (Ellegren and Galtier 2016; Galtier and Rousselle 2020; Nei and Tajima 1981). However, it is important to note that disparate taxa and species have different conditions under which they thrive, including species with low N_e that are able to maintain their population viability.

Several previous studies have hypothesized that N_e in eusocial insects should be relatively low when compared to other, non-eusocial taxa. For example, Romiguier et al. (2014) found that eusocial insects showed a generally elevated ratio of nonsynonymous to

synonymous changes and reduced genetic polymorphism, as would be expected for species with low N_e. Moreover, this trend was correlated with the level of social complexity. Imrit et al. (2020) also found that social insects displayed patterns of relaxed negative selection, which would be consistent with a low N_e for social species. Investigations into the molecular evolution and genetic diversity of bees (Lozier 2014; Maebe et al. 2019), social spiders (Settepani et al. 2016; Tong et al. 2020), ants (Galtier and Rousselle 2020), and social shrimp (Chak et al. 2020) used different means of estimating genetic variation but also came to the general conclusion that social species may show relatively low N_e (but see Bromham and Leys 2005).

Our method for estimating N_e in *V. maculifrons* relied on measuring changes in allele frequencies between generations in contemporary populations. This technique has been used to successfully estimate N_e in a wide range of non-eusocial, animal taxa including vertebrates such as fish (Francisco and Robalo 2020; Molnar et al. 2020), birds (Olah et al. 2020; Palinkas-Bodzsar et al. 2020), snakes (Wood et al. 2020), and wolves (Jansson et al. 2012), as well as invertebrates such as oysters (Hedgecock and Pan 2021), sea urchins (Calderon et al. 2009), coral (Ledoux et al. 2020), flies (Barker 2011; Bergamo et al. 2020; Echodu et al. 2011; Rasic et al. 2015; Saarman et al. 2017), butterflies (Saarinen et al. 2010), and crickets (Kanuch et al. 2020). Interestingly, these previous investigations in non-eusocial taxa have uncovered estimates of N_e similar to those we uncovered in *V. maculifrons*. As such, it is unclear whether our estimates of N_e in *V. maculifrons* differ substantially from those found in other species, including non-eusocial insects. However, the ratio of N_e/N may differ between eusocial species, such as *V. maculifrons*, and non-eusocial taxa. Thus further empirical estimates of N_e, and N, from other eusocial insects

are needed to test the theoretical arguments surrounding the effects of eusociality on effective population size.

It is also important to consider that disparate analytical techniques can yield varied estimates of N_e and need to be interpreted carefully. We suggest that more studies investigating the effects of sociality on N_e would be of considerable value. Moreover, such studies should discuss the methods used for assessing N_e and compare applicable results. In this way, the existing theoretical and simulation-based models stand to benefit from the addition of more empirical data and analysis from natural populations.

3.5.2 Changes in mating system over time

The principal result from our investigation of polyandry was that all metrics of queen mate number were statistically the same across years. The total number of mates per queen, as well as the effective number of mates per queen, did not differ significantly over time (Figure 3-2). The skew among *V. maculifrons* male mates (Figure 3-3) and the associated relatedness among nestmate workers also did not differ significantly among years. Moreover, these values did not differ from previous investigations of nestmate relatedness in this taxon (Hoffman et al. 2008; Ross 1986).

Thus, we hypothesize that *V. maculifrons* mate number is subject to stabilizing selection in this population (Sutter et al. 2019), but *V. maculifrons* queens are under strong selection to mate multiple times. Indeed, the queens of all species of *Vespula* are known to mate multiply (Foster and Ratnieks 2001; Goodisman et al. 2002; Hoffman et al. 2008; Strassmann 2001; Wenseleers et al. 2005), and observed mate number in *V. maculifrons* in our study was never below three. Previous studies in Vespula have suggested that

polyandry may have positive effects on colony reproductive output (Dobelmann et al. 2017; Goodisman et al. 2007; Loope et al. 2014) and defense against pathogens (Saga et al. 2020). Thus, successful *V. maculifrons* queens engage in behaviors that assure multiple mating, although the selective reasons for why *Vespula* queens mate multiply continue to be investigated.

Skew among the male mates of polyandrous social insect queens is of interest because male skew indicates that some males do better than others in reproductive contests. Moreover, skew is directly related to worker relatedness (Baer 2016; Jacobs and Schrempf 2017; Jaffe 2014; Jaffe et al. 2012). Our results indicate that skew is generally nonsignificant in *V. maculifrons* (Figure 3-3, Table A-3). That is, the male mates of queens divide reproduction more or less equally. This is consistent with hypotheses that highly polyandrous eusocial insect taxa tend to show relatively low skew among male mates (Jaffe et al. 2012).

Our research suggests that the selective and behavioral processes operating on queen mate number in *V. maculifrons* have not changed in our sampled years. This may be the case for several reasons. It is possible that *V. maculifrons* is living in a more or less stable environment and directional selection is not currently operating on any aspects of queen mating behavior (Kraus et al. 2004). *V. maculifrons* is native to the region studied. Thus, mate number may be adapted to the prevailing selective conditions in this population.

Notably, previous investigations in other eusocial insect taxa have found that mate number can vary under different environmental conditions. Abiotic environmental variation such as temperature or rainfall may affect reproductive behaviors in social

arthropods (Aviles and Guevara 2017; Dew et al. 2018; El-Niweiri and Moritz 2011; Field et al. 2010; Groom and Rehan 2018; Purcell 2011; Schurch et al. 2016; Shen et al. 2017). In addition, geographic differences in queen mate number in eusocial bees may arise from environmental differences affecting mating behaviors (Crowther et al. 2019; DeFelice et al. 2015). This may be particularly relevant in cases where invasive and native populations are compared (Delaplane et al. 2015; Inoue et al. 2012; Tsuchida et al. 2019). Notably, however, other studies have failed to find evidence for variation in queen mate number in different geographic regions (Ding et al. 2017; Rattanawannee et al. 2012; Tarpy et al. 2010; Tarpy et al. 2015).

Surprisingly, few studies have investigated variation in queen mate number over time in eusocial species. Studies in bumblebees (Paxton et al. 2001) and honeybees (Jara et al. 2015) failed to find evidence for temporal variation in queen mate number. Although a separate investigation in honeybees did show differences in levels of polyandry in different seasons (Chapman et al. 2019) suggesting that queen mate number may vary in response to seasonal conditions.

3.5.3 Conclusions

In this study, we estimated the effective population size in a eusocial insect. To the best of our knowledge, this is the first estimate of N_e in a eusocial taxon obtained from temporal information. We found that our estimate of N_e in *V. maculifrons* was similar to estimates in other, non-eusocial species. However, we believe that the ratio of N_e/N in eusocial taxa is likely to be considerably lower than in many non-eusocial species. We also found no significant changes to the mating system of *V. maculifrons* queens over time. The

lack of variation in queen mate number across a 13-generation timeframe suggests a possible lack of directional selection acting on the mating system. Importantly, changes in mating system can have strong effects on effective population size. Thus, consideration of the effects of mating system and family structure on N_e may be important. Overall, our results provide novel insights into the evolution of a eusocial species using temporal genetic analyses. However, additional investigations are needed across eusocial and non-eusocial taxa to develop robust comparisons across species. Such future analyses into temporal population genetic variation, as well as further investigation of temporal changes in behavior, will continue to provide substantial insight into the evolutionary processes affecting social systems.

Funding

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CHAPTER 4. GENE DUPLICATION IN THE HONEYBEE: PATTERNS OF DNA METHYLATION, GENE EXPRESSION, AND GENOMIC ENVIRONMENT[3]

4.1 Abstract

Gene duplication serves a critical role in evolutionary adaptation by providing genetic raw material to the genome. The evolution of duplicated genes may be influenced by epigenetic processes such as DNA methylation, which affects gene function in some taxa. However, the manner in which DNA methylation affects duplicated genes is not well understood. We studied duplicated genes of the honeybee *Apis mellifera*, an insect with a highly sophisticated social structure, to investigate whether DNA methylation was associated with gene duplication and genic evolution. We found that levels of gene body methylation were significantly lower in duplicate genes than in single copy genes, implicating a possible role of DNA methylation in post-duplication gene maintenance. Additionally, we discovered associations of gene body methylation with the location, length, and time since divergence of paralogous genes. We also found that divergence in DNA methylation was associated with divergence in gene expression in paralogs, although the relationship was not completely consistent with a direct link between DNA methylation and gene expression. Overall, our results provide further insight into genic methylation and how its association with duplicate genes might facilitate evolutionary processes and adaptation.

[3] Dyson, C. J., & Goodisman, M. A. (2020). Gene duplication in the honeybee: patterns of DNA methylation, gene expression, and genomic environment. *Molecular Biology and Evolution*, *37*(8), 2322-2331. doi:10.1093/molbev/msaa088

4.2 Introduction

Gene duplication is a fundamentally important process that introduces new genetic material into the genome (Conant and Wolfe 2008). New genes generated through duplication events can be expressed in novel ways (Holland 1994; Li, et al. 2018). For example, after duplication, one paralog (duplicate gene copy) can be removed from the selective pressure of its ancestral function and be expressed in a divergent fashion from its sister paralog (Stephens 1951; Nei 1969; Ohno 1970; Otto and Whitton 2000;).

Paralogous genes can evolve in several ways following duplication events. The majority of duplicate gene copies become nonfunctionalized and are removed through negative selection (Lynch and Conery 2000). However, in some circumstances, both paralogs persist. In such cases, one possible outcome is the conservation of ancestral function in both duplicated genes, allowing for amplification of that function through gene dosage effects. Alternatively, a duplicated gene can serve a novel function by gaining a new expression profile. Finally, paralogs may experience subfunctionalization, which requires expression of both copies to fulfill the original ancestral function and level of expression (Ohno 1970; Force, et al. 1999). The distinct fates of duplicate genes provide the mechanisms for paralogs to display distinct expression profiles, allowing for the possibility of adaptation and evolution of organismal function.

One mechanism by which the expression of duplicate genes could be regulated is through the effects of DNA methylation (Berger, et al. 2009). DNA methylation is an heritable epigenetic modification that is found across a wide array of species, including animals, plants, bacteria, and fungi (Suzuki and Bird 2008; Feng, et al. 2010; Niederhuth, et al. 2016; Bewick, et al. 2019a). Associations between DNA methylation and gene expression have been uncovered previously. For example, methylation of certain targeted

genomic regulatory elements, namely the promoter regions of genes, has been causatively associated with a repression of gene expression levels in vertebrate systems (Jones 2012).

The function of methylation of gene bodies, which is the predominant target of DNA methylation in insects, is less clear. Gene body methylation has been proposed to affect gene expression through regulation of transcription elongation and alternative splicing (Bird 2002; Lorincz, et al. 2004; Zilberman and Henikoff 2007; Luco, et al. 2010; Maunakea, et al. 2010; Shukla, et al. 2011). DNA methylation in many insect species is found at much lower overall levels than in vertebrate and plant species, and is mainly localized to CpG dinucleotides in intragenic regions, rather than being found across the genome (Wang, et al. 2006; Zemach, et al. 2010). Additionally, the sparse DNA methylation in holometabolous insects typically targets phylogenetically conserved "housekeeping" genes (Foret, et al. 2009; Elango, et al. 2009; Lyko F. 2010; Sarda, et al. 2012). These methylated genes are associated with constitutive expression across tissues and phenotypes, and they are typically found ubiquitously across species (Hunt, et al. 2010; Bonasio, et al. 2012; Glastad, et al. 2014; but see Bewick et al. 2019a, b). Thus, gene body methylation is generally positively associated with levels of gene expression and negatively associated with gene expression bias. However, the causal connection, if any, between gene expression and gene body methylation remains unclear (Simmen, et al. 1999; Suzuki and Bird 2008; Feng, et al. 2010; Zemach, et al. 2010; Bewick et al. 2019a, b).

The potential effects of DNA methylation on the evolution of duplicate gene function have been investigated previously (Rodin and Riggs 2003; Qian, et al. 2010; Ramirez-Gonzalez, et al. 2018). These prior studies focused on methylation of promoter regions, which has been linked to a downregulation in gene expression in vertebrate systems. Results from these investigations suggest that it would be possible for epigenetic modification to maintain paralog expression after a duplication event, allowing for subsequent functional modification and differential expression. Additionally, previous

work indicates promoter DNA methylation as a causative factor in tissue-specific expression of duplicate genes (Keller and Yi 2014). This suggests that DNA methylation could play a role in evolution of duplicate genes in eukaryotic species.

The study of gene duplication and epigenetics holds particular interest in social insects. Social insects are remarkable because they display a caste system, where members of the same species share a common genotype yet express strikingly different phenotypes (Wheeler 1986; Normark 2003; Simpson, et al. 2011). The caste system arises through phenotypic plasticity, which occurs through flexibility in gene expression and allows for the formation of phenotypically distinct classes within species (Whitman 2009). Social insects represent interesting systems for studying gene duplication, because gene duplication may have important effects on the elaboration of caste systems. For example, selection should favor each caste member to evolve a phenotype in a way most beneficial to its own fitness requirements. However, if castes have different selective requirements for expression at a single locus, genetic conflicts can emerge, which may result in a decreased fitness for both castes (Rice and Chippindale 2001; Bonduriansky and Chenoweth 2009; Connallon and Clark 2011; Pennell and Morrow 2013; Pennell, et al. 2018). The evolutionary constraints arising from this conflict can be mitigated through gene duplication (Ellegren and Parsch 2007; Gallach and Betran 2011). The insertion of novel genetic material into the genome through duplication allows for the evolution of different expression profiles for a single gene copy, providing the means to reach the fitness maxima for each class.

The purpose of this study is to gain a greater understanding of consequences of gene duplication by identifying how evolutionary and epigenetic processes affect duplicate genes. We study this question in the honeybee *Apis mellifera*, which displays a defined caste system where the expression of genes in multiple copy could hold significance to social evolution. We are specifically interested in understanding if DNA methylation is

associated with gene duplication. To examine this issue, we investigate the association between levels of genic methylation and gene duplication status in a variety of genic contexts. Additionally, we investigate divergence in paralogs – from each other and from orthologous gene copies – to provide a framework for understanding the evolution of duplicated genes. Our research provides further insight into questions regarding the nature of DNA methylation in invertebrate systems and the role of gene duplication in a social insect.

4.3 Results

4.3.1 DNA methylation is associated with caste-biased gene expression and gene copy number

We compared the methylation levels of 5235 single-copy genes, 734 duplicated genes, and 271 genes in families of three or larger in the honeybee, *A. mellifera*. We found that the mean percentage of DNA methylation differed significantly among gene family classes. Singletons were significantly more highly methylated than duplicates, which were significantly more highly methylated than genes in larger families ($F(2,6190) = 177.29$, $p < 0.0001$, Figure 4-1, Table B-1).

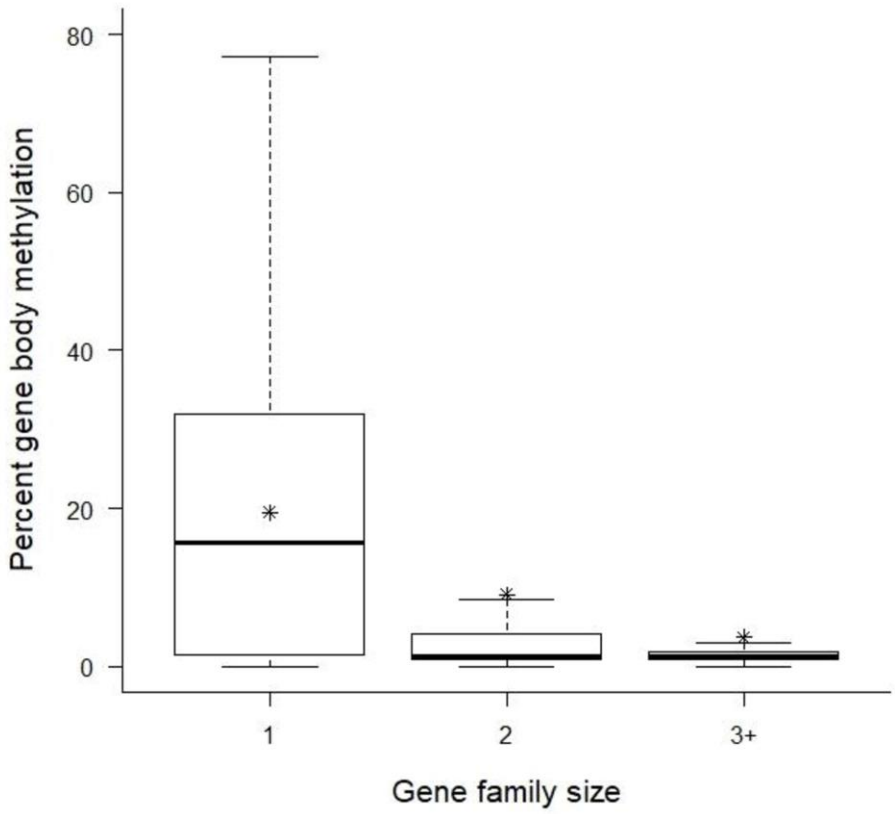

Figure 4-1. Percentage of methylated CpG dinucleotides in gene families of size 1 (singletons), 2 (duplicates), and 3 or more. Box represents first quartile, median, and third quartile values, while whiskers represent values within 1.5 x the interquartile range. Stars represent mean gene body methylation percentage for each group. All three means differed significantly ($p < 0.0001$).

We also investigated associations between gene duplication and DNA methylation using $\chi 2$ tests of independence. We found that the methylation status and the duplication status of paralogs were significantly associated ($\chi 2$ (1, N = 5936) = 396.37, $p < 0.001$, supplemental table S2, S3). Specifically, there were substantially more duplicated genes showing low levels of methylation, and fewer duplicate genes showing high levels of methylation, than expected. This result further indicates that patterns of DNA methylation depend on whether a gene is a duplicate or a singleton.

We determined whether relative divergence (D_r) in methylation between paralogs differed from D_r in methylation between randomly paired "pseudoparalogs." We found that randomly-paired duplicated genes displayed a mean D_r of 0.568 and a standard deviation

of 0.015. Similarly, the mean methylation D_r of randomly paired singletons was 0.549 with a standard deviation of 0.006. In contrast, the mean methylation D_r between actual duplicated genes was 0.242, which fell significantly below both other distributions of D_r (Figure B-1, $p < 0.0001$). This indicates that true paralogs are significantly less diverged in methylation than would be expected for two randomly selected duplicates or singletons.

Finally, we considered associations between gene duplication status, methylation level, and gene expression bias between castes in *A. mellifera*. We found a strong association between caste-biased expression and gene body methylation in *A. mellifera* singletons. Specifically, we found an excess of genes with low methylation levels showing caste-biased expression, and an excess of highly methylated genes showing unbiased expression between castes. However, in duplicated genes, this trend was absent in five of the six analyses (Table 4-1, Table B-4) indicating that the association of gene body methylation with gene expression depends on whether the focal gene is a singleton or a duplicate. Importantly, the observed differences in significance of trends for duplicate and singleton genes were still present even when controlling for sample size differences between singleton and duplicate genes. We further examined the differences in expression bias between singleton and duplicate genes by measuring associations in separately analyzed gene sets consisting of low and high DNA methylation genes. We found that high-methylation genes generally showed a stronger association between copy number and caste-biased expression. In contrast, the association was much weaker and usually non-significant in genes classified as low-methylation (Table B-5). These results, once again, demonstrate differences in the associations between gene expression bias and gene duplication status for genes showing differences in DNA methylation levels.

Table 4-1. χ2 tests of independence between DNA methylation level and caste-biased gene expression for singletons and duplicate genes in six gene expression datasets.

	Singleton		Duplicate	
Dataset	χ^2	p-value	χ^2	p-value
Drone-Queen[a]	309.65	***	2.611	NS
Drone-Worker[a]	413.97	***	15.34	***
Queen-Worker[a]	399.25	***	0.5792	NS
Drone-Queen[b]	304.35	***	0.0084	NS
Drone-Worker[b]	27.85	***	1.660	NS
Queen-Worker[b]	347.96	***	0.078	NS

[a]*Ashby, et al. 2016*

[b]*Vleurinck, et al. 2016*

** p < 0.05; ** p < 0.01; *** p < 0.001; NS = Not significant*

4.3.2 Divergence in gene expression and DNA methylation of paralogs demonstrates evolution of gene function

We next investigated the patterns of DNA methylation and gene expression for pairs of paralogous genes in *A. mellifera* and their corresponding outgroup orthologs in a related bee species, *Ceratina calcarata*. We specifically identified 92 genes that were in single copy in *C. calcarata* but which were duplicated in *A. mellifera*. We then compared patterns of methylation and gene expression between the orthologous *C. calcarata* copy (CcalA) and the paralogous *A. mellifera* duplicates (AmelA1 and AmelA2) in order to gain a greater understanding of how gene methylation and expression evolved over time.

We found that methylation levels were significantly correlated for all three gene copies (CcalA-AmelA1, Spearman $\rho = 0.6390$, $p < 0.0001$; CcalA-AmelA2, Spearman $\rho =$

0.5291, $p < 0.0001$; AmelA2-AmelA1, Spearman $\rho = 0.4995$, $p < 0.0001$). We also found that expression levels were significantly correlated for the *C. calcarata* ortholog and each of the *A. mellifera* paralogs, and marginally correlated for the two *A. mellifera* paralogs themselves (CcalA-AmelA1, Spearman $\rho = 0.2624$, $p = 0.0115$; CcalA-AmelA2, Spearman $\rho = 0.4061$, $p < 0.0001$; AmelA2-AmelA1, Spearman $\rho = 0.1750$, $p = 0.0953$). These results indicated that both levels of expression and levels of methylation were relatively conserved across species.

Additionally, we found that levels of methylation and levels of gene expression were correlated for each of the individual *A. mellifera* gene copies (AmelA1, Spearman $\rho = 0.3142$, $p = 0.0023$; AmelA2, Spearman $\rho = 0.3687$, $p = 0.0003$) but not for the CcalA ortholog (Spearman $\rho = 0.0564$, $p = 0.595$). Notably, however, a significant correlation between methylation and expression in CcalA did emerge when the sample size was increased to include all genes, reinforcing associations found in previous studies (N=11535, Spearman $\rho = 0.1059$, $p < 0.0001$, Rehan, et al. 2016). Consequently, overall, we found evidence for a correlation between gene expression and DNA methylation among gene copies.

We next investigated D_r for methylation and expression in paralog and ortholog comparisons. We found that there was no significant correlation between D_r in methylation and expression between either paralog and the ortholog (AmelA1-CcalA, Spearman $\rho = 0.0201$, $p = 0.8498$; AmelA2-CcalA, Spearman $\rho = 0.0490$, $p = 0.6448$). This indicates that divergence in methylation between the ortholog and paralog was not predictive of divergence in expression between the ortholog and the paralog.

We next considered if D_r in methylation for the *A. mellifera* paralogs was correlated with D_r in expression of the *A. mellifera* paralogs. We found that these measures were significantly correlated (Spearman $\rho = 0.3638$, $p = 0.0004$) indicating that methylation

differences in *A. mellifera* paralogs were positively related to expression differences. We then compared divergence in methylation and expression between species by examining the relationship between D_r in methylation and D_r in expression of the *C. calcarata* ortholog and the mean of the *A. mellifera* paralogs (CcalA – (AmelA1 + AmelA2)/2)/(CcalA + (AmelA1 + AmelA2)/2). We found that D_r of methylation between the ortholog and the mean of the two paralogs was marginally negatively correlated with D_r of expression between the ortholog and the mean of the two paralogs (Spearman ρ = -0.2245, p = 0.0324). Interestingly, however, we found that there was no correlation between the species level D_r estimates and the D_r of the paralogs themselves for either methylation (Spearman ρ = 0.1846, p = 0.0781) or gene expression (Spearman ρ = -0.1486, p = 0.1575), indicating that the intra-paralog divergence in gene expression or DNA methylation was not associated with the *A. mellifera – C. calcarata* divergence.

Finally, we examined the distribution of D_r values for the *C. calcarata* ortholog and the *A. mellifera* paralogs. We found that the distribution of D_r estimates for both DNA methylation and gene expression differed significantly from Gaussian (DNA methylation, μ = -0.0177, σ = 0.3896, W = 0.9704, p = 0.0006, Figure 4-2A; Gene expression, μ = 0.0085, σ = 0.4088, W = 0.9811, p = 0.0137, Figure 4-2B). This result suggested a non-random distribution of relative divergence estimates for the paralogous and orthologous gene copies.

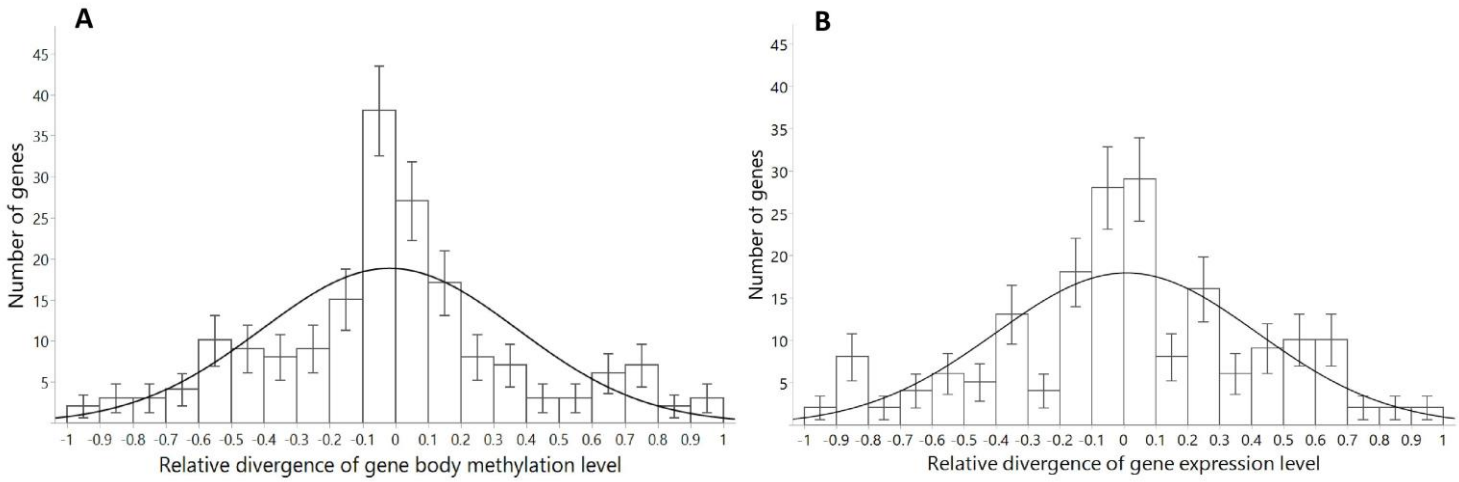

Figure 4-2. Distribution of relative divergence (D_r) values for (A) gene methylation and (B) gene expression between *Apis mellifera* duplicates and their *Ceratina calcarata* singleton orthologs. Overlaid curves estimate normal distribution, and error bars represent standard error of each bin of D_r values. Both distributions of D_r values deviated significantly from Gaussian (methylation, p = 0.0006; expression, p = 0.0137).

4.3.3 DNA methylation varies with location, length, and age of duplicate genes

We next investigated DNA sequence evolution in the context of gene duplication. ANOVA was used to determine the strength of association between sequence divergence, as measured by dS, and the methylation divergence of all paralogs. We found that paralogs with fewer synonymous substitutions per synonymous site, which were putatively younger duplicate pairs, tended to be more divergent in their methylation than older duplicate pairs ($F(2,250) = 19.1613$, $p < 0.0001$, Figure 4-3A). We also found a negative correlation between dS and the overall mean percent methylation of paralogs ($F(2,280) = 5.1602$, $p = 0.0063$, Figure 4-3B). Together, these results indicated that older pairs of duplicate genes tended to have lower and more similar levels of DNA methylation than younger pairs.

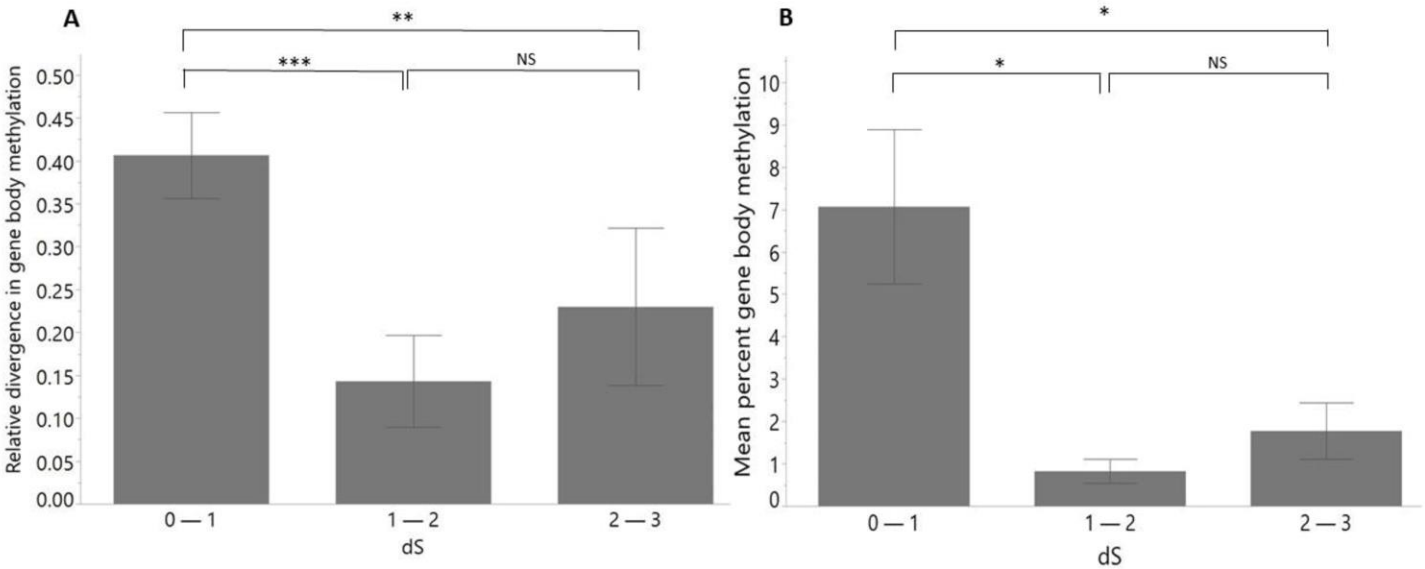

Figure 4-3. (A) Relative divergence in DNA methylation levels for duplicated genes displaying different synonymous substitution rate ratios, indicating that younger duplicate gene pairs have more dissimilar levels of methylation than older pairs. Bars represent mean value for each bin, and error bars represent 95% confidence interval. (B) Mean percent gene body methylation for duplicate genes displaying different synonymous substitution rate ratios, indicating that pairs of genes that duplicated more recently tend to have higher overall methylation levels than pairs of genes that duplicated longer ago. Bars represent mean value for each bin, and error bars represent 95% confidence interval. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; NS = Not significant.

We next tested if the methylation level of genes differed across the 16 chromosomes of the *A. mellifera* genome. We found that genes on 14 of the 16 chromosomes did not differ significantly in their mean methylation. Of the remaining two chromosomes, genes from one chromosome (chromosome 3) did not differ significantly from the means of genes on the 13 other chromosomes. Only genes on chromosome 13 were found to differ significantly from the means of the majority of other chromosomes (Figure B-2).

We examined whether paralogous genes located on the same chromosomes (syntenic) tended to show lower levels of methylation divergence than paralogous genes located on different chromosomes (non-syntenic). We found that non-syntenic paralogs

showed a much higher D_r in their methylation level than syntenic paralogs ($F(1,332) = 85.9705$, $p < 0.0001$, Figure B-3). To further examine whether this association was unique to duplicated genes, we compared the divergence of randomly paired singleton "pseudoparalogs" that were syntenic or non-syntenic. We found that D_r of methylation of these pseudoparalogous singletons did not significantly depend on whether they were found on the same or different chromosomes ($F(1,365) = 0.6990$, $p=0.5480$, Figure 4-4). Thus, the relatively low divergence in methylation of syntenic duplicate genes was not due solely to their residing on the same chromosome.

We also investigated the frequency with which duplicates resided on the same or different chromosome. We found that randomly-paired singletons tended to fall on the same chromosome with a probability of approximately 1 in 16, as expected considering the sixteen linkage groups to which they could belong. True duplicates, however, tended to be collocated on the same chromosome almost 50% of the time (Figure 4-4). A more granular inspection of the influence of chromosomal location on methylation showed that syntenic and non-syntenic singleton pseudoparalogs had roughly the same D_r. True duplicate pairs, on the other hand, showed drastically lower mean D_r levels for pairs on the same chromosome (Figure 4-4).

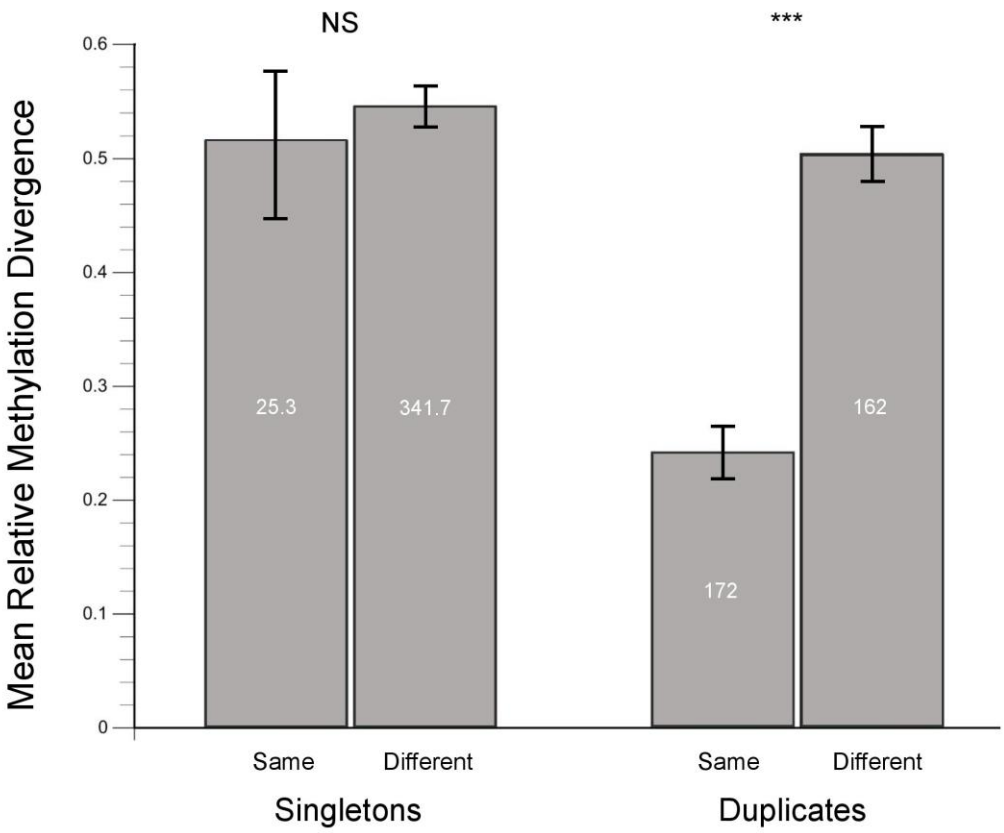

Figure 4-4. Mean relative divergence in methylation for singleton pseudoparalogs (Singletons) and true duplicate gene pairs (Duplicates) on the same- and different-chromosomes. Interior-bar numbers represent number of genes in that category and error bars represent standard error of the mean (mean of 10 trials for Singletons). *** p < 0.0001; NS = not significant.

Finally, we examined whether gene body methylation varied as a factor of gene length in both singletons and duplicated genes. We found no significant difference in the lengths of singletons and duplicated genes ($F(1,5967) = 0.5092$, $p = 0.4755$). Additionally, when examining genes up to 40kb, applicable to over 95% of *A. mellifera* genes examined in this study, we confirmed previously uncovered negative correlations between gene body methylation and gene length in both singletons ($F(3,5037) = 227.3255$, $p < 0.0001$) and duplicate genes ($F(3,662) = 5.6724$, $p = 0.0008$). Interestingly, however, ANOVA analysis of all genes binned by length in bins of 40kb showed that the mean gene body methylation of shorter singleton genes was significantly higher than that of longer singletons (F(7,5225)

= 27.1343, $p < 0.0001$), but this relationship was not significant in duplicate binned genes ($F(7,694) = 1.1707$, $p = 0.3173$, Figure B-4).

4.4 Discussion

4.4.1 DNA methylation is associated with gene copy number in A. mellifera

We analyzed the levels of gene body methylation in singleton and duplicate genes of the honeybee in order to understand whether epigenetic factors may be associated with the fate of paralogs. We found a significant difference between the mean CpG methylation levels of singleton and duplicate genes (Figure 4-1). Singletons consistently had higher levels of gene body methylation than duplicates, which were themselves more highly methylated than genes in larger gene families. This striking result suggests that gene body methylation may be relevant to gene duplication in insects.

Prior studies in methylation of duplicate genes have mainly been limited to vertebrate and plant species. For example, duplicated genes showed higher levels of DNA methylation of promoters than singletons (Chang and Liao 2012; Xu, et al. 2018). These differences in promoter methylation were viewed as potentially indicating that DNA methylation was involved in gene dosage rebalancing after a duplication event. In vertebrate systems, DNA methylation of the promoter region has been causatively linked to decreased gene expression via gene silencing, through which transcriptional regulation of gene dosage can maintain the optimal expression levels of duplicated genes (Weber, et al. 2007; Baylin and Jones 2011). Epigenetic silencing of protein-coding genes through promoter methylation has also been demonstrated in plants and suggested as an advantageous means to regulate expression levels (Lee and Chen 2001). The findings of differences in promoter methylation of duplicate and singleton genes in vertebrates and

plants demonstrate a possible epigenetic role in the regulation of paralogs post-duplication in these taxa.

Chang and Liao (2012) also investigated patterns of gene body methylation in a vertebrate system and, in contrast to our investigation, found higher gene body methylation in duplicates than singletons. This previous study focused on DNA methylation in vertebrate genomes, which show very different methylation patterns than insects. Thus, the contrasting results could arise from the distinct epigenetic systems in the two taxa. Nevertheless, the presence of differential gene body methylation in singleton and duplicate genes of *A. mellifera*, akin to the differential promoter methylation in plant and vertebrate models, supports the idea of gene body methylation being associated with duplicate gene evolution.

The function of gene body methylation, which is the predominant form of methylation found in insects, is poorly understood (Zemach, et al. 2010; Zilberman 2017). It has been suggested that gene body methylation is simply a byproduct of the methylation of other genomic regions, and therefore does not serve a major function (Roudier, et al. 2009; Bewick, et al. 2016; Bewick and Schmitz 2017). Additionally, recent work in insects has shown that gene expression and levels of RNA transcription are not consistently associated with gene body methylation (Bewick, et al. 2019b). However, it has also been proposed that gene body methylation does serve some function in invertebrates, including increasing the accuracy of splicing, providing epigenetic variation to resist selection effects, regulating developmental processes, or controlling erroneous transcription (Lorincz, et al. 2004; Zilberman and Henikoff 2007; Maunakea, et al. 2010; Harris, et al. 2019). Regardless, more research is needed to understand what function gene body methylation serves in invertebrate systems.

We investigated the relative divergence in gene body methylation between paralogs in order to determine whether they were more- or less-similarly methylated than other pairs of genes. Our results indicated that duplicate genes tended to show relatively similar patterns of DNA methylation. This similarity has been shown to exist previously for other epigenetic marks such as histone modifications in yeast, where duplicate genes share more common promoter and open-reading-frame histone code patterns than random singleton pairs (Zou, et al. 2012). Because paralogous genes are – by nature of gene duplication – likely to be more similar in sequence than random pairs of genes, the encoded heritable gene body methylation profile of duplicate genes is also likely to be similar.

Changes to the methylation profile, however, could have consequences on divergence of paralog function. DNA methylation of promoters in vertebrate systems has been suggested to be an important factor in the initial divergence in paralogs through repressive effects on gene expression (Rodin and Riggs 2003; Fang, et al. 2018). Specifically, promoter methylation could mask one paralog from selection by, in essence, turning the gene off. Under this model, the masked paralog could then accumulate mutations advantageous to its functional divergence. However, any functional significance of insect methylation of gene bodies is still unclear, and is associated with increased and constitutive expression rather than the silencing of expression associated with promoter methylation. Thus, the previously-developed models may not directly apply to insect systems and a new interpretation is necessary to understand the methylation of duplicates in other biological systems.

4.4.2 Caste-biased expression of genes depends on gene copy number

Previous studies have shown that singleton genes in *A. mellifera* have lower levels of caste- and tissue-biased expression than duplicate genes (Chau and Goodisman 2017). Moreover, singletons consistently have higher levels of gene body methylation, indicating

that genes with higher DNA methylation levels are more likely to serve as the highly conserved housekeeping genes (Elango 2009; Hunt, et al. 2010; Bonasio, et al. 2012; Glastad, et al. 2013). Our data reinforce these results by demonstrating strong associations between gene duplication status and biased gene expression. In particular, duplicates tended to display biased gene expression between castes.

Additionally, we found a strong association between caste-biased gene expression and DNA methylation for singleton and duplicate genes in *A. mellifera*. This general correlation between gene body methylation and gene expression bias has been uncovered many times previously in insects (Kucharski, et al. 2008; Foret, et al. 2009; Lyko F. 2010; Herb, et al. 2012; Li-Byarlay, et al. 2013; Glastad, et al. 2016; but see Bewick, et al 2019a, b). However, our work investigated the interactions between these previously identified associations within gene body methylation, caste-biased expression, and duplication status. We found that the established associations between caste-biased expression and gene body methylation levels were much stronger in single-copy genes than in duplicated genes (Table 4-1). Indeed, in all but one comparison, there was no statistical significance to the relationship between caste-biased expression and gene body methylation in duplicated genes. In other words, duplicate genes and singletons showed different relationships between gene body methylation and expression bias between castes. This suggests that DNA methylation, while differentially applied to single- and multiple-copy genes, does not play a clear causative role in the caste-biased expression of a gene that has been duplicated.

To further probe the importance of gene body methylation to caste-biased patterns of expression, we removed methylation as an explanatory variable. We determined that the association between duplication status and caste-biased expression was much weaker in genes showing low methylation than in genes with high methylation levels. Duplicated genes tended to show much lower levels of genic methylation on average (Figure 4-1) and, accordingly, also lacked associations between genic methylation and caste-biased

expression (Table 4-1). These analyses demonstrate an association between gene body methylation and biased expression of duplicate genes.

4.4.3 Divergence of paralogs reveals evolution of DNA methylation and gene expression

We compared DNA methylation and gene expression in *A. mellifera* paralogs to single-copy outgroup orthologs from a related bee species, *C. calcarata*. We found that levels of gene expression were correlated to levels of DNA methylation in *A. mellifera*, a result which has been noted previously (e.g., Kucharski, et al. 2008; Foret, et al. 2009; Lyko F. 2010). We also found that levels of DNA methylation for the *C. calcarata* ortholog and *A. mellifera* paralogs were highly correlated. This demonstrates the general stability of gene body DNA methylation patterns across insects (Hunt, et al. 2010; Zemach, et al. 2010; Sarda, et al. 2012; Glastad, et al. 2014). Patterns of gene expression were also highly correlated between the outgroup ortholog and each paralogous gene copy, again indicating general conservation of gene expression patterns over time. Interestingly, however, the gene expression levels were not significantly correlated between the two *A. mellifera* paralogous gene copies. This result suggests that the paralogous gene copies have diverged in levels of expression, presumably as some duplicated genes gain novel expression profiles and functions (Holland 1994; Li, et al. 2018).

Next, we examined the relative divergence in methylation and expression between paralogs and outgroup orthologs to understand if methylation and expression evolution were associated. We found that relative divergence of gene body methylation was correlated with relative divergence of gene expression for the *A. mellifera* paralogs. These results suggest a connection between gene body methylation evolution and gene expression evolution.

To further investigate this connection between DNA methylation and gene expression, we next compared relative divergence of methylation between the *A. mellifera*

paralogs and the outgroup ortholog against relative divergence of gene expression between *A. mellifera* paralogs and the ortholog. We predicted a positive association between these divergence estimates, which would indicate that as methylation divergence increased between orthologous copies so did expression divergence. Surprisingly, however, we uncovered a marginally negative association between these methylation and divergence estimates, suggesting that gene body methylation and gene expression were not necessarily always directly linked (Roudier, et al. 2009; Bewick, et al. 2016; Bewick and Schmitz 2017; Bewick, et al 2019a, b).

Finally, we investigated the distribution of relative divergence estimates between *C. calcarata* orthologs and *A. mellifera* paralogs. We reasoned that the distribution of these estimates should be more or less Gaussian if evolution of gene expression and DNA methylation occurred predominantly through random processes. However, we found that the distributions of relative divergence estimates for both gene expression and DNA methylation differed significantly from a normal distribution. Interestingly, these differences came in the form of an excess of values around the mean, rather than an excess of values in the tails (Figure 4-2). We suggest that the lack of genes at intermediate values could indicate selective effects removing duplicated genes that diverge at marginal levels of methylation or expression. Instead, selection may act to preserve genes showing conserved or extreme patterns of genic methylation or gene expression.

4.4.4 Patterns of DNA methylation between duplicate genes change over time

We investigated whether gene body methylation divergence between paralogs changed over time. We found that putatively younger pairs of duplicate genes generally had higher levels of DNA methylation divergence than older pairs (Figure 4-3). This suggests that the DNA methylation level of a duplicated gene becomes more similar to its sister paralog as the pair ages. This result is surprising, since one might expect patterns of

methylation to diverge as a pair of duplicate genes ages. The finding that gene body methylation levels in *A. mellifera* become more similar as duplicate pairs age (i.e., diverge in DNA sequence) runs contrary to our expectations.

A previous study of human duplicate genes found no significant correlation between gene body methylation divergence and evolutionary time but did uncover a positive association between promoter methylation divergence and evolutionary time (Keller and Yi 2014). Moreover, studies of DNA methylation in *Arabidopsis* demonstrated a positive correlation between gene body methylation divergence and sequence divergence of paralogs (Wang, et al. 2014), possibly indicating the evolution of differential expression between paralogs in *Arabidopsis*. The observed differences in patterns of paralog methylation divergence in different species could reflect the distinct methylation contexts in which they are seen; insect, vertebrate, and plant systems all show differences in levels and targets of DNA methylation, which may affect patterns of DNA methylation divergence.

We also investigated how overall DNA methylation level of paralogs changed over time. We found that older pairs of duplicate genes displayed lower overall levels of gene body methylation. That is, it appears that duplicates lost methylation as they aged. Zhong et al. (2016) demonstrated that promotor methylation decreased with evolutionary time (dS) in zebrafish; in contrast, gene body methylation was significantly higher in older duplicates than in younger duplicates. These differences in methylation patterns between the honeybee and zebrafish could be a result of the different methylation systems in the two taxa. The decrease in overall methylation of duplicates over time could also be a factor in the observed differences in methylation between singleton and duplicate genes. Singleton genes, as defined and measured in this study, are likely to be older than duplicated genes. The interplay between divergence time and DNA methylation adds a

layer of complexity to the system, making it likely that the observed differences result from multiple factors.

4.4.5 Methylation divergence and the genomic location of paralogs

We investigated the methylation levels of genes on the sixteen *A. mellifera* chromosomes to determine whether differential patterns of gene body methylation between chromosomes could explain the methylation divergence between paralogous genes. We found that most chromosomes showed similar levels of DNA methylation, with only one out of sixteen chromosomes showing a significant difference in genic methylation level from the majority of the others (Figure B-2). This suggests that any epigenetic 'chromosomal effect' on DNA methylation of genes, which could differentially affect the function of genes residing on different chromosomes, is limited and does not account for potential divergences in gene body methylation between non-syntenic paralogs (Figure 4-4).

In contrast, we found that duplicated genes did show significantly higher methylation divergence when located on different chromosomes rather than when located on the same chromosome. This result reinforces previous work in *Arabidopsis* and *Oryza* that showed that retrotransposed and dispersed paralogs, which are often found on different chromosomes, had a higher divergence in gene body methylation than tandem duplications, which are typically located on the same chromosome (Wang, et al. 2014; Wang, et al. 2017). In vertebrates, it has been demonstrated that putatively younger duplicate gene pairs (by measure of synonymous substitution rate ratio) are more likely to be syntenic than older duplicate pairs (Rodin, et al. 2005). If younger duplicates in insects also tend to be syntenic, then higher divergence in gene body methylation of non-syntenic paralogs could be indicative of their evolutionary divergence as the pair ages.

4.4.6 Gene length and gene body methylation is associated with gene copy number

We found that genes with a lower genic methylation level were significantly longer than those with higher methylation. Previous studies also demonstrated a negative correlation between gene length and gene body methylation in *A. mellifera* (Zeng and Yi 2010). The novel result in our study, however, is the degree to which this correlation seemingly differed between singletons and duplicated genes (Figure B-4). Duplicated genes did not show a significant correlation between length and level of DNA methylation when long genes were included in the analysis. The correlation was quite strong, however, in singletons. In contrast, when excluding longer genes, we found that the negative correlation between length and methylation was present in all genes, as previously demonstrated (Zeng and Yi 2010).

Previous studies have shown that genes with lower levels of promoter methylation, and subsequently increased transcription, tend to be shorter than genes with higher levels of promoter methylation (Takuno and Gaut 2012; Zhong, et al. 2016). It has been proposed that constitutively expressed and biologically essential housekeeping genes could evolve to be shorter than tissue-biased genes in order to maximize the efficiency of transcription and increase expression (Eisenberg and Levanon 2003; Urrutia and Hurst 2003). *A. mellifera* housekeeping genes are associated with high levels of gene body methylation and moderate to high levels of expression (Elango 2009; Hunt, et al. 2010; Bonasio, et al. 2012; Glastad, et al. 2014). We found that shorter genes are associated with higher levels of gene body methylation, and could therefore be representative of these conserved housekeeping genes. Duplicated genes, conversely, would be associated with divergent function and biased expression, as has been shown in *A. mellifera* previously (Chau and Goodisman 2017).

4.5 Conclusions

This study revealed that patterns of gene body methylation differed between single-copy genes and duplicated genes. We also discovered associations in duplicate gene methylation in other contexts including gene pair divergence time, location, and length. Our work demonstrates the potential of gene body methylation to affect the regulation and evolution of paralogs. Future studies could investigate whether patterns of DNA methylation differ between duplicates and singletons in other taxa that possess different systems of DNA methylation. Similarly, controlled experimental systems allowing more targeted probing into associations between duplicate gene methylation and gene function will be important. Overall, further research on the effects of epigenetic marks in diverse biological systems will be crucial to understanding the evolution of duplicate genes and the mechanisms of gene regulation.

4.6 Materials and Methods

Full details of materials and methods are provided in supplemental information file. Gene family information from OrthoDB v9.1 (Zdobnov, et al. 2017) was used to identify genes that were found in single- and multiple-copy in *A. mellifera*. Following identification of gene families, genes with the lowest pairwise dS value in the family were identified as duplicate pairs within those larger family contexts (Supplemental Materials and Methods 1). Whole-genome bisulfite sequencing data from three studies of DNA methylation in the honeybee (Herb, et al 2012; Lyko F. 2010; Li-Byarlay, et al. 2013) were downloaded from the European Nucleotide Archive and processed (Leinonen, et al. 2010; Andrews 2010; Krueger 2015; Martin 2011; Munoz-Torres, et al. 2010). Trimmed FastQ files were aligned to the indexed reference using Bismark and Bowtie2 (Langmead and Salzberg 2012; Table B-6). Methylation calls from Bismark were imported into SeqMonk (Andrews) and reading frames (probes) were created at the level of individual genes (Elsik, et al. 2014). Sequenced

RNA data was obtained from previous studies that examined gene expression differences in *A. mellifera* between drone, worker, and queen larvae (Ashby, et al. 2016) and drone, worker, and queen pupae (Vleurinck, et al. 2016).

ANOVA was used to determine if the mean methylation level of genes belonging to families of different sizes differed significantly. $\chi 2$ tests of independence were used to test the association between "duplication status" and "methylation status" of duplicated genes (Social Science Statistics, 2018). We then tested if the relative methylation divergence between duplicate gene pairs (Keller and Yi 2014) differed from null expectations using a pooled randomization test (Supplemental Materials and Methods 1). For sequence divergence analyses, multiple protein alignments between duplicate genes were generated using MUSCLE aligner (Edgar 2004). Codon alignments were then created using PAL2NAL (Suyama, et al. 2006) and used to calculate synonymous substitution rate ratios between paralogs using the PAML yn00 package (Yang 2007).

OrthoDB v9.1 (Zdobnov, et al. 2017) was used to identify *A. mellifera* duplicate genes that had single copy orthologs in the bee *Ceratina calcarata* (Supplemental Materials and Methods 1). *C. calcarata* de novo genome, methylome, and transcriptome files were obtained from previous studies (Rehan, et al. 2014; Rehan, et al. 2016). Spearman's rank correlations were used to determine whether relationships existed between the methylation/expression levels of the three orthologous genes and to find correlations between relative divergence of percent genic methylation and levels of expression (Supplemental Materials and Methods 1). The distributions in the relative divergence of methylation and expression between *C. calcarata* and each *A. mellifera* paralog was tested against a normal distribution using a Shapiro – Wilcoxon Goodness of Fit test.

Metadata regarding chromosomal location was extracted from OrthoDB gene annotations for downstream analysis using custom perl scripts. ANOVA was used to

determine whether the 16 *A. mellifera* chromosomes differed in their mean cytosine methylation percentage (Supplemental Materials and Methods 1). ANOVA was used to determine if the divergence of syntenic duplicates differed significantly from the divergence of non-syntenic duplicates. A randomization trial of pooled singletons was used as a control for this analysis. ANOVA was used to test the difference in the mean gene body methylation level of each length bin.

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CHAPTER 5. THE DISTRIBUTION OF DNA METHYLATION ACROSS INSECT CHROMOSOMES

5.1 Abstract

DNA methylation is a widespread epigenetic modification that has the potential to change the manner and degree to which genes are expressed. In insects, DNA methylation is sparse and mainly located in the bodies of genes, rather than being targeted to promoter or intragenic regions as seen in other taxa. However, little is known about the global distribution of methylation across insect genomes. Analysis of levels of methylation across chromosomes could provide further insight into how DNA methylation functions in insect species. Moreover, differences in the patterns of methylation within the genomes of phylogenetically distinct clades could help elucidate the evolution of DNA methylation in insects. Here we examine the distribution of DNA methylation within genomes across several insect species. Our goal is to determine whether patterns of DNA methylation are similar across chromosomes in insect taxa or if global methylation distribution varies phylogenetically. We found that the mean levels of genic methylation differed significantly across chromosomes in all insect species, and that the magnitude of this difference showed a potential phylogenetic signal. We also found that the variance in genic methylation differed across chromosomes in all tested species, however there was no clear patterning phylogenetically for methylation variance. Additionally, we used CpGo/e as a proxy for DNA methylation data to incorporate a larger sample of species and further investigate the distribution of methylation within insects. These analyses resulted in examinations of 25 insect species, out of which all species differed in mean genic CpGo/e across

chromosomes, and 22 out of 25 differed in variance of genic CpGo/e across chromosomes. Our research seeks to provide a deeper understanding of the specific targets and chromosomal distributions of DNA methylation in insects and to yield important insights into the evolution and maintenance of DNA methylation patterns.

5.2 Introduction

Many insect species display a remarkable capacity for the expression of discrete phenotypes from a shared genome (Wheeler 1986; Normark 2003; Simpson, et al. 2011). In some taxa, these differentiated phenotypes – or polyphenisms – enable the development of castes with different morphological and behavioral characteristics (Whitman 2009). While some of the developmental genetic underpinnings of this phenotypic plasticity have been elucidated, the mechanism of control for differential expression and subsequent changes in phenotype remain a field of debate in insect biology.

One area of interest in furthering understanding of the evolution and regulation of phenotypic plasticity is epigenetics. Epigenetic – "above genome" – information is a means of altering the genome without directly changing the encoding of the DNA. As such, the possible implications of epigenetic changes, and particularly DNA methylation, in phenotypic plasticity have been a target of study across taxa.

DNA methylation is a heritable epigenetic mark involving the addition of a methyl group to a nucleotide – typically cytosine – that is found in plants, animals, and bacteria (Suzuki and Bird 2008; Feng, et al. 2010; Niederhuth, et al. 2016; Bewick, et al. 2019a). While epigenetic modifications do not fundamentally change the DNA sequence of the genome, they been found in some taxa to have downstream effects on expression and

sequence evolution. In many vertebrate systems, for example, causative associations have been seen between DNA methylation of targeted genomic features such as promotor regions and the subsequent repression of the expression of genes controlled by those regulatory elements (Jones 2012).

The function of DNA methylation in taxa such as the insects is less clear, as functional analyses have not shown evidence of consistent, causal effects on gene expression or individual development. In general, the overall levels of methylation across insect genomes tend to be much lower than is seen in vertebrate species (Yi 2017). Even within Insecta, cytosine methylation varies widely and without truly consistent phylogenetic patterning. Some orders, such as Diptera, show negligible levels of methylation of the genome, while others such as Blattodea have some of the highest genomic methylation levels. The Blattodean *Reticulitermes virginicus*, however, does not show evidence of significant DNA methylation in its genome (Bewick, et al. 2017).

Additionally, DNA methylation in insects is typically localized to intragenic regions rather than the global distribution seen in animals, and in the context of CpG dinucleotides rather than the other contexts that show evidence of methylation most commonly in plants (Wang, et al. 2006; Zemach, et al. 2010). These patterns in insect methylation also do not correlate with levels of sociality, as might be expected if there was a directive causative mechanism to phenotypic plasticity (Bewick, et al. 2017). Overall, DNA methylation varies across species and taxa within insects, making the drawing of conclusions regarding any possible functional role difficult.

However, many insect species still possess the necessary enzymatic "toolkit" for maintaining historical DNA methylation within the genome, as well as for the *de novo* methylation of new targets (Bewick et al., 2017; Provataris, et al., 2018; Lewis et al., 2020). Alternative hypotheses for the continued presence of DNA methylation in insects posit that it may affect the integrity of transcription enzyme binding and the promotion of alternative gene splicing, although there is evidence that these roles are not consistently found across species (Lorincz, et al. 2004; Zilberman and Henikoff 2007; Maunakea, et al. 2010; Harris, et al. 2019).

High levels of DNA methylation in insects are associated with constitutive expression of highly conserved genes (Foret, et al. 2009; Elango, et al. 2009; Lyko F. 2010; Sarda, et al. 2012). In some systems, correlations have been found wherein differentially methylated genes have been associated with differential expression. However, a true causative link between gene expression and DNA methylation has been disputed in several studies (Roudier, et al. 2009; Bewick, et al. 2016; Bewick and Schmitz 2017). Significantly, some evolutionary targets of DNA methylation such as differentially methylated genes between castes and genes that have been duplicated have lower overall levels of genic methylation indicating the possibility of a historical function for the maintenance of DNA methylation systems in insects (Chau and Goodisman 2017; Dyson and Goodisman 2020).

One key component to furthering our understanding of what role DNA methylation possibly serves in the insect genome is to elucidate the patterns and contexts in which it is consistently found. As previously mentioned, while associations have been discredited in some circumstances such as phylogenetic clade, caste differentiation, and level of sociality,

other contexts such as gene length and gene family size do show promising results. Therefore, established correlations in genic methylation in insects lead us to hypothesize that DNA methylation could be targeted to genes mechanistically and in broader genetic contexts, rather than based strictly on the functional role of the gene. That is, factors such as age, orthology, and location of genes represent intriguing areas of research into potential roles of gene body methylation in the absence of clear expression-based causation.

In order to further examine patterns of DNA methylation distribution in insect genomes, we investigated differences in levels of DNA methylation across chromosomes within insect species. Chromosomes are structures located within the nucleus of cells of plants and animals that contain the genetic code along with its epigenetic modifications. Different chromosomes, or even different regions within each chromosome, can contain vastly different sets of genes whose expression can functionally effect phenotype in different ways (Devbhandari, S., et al. 2017; Kurat, C. F., et al. 2017; Jørgensen, S., et al. 2013; Sexton and Cavalli 2015). Because of this, the presence and patterning of epigenetic modifications across the landscape of chromosomes could indicate differential functional effects.

We expect that if DNA methylation was non-functional in insects, it would present a relatively uniform distribution across linkage groups. As chromatin environment varies across the chromosomes comprising the genome, differences in chromosomal methylation levels could intimate differences in gene content or other chromosomal features. Overall, our goal for this study is investigating at a more granular level how DNA methylation is distributed within insect genomes. Due to the high variation in levels of cytosine methylation across Insecta, it has proved difficult to ascertain patterns that could further

our understanding of any functional role DNA methylation serves in insects. By examining dispersal patterns within single species, we hope to add to the knowledge of insect genic methylation by identifying how methylation varies across chromatin environments.

5.3 Methods

5.3.1 DNA methylation analyses

We identified methylomes from whole-genome bisulfite sequencing experiments from 7 insect species representing three orders: Hymenoptera (*Apis mellifera, Bombus terrestris, Solenopsis invicta, Cerapachys biroi, Nasonia vitripennis*), Hemiptera (*Acyrthosiphon pisum*), and Lepidoptera (*Bombyx mori*).

Chromosome-level genome assemblies with high quality gene annotation datasets were identified for each of the 7 species for more granular analysis of DNA methylation patterning. These 7 species represented all published insects with all three datasets (methylome, chromosome-level genome, and gene annotation) available. BS-seq data were aligned to genomes using Bismark with the ambiguous filter, in which reads that fail to align uniquely or align multiple times with the same alignment score were discarded. This was to avoid biasing of the data from gene duplications, which have been shown in social insects to have associations with levels of DNA methylation (Dyson and Goodisman 2020).

DNA methylation alignments were visualized and filtered using SeqMonk. Probes were created at the gene level using aforementioned gene annotation sets. Genic methylation percentage levels were extracted by calculating the number of cytosines labeled as “methylated” in the context of total cytosine number within the gene. Genes

were then assigned to chromosomes based on annotation dataset information to determine chromosomal methylation levels.

5.3.2 DNA methylation of chromosomes

We analyzed the distribution of DNA methylation across chromosomes within each species by using ANOVA to test for differences in the mean methylation levels of genes contained within the chromosomes. These p-values were transformed as -log(p) to find the relative degree of significance across species. The magnitude of how strongly the methylation of chromosomes differed in each species was assessed for phylogenetic patterning using ANOVA and two sample t-tests.

To test the variance in the genic methylation levels of chromosomes, we used a Levene's Test for Equality of Variances to determine whether there was homogeneity in the variation of the DNA methylation of genes across chromosomes for each species. The Coefficient of Variation (CV) was used to compare levels of variance across chromosomes and species. This value is representative of the relative dispersion of genic methylation values around each chromosomal mean. CV is calculated as: σ / μ, or the standard deviation divided by the mean for each population.

5.3.3 Analysis of CpGo/e

In order to expand the phylogenetic scope of the analyses, we used CpGo/e as a metric for estimating DNA methylation in the original 7 species as well as in 18 additional insect species. Methylated cytosines found in a cytosine-guanine context (CpG) within the genome are highly prone to undergoing deamination reactions, which remove an amino

group from 5-methylcytosine resulting in a thymine residue (Bhutani, et al. 2011). Because of the frequency of the occurrence of this transformation reaction, levels of DNA methylation within the genome can be estimated based on the depletion of CpG dinucleotides within the DNA sequence, without the need for methylome data from bisulfite sequencing.

This is most commonly calculated as simply the ratio [$CpG_{Observed}$ / $CpG_{Expected}$], where CpG_O is the frequency of CG dinucleotides within the genome and CpG_E is the product of the frequencies of cytosine and guanine nucleotides to provide an estimate of the expected frequency of the dinucleotide pair. Often, this ratio is then adjusted to account for L, the length of the sequence being analyzed. Through the measurement of this C to T transformation based on CpG frequency ratio, it is possible to ascertain an estimate of historical DNA methylation within a genome using only the sequence of the genome itself (Yi and Goodisman 2009).

We identified all insect species with publicly available chromosome level genomes and high-quality gene annotations that could be used in CpGo/e analyses. In addition to the 7 species used in the initial methylation analyses, this included 3 Hemipterans (*Apolygus locorum, Nilaparvata lugens, Rhopalosiphum maidis*), 6 Hymenopterans (*Aphidius gifuensis, Belocnema treatae, Monomorium pharaonic, Vespula germanica, Vespula pennsylvanica, Vespula vulgaris*), 8 Lepidopterans (*Zerene cesonia, Danaus plexippus plexippus, Manduca sexta, Aricia agestis, Pararge aegeria, Spodoptera frugiperda, Spodoptera litura, Trichoplusia ni*), and 1 Dipteran, *Drosophila melanogaster*, which was included as a control group due to its established lack of significant DNA methylation across the genome.

We used Notos to calculate the CpGo/e ratios of each gene from each chromosome within the genomes of 25 insect species from 4 orders (Bulla, et al. 2018). In order to confirm the association between quantifiable DNA methylation from bisulfite sequencing data and calculations of CpGo/e, we used the 7 species from initial DNA methylation analyses to test for the correlation. We expected to see a negative correlation between "methylation percentage" and "CpGo/e" of genes, as a higher level of DNA methylation would correspond to more depletion of CG dinucleotides within the genome, and therefore a lower CpGo/e ratio.

To determine differences in mean chromosomal methylation from the expanded sample space, we used ANOVA and a Welch's test to determine significance of differences across chromosome mean CpGo/e levels while allowing for differences in the standard deviation of CpGo/e between chromosomes. Additionally, we used a Levene's test for equality of variances in chromosomal CpGo/e across linkage groups.

5.4 Results

5.4.1 DNA methylation of chromosomes

We calculated the levels of genic DNA methylation in 7 insect species. The resulting percentages of cytosine methylation reflect what has been found previously in these species (Figure 5-1). We analyzed the distribution of DNA methylation across chromosomes within each species by using ANOVA to test for differences in the mean methylation levels of genes contained within the chromosomes. We found that in all 7 species, differences in mean genic methylation level of chromosomes were highly significant (Table 5-1, Figure 5-2).

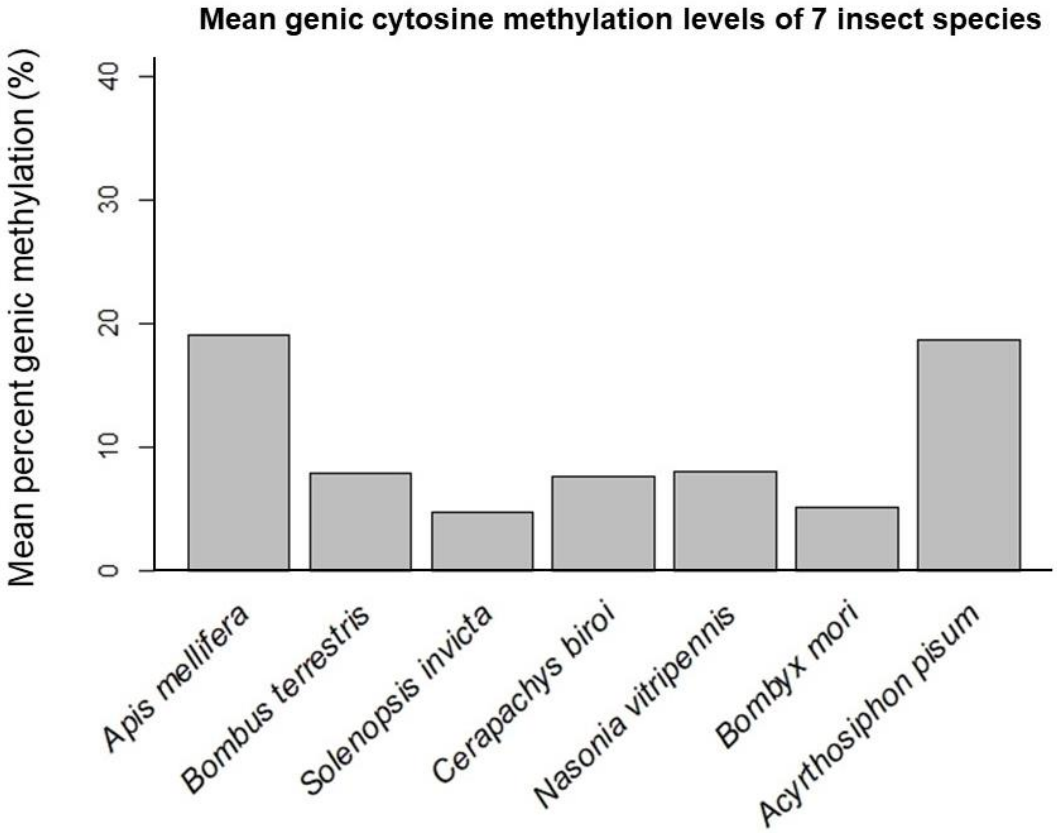

Figure 5-1. Mean genic DNA methylation level in 7 insect species analyzed. Levels are expressed as percentage of cytosines methylated within genes.

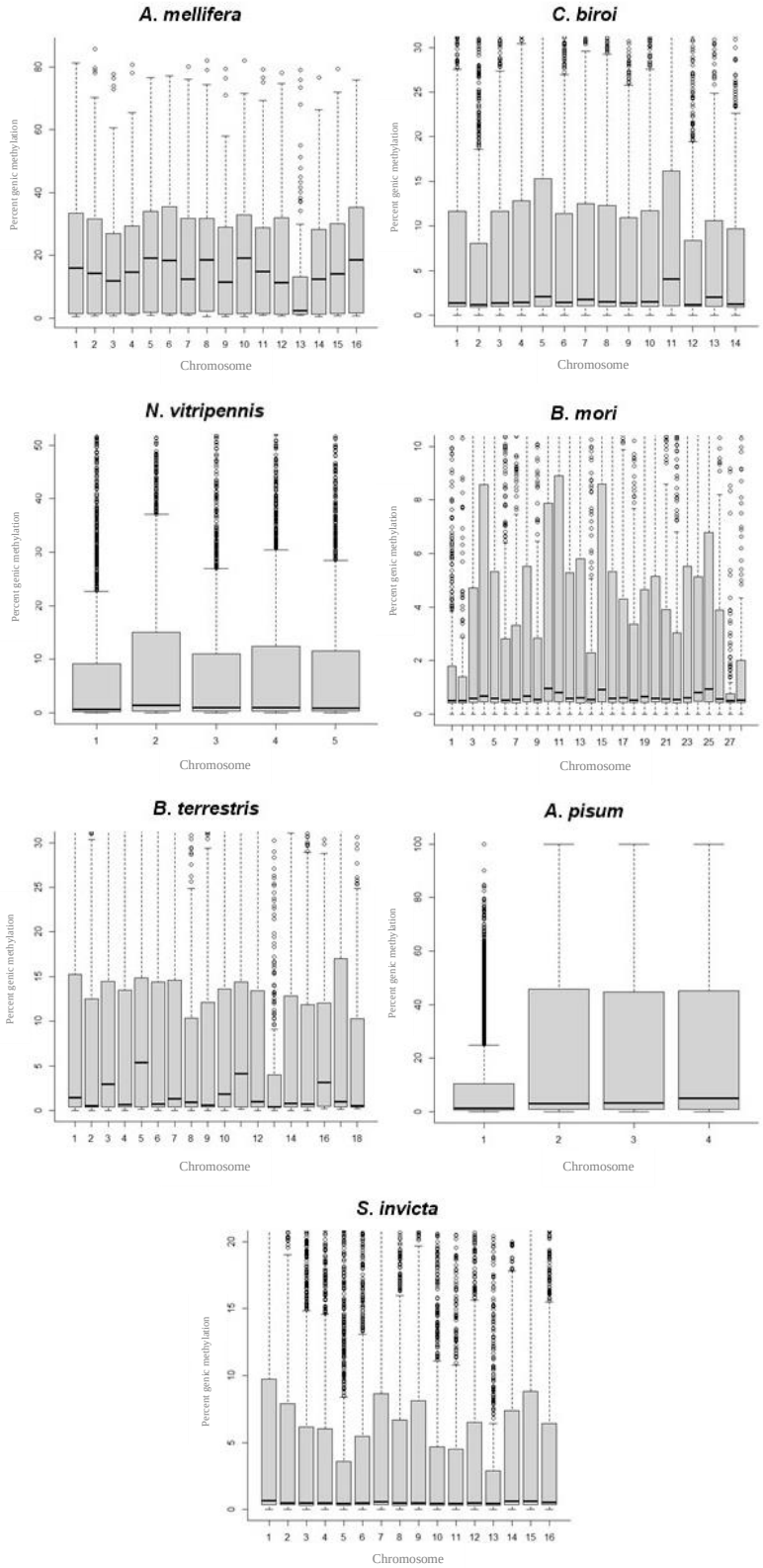

Figure 5-2. Distribution of cytosine methylation of genes in 7 insect species across chromosomes.

When standardized significance values from differences in mean chromosomal methylation level were compared phylogenetically, a rudimentary pattern emerged from this small sample in which the order Hymenoptera clustered with lower -log(p) values (i.e. more similar methylation levels of chromosomes within each species) while more distant orders Lepidoptera and Hemiptera had higher values of -log(p) (i.e. less similar methylation levels of chromosomes, Figure 5-3). These differences between the orders were significant using ANOVA ($F(2,7) = 1504.87$, $P < 0.0001$).

Table 5-1. Genic methylation analysis of differences between chromosomes in 7 insect species.

Species	Order	Chrom count	Gene count	ANOVA F	ANOVA P	-log(p)	Levene's F	Levene's P	CV
Acyrthosiphon pisum	Hemiptera	4	11196	228.49	6.90E-144	143.20	990.00	0	139.36
Bombyx mori	Lepidoptera	28	8326	3.25	2.61E-08	7.85	7.50	2.05E-28	224.04
Apis mellifera	Hymenoptera	16	9060	8.29	3.89E-19	18.41	17.23	1.13E-45	143.82
Bombus terrestris	Hymenoptera	18	5808	4.37	4.61E-09	8.34	8.51	5.62E-22	173.60
Solenopsis invicta	Hymenoptera	16	9400	3.36	1.05E-05	4.98	8.05	1.88E-18	215.90
Cerapachys biroi	Hymenoptera	14	9432	6.39	3.42E-12	11.47	11.56	2.99E-25	153.97
Nasonia vitripennis	Hymenoptera	5	5100	14.14	3.56E-09	8.45	22.58	1.63E-14	157.65

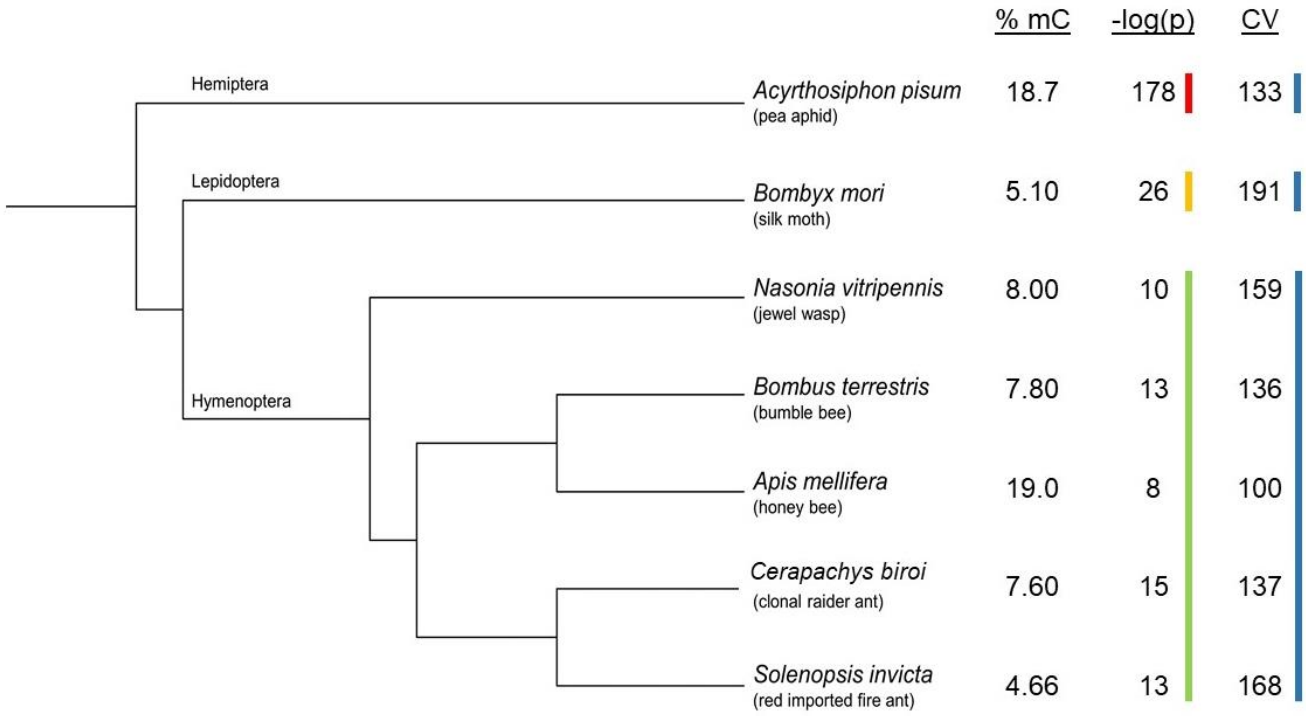

Figure 5-3. Patterns of DNA methylation across chromosomes in seven insect species from 3 orders. A weak phylogenetic pattern emerged in the level of significance of mean genic methylation differences across chromosomes, but this signal was not found in the variation of genic methylation across chromosomes. % mC: percentage of cytosines within genes methylated for each species, -log(p): transformed p-value from ANOVA of mean genic methylation of chromosomes within species, CV: Coefficient of Variation for dispersion of genic methylation levels of chromosomes. Presence of different colors beside taxa indicate taxon means that differ significantly from other groups.

In order to test the variance in the genic methylation levels of chromosomes, a Levene's Test for Equality of Variances was used. We found that, in all 7 species tested, there were significant differences in the dispersion of genes around the mean of each chromosome (Table 5-1).

The Coefficient of Variation (CV) was calculated to compare levels of variance across chromosomes and species. When the variance in chromosomal methylation was

compared across the phylogeny, we did not detect the same patterning across orders as had been seen in mean methylation differences (ANOVA: ($F(2,7) = 1.7137$, $P = 0.29$).

5.4.2 CpGo/e analyses

We found that in all species with available bisulfite-sequencing DNA methylation data, there was a negative correlation between cytosine methylation and CpGo/e for each gene (Figure 5-4, Figure C-1). While the correlation is not expected to be directly linear at more extreme levels of DNA methylation due to the precision of CpGo/e calculation, a linear fit showing the general correlation was highly significant ($P < 0.001$), confirming the suitability of CpGo/e data for use in these analyses.

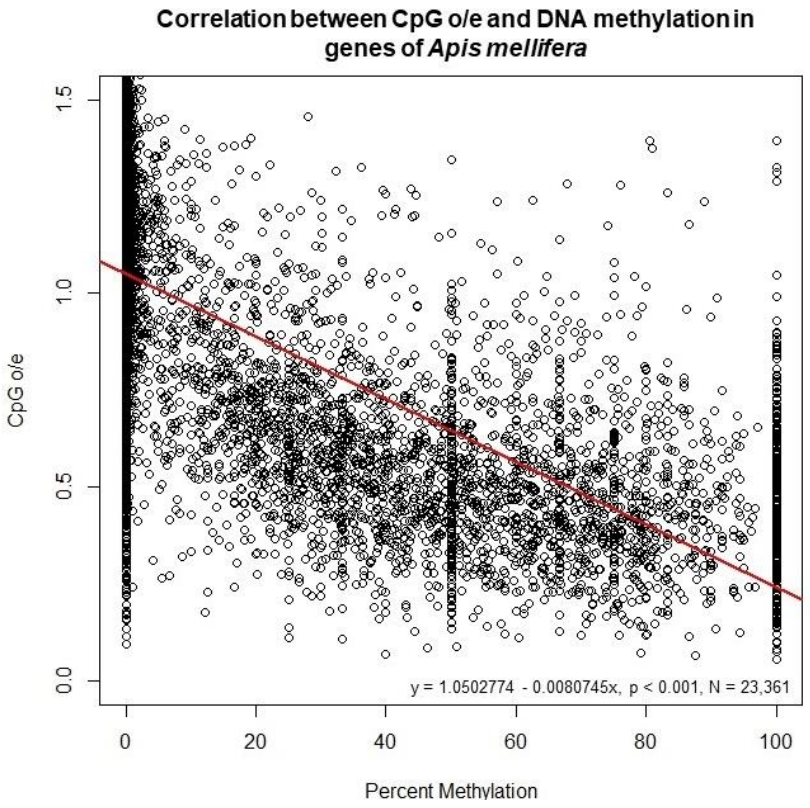

Figure 5-4. Negative correlation between DNA methylation and CpGo/e of genes in *Apis mellifera*. This relationship allows the use of CpGo/e as a proxy for DNA methylation in the absence of methylome sequencing. Inset equation for linear fit shows significance of correlation.

We analyzed the CpGo/e levels of genes within chromosomes of 25 insect species, including the 7 used in the preliminary DNA methylation analyses (Figure 5-5). We used a Welch's test to determine significance of differences across chromosome mean CpGo/e levels while allowing for differences in the standard deviation of CpGo/e between chromosomes. We found that in 24 out of 25 species, there was a significant difference in the mean CpGo/e (and putatively, cytosine methylation) across chromosomes, while *A. pisum* showed differences that were, ultimately, insignificant (P = 0.0716).

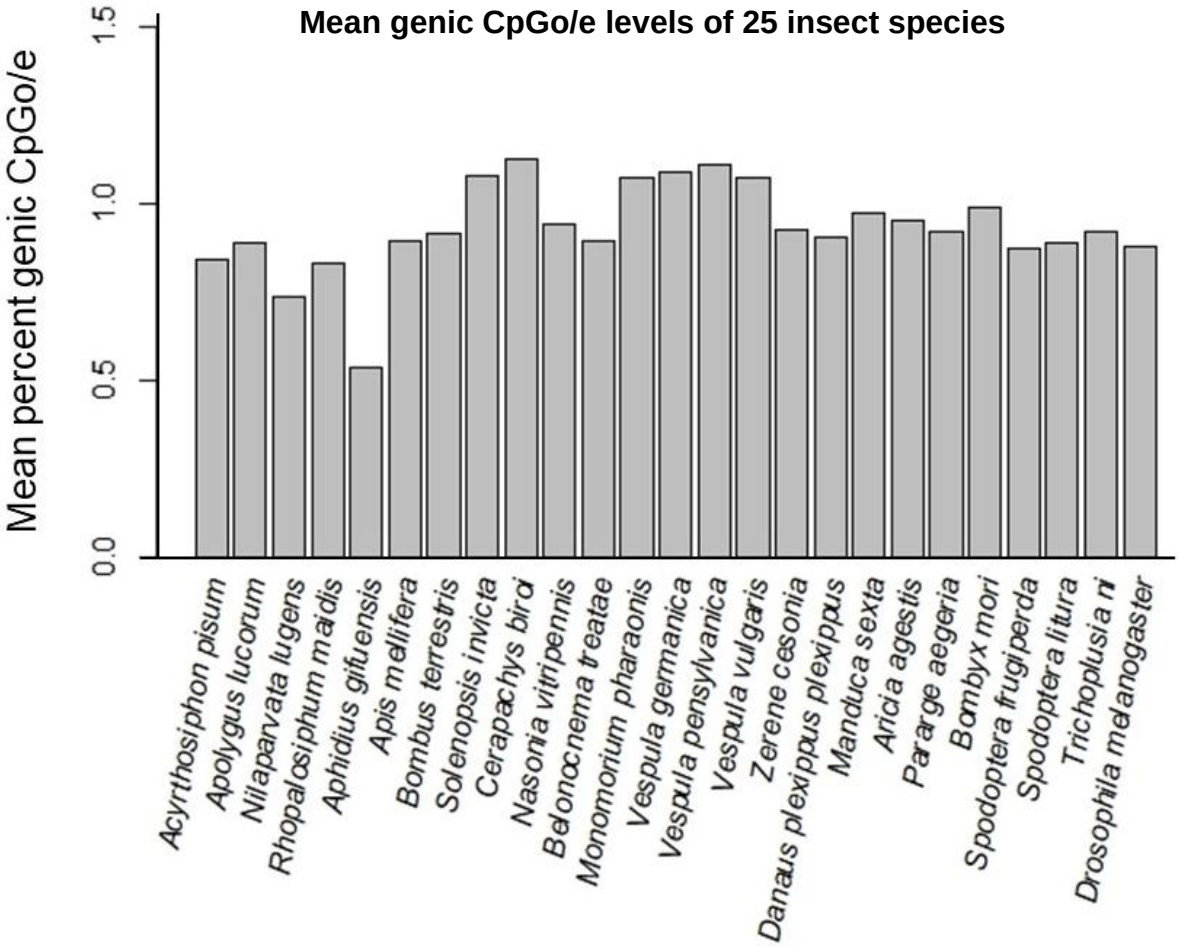

Figure 5-5. Mean CpGo/e of genes in 25 insect species analyzed.

Additionally, we used a Levene's test for equality of variances in chromosomal CpGo/e across linkage groups and found that most species showed significant differences in CpGo/e variances between chromosomes. However, 2 species (*A. pisum and A. gifuensis*) representing two orders did not show differences in variance levels. This indicates that in these two species the levels of methylation were distributed consistently across chromosomes, while in the majority of insects examined methylation was not evenly distributed with some chromosomes having large ranges of CpGo/e and others having tighter spreads.

5.5 Discussion

5.5.1 Variation in DNA methylation distribution within insect genomes

Our goal for this study was to examine DNA methylation within insect genomes and to determine whether the distribution of methylation levels in genes was similar across chromosomes. Due to the presence of differing chromatin environments and gene clusters, we expected that a hypothesized system of DNA methylation in insects with no function or role in the genome would lead to a more-or-less uniform distribution of the epigenetic mark across chromosomes.

Instead, when examining distributions of DNA methylation, we found differences in the mean genic methylation level of chromosomes in all seven species tested. This intimates a non-random distribution of genic methylation throughout the genome. Additionally, the degree to which genic methylation differed across chromosomes within insect species seemed to show a rudimentary correlation with insect phylogeny. However, it should be noted that the result was statistically significant in every species tested, and

conclusions drawn from the magnitude of significance values can be difficult to rely on. Regardless, the possibility of non-uniform distributions of DNA methylation sorting phylogenetically are promising to future projects that have targets resulting in higher precision from which further conclusions can be drawn.

We next investigated whether the degree of dispersion of genic methylation levels differed across chromosomes. That is, does a genome contain one or more chromosomes with relatively tight spreads of methylation levels (i.e. similar levels of methylation in genes on that chromosome) while others have a wider spread that could indicate a more stochastic distribution. Assuming uniformity of distribution, we would expect to find that the variance of DNA methylation of genes on each chromosome would not be significantly different within a species. Instead, we found that all species showed significant differences between chromosomes in the dispersion of genic methylation. This indicated that at least one chromosome within the genome of each insect species showed a different degree of dispersion in DNA methylation levels from the rest of the chromosomes. This is significant in that some types of genes have shown associations with distinct levels of DNA methylation, such as the higher levels seen in constitutively expressed "housekeeping" genes, and their localization could be of import to the distribution of DNA methylation in the genome (Foret, et al. 2009; Elango, et al. 2009; Lyko F. 2010; Sarda, et al. 2012). Additionally, genes that have been duplicated have been shown to generally have lower levels of methylation than singleton genes and have a propensity to be located within the same linkage group as their sister paralog(s) (Dyson and Goodisman, 2020). The unequal variation in DNA methylation levels across chromosomes could be representative of these models and show how clusters of genes or regulatory elements in different linkage group

affect genome evolution. However, variance of DNA methylation on chromosomes did not show the same rudimentary phylogenetic pattern as seen in mean genic methylation level, indicating that this mechanism of distribution is universal across the sampled species.

Previous studies in other systems have investigated the effect of the chromatin environment on the functionality of DNA methylation and shown evidence that epigenetic marks do vary among chromosomes and across different areas within chromosomes (Duncan, et al. 2020). A significant point of interest has been the way that DNA is "packaged" within the nucleus and how tightly packed and difficult to access heterochromatin differs as an epigenetic target from the related euchromatin, which is more easily transcribed and typically contains more genes. Additionally, DNA methylation has been shown to serve a crucial role in the sex-specific expression by differential methylation of sex chromosomes, which can contribute to dosage compensation effects in many systems (Mathers, et al., 2019). Another key region of the chromosome, the telomere, has been shown in humans to be lacking in DNA methylation relative to other areas within the linkage group (Toubiana, et al. 2020). However, those same studies showed evidence that the promotor regions immediately flanking the telomere were typically highly methylated and levels were correlated with the length of the telomere. While the system in which this result was seen differs significantly from the context of insect methylation, it is indicative of the possible role that DNA methylation distribution can serve in the maintenance and evolution of the chromosome.

These preliminary results, albeit from a relatively small sample size of species, indicate that genic methylation is not uniformly distributed across the chromosomes of insect genomes. Instead, imbalances in genic methylation levels potentially indicate that

targets of genic methylation are related to the chromatin environment in which genes are found. While difficult to ascertain the determination of these targets without a broader knowledge of any putative function of DNA methylation in insects, further investigation into the chromatin environments and specific gene content of chromosomes with genic methylation levels most aberrant from the genome mean is merited.

5.5.2 Distribution of genic CpGo/e levels across insect chromosomes

Due to the relatively small number of insect species available with high quality methylomic, genomic, and gene annotation data, we used CpGo/e to expand our analyses to a larger sample of insect taxa. We found that in 24 of 25 species investigated, there were significant differences in the mean levels of genic methylation across chromosomes. *Acyrthrosiphon pisum*, however, was marginally non-significant in this statistic ($P = 0.072$), which was surprising as it contradicts the result based on quantitative bisulfite-sequencing data obtained previously and possibly casts doubt on the suitability of using CpGo/e as a proxy for DNA methylation in these analyses. However, because CpGo/e is calculated strictly off of the genome sequence itself and is then used to make biological assumptions about epigenetic information, the quality of the genome is of utmost importance to these precise calculations. Therefore, it is possible that the particular assembly used for analysis of *A. pisum* was not suitable for CpGo/e calculation due to its higher number of gaps and unresolved bases.

Additionally, in examining the variance in genic methylation across chromosomes, we found that 22 of 25 species showed significant differences in genic methylation dispersion. This included 2 newly added species, *Aphidius gifuensis* and *Drosophila*

melanogaster, as well as *A. pisum*, again contradicting previous results from this species. This result is intriguing in that it is the first evidence from our study (aside from *A. pisum* which could perhaps be explained by the quality of the genome files) suggesting an insect species with a similar variance dispersion of genic methylation levels across its chromosomes. *D. melanogaster* has been shown to be lacking in significant DNA methylation of its genome, and as such non-significant differences in dispersion of methylation are not unexpected (Lyko et al., 2000). *A. gifuensis*, however, is a Hymenopteran insect with low levels of DNA methylation present in its genome (Bewick et al., 2017). It therefore is the only representative with established DNA methylation from the insect cohort studied here that showed a lack of significant variation differences across chromosomes. Future comparative studies into the divergence of results from this insect species as compared to the other analyzed species could provide substantial insight into the mechanistic distribution of DNA methylation in insects.

5.5.3 Precision in CpGo/e analyses

A particularly intriguing result arose from these preliminary CpGo/e analyses, which has served to give some pause and possibly merits further inspection into these techniques. *Drosophila melanogaster*, which lacks key components of the DNA methylation enzymatic toolkit to develop and maintain cytosine methylation within the genome, is often used as a control group in insect methylation studies due to this absence of significant, detectable methylation (Lyko et al., 2000). Indeed, we found using CpGo/e analyses that methylation levels across the two sex chromosomes (X, Y) and three autosomes (2, 3, and 4) in *D. melanogaster* were 0.93, 0.96, 0.88, 0.88, and 0.91, respectively. These CpG ratios are similar to what has been seen previously in this system

with no significant functional DNA methylation (Hunt et al., 2010). However, we also found that these very small differences between chromosomes were significant, which raises an issue wherein the "lack of DNA methylation" within this control species was different across chromosomes as measured by CpGo/e, which was not expected (Figure 5-6). While this could be an issue of the sample size (*D. melanogaster* has only 3 autosomes that were used in preliminary analysis), it could also be an indication of a limitation in the methodology.

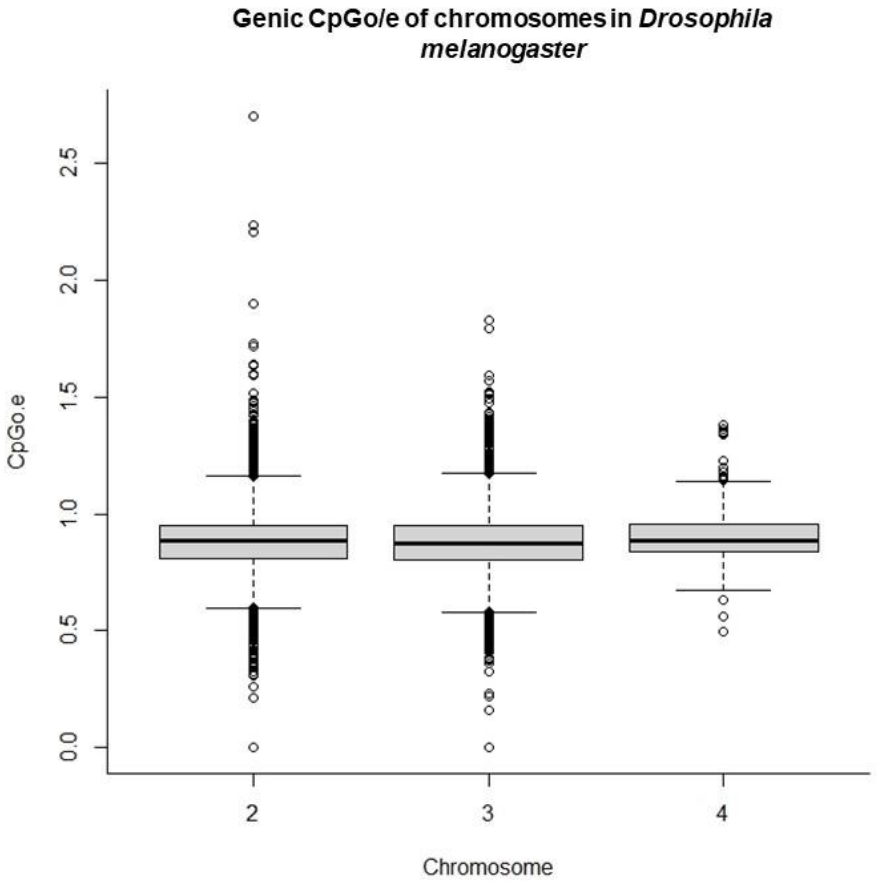

Figure 5-6. CpGo/e of genes in autosomes of *Drosophila melanogaster*. Differences in mean levels of genic methylation across chromosomes were found to be significant (P < 0.001).

The small differences in $CpG_{Observed}$ and $CpG_{Expected}$ potentially expose a flaw in the confidence of using CpGo/e for more precise calculations. While the accumulation of deamination reactions is typically thought to be the main culprit of CpG suppression within the genome, in systems where the number of CpG dinucleotides is reduced by other, non-methyltransferase facilitated means, it could be more difficult to align CpGo/e data with more robust techniques of DNA methylation quantification. These non-methylation decreases in CpG could occur through spontaneous mutations from transposable element movement or from an accumulation of random mutations within the genome, as has been shown to be beneficial in several RNA virus systems (Takata, et al. 2017). Alternatively, isochores, or long stretches of DNA sequence in a genome that show a uniformity of GC content, could potentially bias estimations that are based strictly on calculating nucleotide frequencies due to differences in mutation and recombination rates that could arise within these units. While such CpG suppressions are also occurring within systems that do have a robust system of DNA methylation, it is possible that their effect is small enough to amount to mere "noise" in the context of deamination CpG ratio effects. As such, it is difficult to draw conclusions from CpGo/e data due to the amount of "noise" that exists, particularly in taxa and genes with very low levels of methylation.

5.6 Conclusions

We examined the distribution of DNA methylation across chromosomes in 7 insect species with available sequencing data and found that mean chromosomal levels of genic methylation differed significantly in all species. Additionally, all 7 species showed differences between chromosomes in the dispersion of genic methylation levels, indicating that some chromosomal units had more closely clustered levels of DNA methylation of

their genes. As chromosomes could have different characteristics and contain genes with divergent functions, a non-uniform distribution of genic DNA methylation could be indicative of a significance to methylation in the insect genome that is based on targeted types or clusters of genes, rather than strictly genes with particular functional roles. A further examination of target genes that are found on chromosomes most highly deviated from the global genic methylation mean could serve to cast additional light on the subject of insect epigenetics. Additionally, investigations into the orthology across species of such aberrant chromosomes or the genes they contain could provide insight into a possible functional history of DNA methylation within Insecta.

CHAPTER 6. CONCLUSIONS

This thesis consists of four studies that expand our understanding of phenotypic plasticity in eusocial insects. Chapters two and three examine plasticity in two social wasps, *Vespula squamosa* and *Vespula maculifrons* at the colony level in response to evolutionary time and changes to environmental conditions. Chapters four and five focus on molecular mechanisms of plasticity by examining DNA methylation patterns in duplicated genes in *Apis mellifera* and across chromosomes within eusocial insects.

Shifts in social form from monogyne colonies with an annual life cycle to a polygyne system that can persist for multiple years mark a striking example of behavioral plasticity in response to abiotic pressures. In chapter two, *Social structure of perennial Vespula squamosa wasp colonies*, we examined this occurrence in perennial colonies of the southern yellowjacket *Vespula squamosa*. We found that every *V. squamosa* perennial colony was headed by multiple queens as evidenced by the genotypes of sampled workers, gynes, and males, indicating a biological shift from monogyny to polygyny in this eusocial species. This variation in colony queen number represents a change in the social phenotype of this social insect (Crozier & Pamilo, 1996; Keller, 1993). Mitochondrial DNA results suggested that all individuals within *V. squamosa* perennial colonies, including the multiple reproductive females, tended to originate from their own parental nest, which would fit with the general expectations of kin selection hypotheses (Crozier and Pamilo, 1996). Overall, in this chapter we sought to investigate whether the formation of perennial *V. squamosa* colonies represents phenotypic plasticity or the evolution of the species through genetic change. Due to the lack of significant genetic variation between annual

and perennial social forms, it appears that the shift to perennial colony life cycles is representative of a plasticity in mating behavior rather than an evolutionary event. However, evidence from more genetic markers is required to make more definitive claims regarding this plasticity.

While changes to the population genetics of social insects have been examined in the context of seasonal climate and habitat destruction, there is a remarkable lack of research into the plasticity of eusocial insects over longer periods of time. In chapter three, *Temporal analysis of effective population size and mating system in a social wasp*, we investigated the mating system of the eastern yellowjacket, *Vespula maculifrons*, over a 13-generation time period to determine if there was evidence of plastic expression of this important behavior. We found no significant changes to the mating system over this timeframe, which suggests that system is not under pressure from directional selection in this species. Additionally, we provided the first estimate of effective population size in this social insect, and found that it was in line with other, non-social organisms rather than reduced as hypothesized based on the genetic bottleneck of queen monopolization of reproduction. However, we believe that the ratio of Ne/N in eusocial taxa is likely to be considerably lower than in many non-eusocial species. Overall, results from this chapter provide novel insights into the evolution of population dynamics within a eusocial species. Additional investigations across other eusocial taxa are necessary to expand these findings to a larger framework of eusociality.

Previous investigations into the molecular basis of intra-locus conflicts that can emerge from plastic expression of a trait by multiple castes or sexes have uncovered associations between caste- and sex-biased expression and gene duplication (Chau and

Goodisman, 2017). In chapter 4, *Gene duplication in the honeybee: patterns of DNA methylation, gene expression, and genomic environment*, we expand on this finding by examining duplicated genes in the context of epigenetic modification. We found that patterns of gene body methylation differed between single-copy genes and duplicated genes. We also uncovered associations between duplicate gene methylation and gene pair divergence time, location, and length. These results suggest the potential of gene body methylation as a mechanism to direct the regulation and evolution of paralogs, which could serve as an important means to evolve and maintain plastic expression in social insects. Future investigations as to whether patterns of DNA methylation differ between duplicates and singletons in social insects that expand to species with different systems of DNA methylation are critical, including insects devoid of significant methylation levels. Similarly, controlled experimental systems allowing more targeted probing into associations between duplicate gene methylation and gene function in cases of plasticity will help to elucidate how DNA methylation can act as a proximate mechanism of duplicate gene evolution.

Examinations of the distribution of DNA methylation in eusocial insects has historically been focused on functional studies regarding differentially expressed genes. However, the association between gene body methylation and gene copy number suggests a possible mechanistic role of DNA methylation in insect genomes, rather than a causal functional role. In chapter five, *The distribution of DNA methylation across insect chromosomes*, we examined genic methylation levels of the genes on chromosomes in 7 insect species. We found that mean chromosomal levels of genic methylation, as well as the variance in the dispersion of genic methylation of each chromosome, differed

significantly within all species. This result is significant as an examination of the gene contexts in which DNA methylation is found in social insects as different chromatin environments consist of different characteristics and contain genes with divergent functions. However, these preliminary findings are from a relatively small sample of the insect phylogeny and would benefit from an expansion to other insect taxa via the production of additional genomic and methylomic datasets. Additionally, further investigations into the orthology across species of chromosomes that diverge the most from the global levels of genic methylation could provide insight into a possible functional history of DNA methylation within Insecta.

The plastic expression of a shared genotype between individuals is one of the most remarkable traits of eusocial insect colonies. However, the majority of research in the field has focused on identifying specific gene targets that are differentially expressed between castes or sexes to enable these polymorphic traits. My research attempts to take a different approach to understanding this phenomenon by investigating potential mechanisms of social plasticity from both a population genetics and a molecular biology perspective. I examined the population dynamics of social insect colonies that had undergone temporal and social form shifts to further understand the evolution of social behaviors. Additionally, I explored the genetic mechanisms by which phenotypic plasticity can be maintained through the methylation of duplicated genes and the distribution of gene body methylation within insect genomes. As genomic technology continues to improve and insect sequencing datasets become more readily available, the results of these studies will aid in our understanding of morphological and behavioral phenotypic plasticity in social insects and further direct the study of the transition to eusociality overall.

APPENDIX A. SUPPLEMENTAL MATERIALS FOR CHAPTER 3

Table A-1. Genotypes of Vespula maculifrons workers at 16 polymorphic DNA microsatellite loci. Year = year collected. Colony = colony number. Individual = individual worker number. -1 = missing data

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2004	8	0408W41	82	86	205	207	-1	-1	290	302
2004	8	0408W42	82	86	-1	-1	-1	-1	290	298
2004	8	0408W43	82	86	-1	-1	-1	-1	290	298
2004	8	0408W44	82	86	-1	-1	263	282	-1	-1
2004	8	0408W45	82	86	-1	-1	-1	-1	296	298
2004	8	0408W46	82	86	-1	-1	263	276	-1	-1
2004	8	0408W47	82	86	-1	-1	-1	-1	-1	-1
2004	8	0408W48	82	86	-1	-1	263	276	-1	-1
2004	8	0408W49	82	86	205	207	263	276	296	298
2004	8	0408W50	82	86	205	207	263	269	-1	-1
2004	8	0408W51	82	86	205	205	269	278	290	298
2004	8	0408W52	82	88	205	213	263	273	290	294
2004	8	0408W53	82	86	205	207	263	282	290	302
2004	8	0408W54	82	86	205	207	263	282	-1	-1
2004	8	0408W55	82	84	211	211	-1	-1	286	296
2004	8	0408W56	-1	-1	205	211	269	273	286	296
2004	10	0410W41	82	84	207	213	263	266	282	284
2004	10	0410W42	82	82	207	213	263	266	274	284
2004	10	0410W43	82	84	-1	-1	266	273	-1	-1
2004	10	0410W44	82	82	207	213	263	266	274	284
2004	10	0410W45	82	84	207	213	266	273	282	284
2004	10	0410W46	82	82	207	213	263	266	274	284
2004	10	0410W47	82	82	207	213	263	266	274	284
2004	10	0410W48	82	84	207	207	266	273	284	312
2004	10	0410W49	82	84	207	207	263	266	281	312
2004	10	0410W50	82	84	207	213	266	273	274	284
2004	10	0410W51	84	86	207	213	266	273	281	286
2004	10	0410W52	82	84	207	207	263	266	281	312
2004	10	0410W53	82	84	207	213	266	273	282	284
2004	10	0410W54	82	82	207	209	263	273	278	281
2004	10	0410W55	82	84	207	209	273	273	278	284
2004	10	0410W56	82	86	207	213	266	273	281	286
2004	13	0413W41	82	86	207	209	263	263	282	286
2004	13	0413W42	84	84	205	209	263	263	284	288
2004	13	0413W43	82	92	207	209	263	263	284	288
2004	13	0413W44	82	92	205	209	263	263	286	288
2004	13	0413W45	82	92	205	209	263	263	-1	-1
2004	13	0413W46	82	92	205	209	263	263	-1	-1
2004	13	0413W47	82	86	205	209	263	274	284	288
2004	13	0413W48	82	92	205	209	263	263	284	288
2004	13	0413W49	82	84	205	209	-1	-1	284	288
2004	13	0413W50	82	84	205	207	263	263	284	288
2004	13	0413W51	82	92	207	209	263	263	284	288
2004	13	0413W52	82	92	207	209	-1	-1	284	288
2004	13	0413W53	82	86	205	209	-1	-1	284	288
2004	13	0413W54	82	84	205	209	263	263	-1	-1
2004	13	0413W55	82	86	205	209	263	274	-1	-1
2004	13	0413W56	82	92	205	209	263	263	286	288

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2004	15	0415W1	86	86	205	207	266	273	276	294
2004	15	0415W2	78	86	205	205	266	278	276	285
2004	15	0415W3	86	88	207	209	278	278	-1	-1
2004	15	0415W4	78	88	205	205	266	273	274	285
2004	15	0415W5	78	88	205	207	266	273	274	298
2004	15	0415W6	86	86	207	209	278	278	274	298
2004	15	0415W7	78	86	205	207	266	273	276	298
2004	15	0415W8	86	86	205	207	266	278	274	294
2004	15	0415W9	86	88	205	205	278	278	274	286
2004	15	0415W10	78	86	205	207	266	273	274	298
2004	15	0415W11	86	88	205	205	278	278	274	286
2004	15	0415W12	78	86	205	205	266	278	274	285
2004	15	0415W13	78	86	205	207	-1	-1	276	298
2004	15	0415W14	78	86	205	205	266	273	276	285
2004	15	0415W15	78	86	205	205	-1	-1	274	285
2004	15	0415W16	78	88	205	205	266	273	274	285
2004	16	0416W41	78	84	205	207	263	263	285	290
2004	16	0416W42	86	88	205	209	263	263	274	285
2004	16	0416W43	86	88	207	209	266	269	282	285
2004	16	0416W44	84	88	205	209	263	266	285	288
2004	16	0416W45	88	88	205	207	263	273	272	285
2004	16	0416W46	78	88	207	209	263	273	285	286
2004	16	0416W47	78	88	205	207	263	263	285	290
2004	16	0416W48	78	88	205	207	263	263	285	290
2004	16	0416W49	82	88	205	209	266	266	284	285
2004	16	0416W50	88	88	207	207	263	266	285	290
2004	16	0416W51	88	88	207	209	263	266	285	290
2004	16	0416W52	84	88	205	207	263	266	285	288
2004	16	0416W53	88	88	207	207	263	266	285	290
2004	16	0416W54	82	88	205	209	263	266	284	285
2004	16	0416W55	82	88	205	209	263	266	284	285
2004	16	0416W56	86	88	205	209	263	263	274	285
2004	18	0418W41	82	84	205	209	269	273	-1	-1
2004	18	0418W42	-1	-1	205	209	269	276	290	296
2004	18	0418W43	84	86	205	209	269	276	290	296
2004	18	0418W44	82	86	205	211	263	269	276	308
2004	18	0418W45	82	84	207	207	273	276	296	296
2004	18	0418W46	82	86	207	209	269	276	290	296
2004	18	0418W47	82	84	207	207	273	276	-1	-1
2004	18	0418W48	82	84	205	209	269	273	276	308
2004	18	0418W49	82	86	205	209	276	269	276	290
2004	18	0418W50	82	86	205	209	-1	-1	276	290
2004	18	0418W51	82	86	205	209	269	276	276	290
2004	18	0418W52	82	84	205	209	273	269	276	308
2004	18	0418W53	-1	-1	205	207	273	276	296	296
2004	18	0418W54	82	86	207	211	263	269	276	308
2004	18	0418W55	82	84	205	209	269	273	296	308
2004	18	0418W56	82	84	205	209	273	269	276	308
2004	19	0419W41	82	82	207	209	-1	-1	-1	-1

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2004	19	0419W42	82	86	205	209	262	278	-1	-1
2004	19	0419W43	82	88	205	209	266	269	282	282
2004	19	0419W44	82	86	205	209	-1	-1	-1	-1
2004	19	0419W45	82	88	205	207	262	266	-1	-1
2004	19	0419W46	82	88	209	209	269	269	-1	-1
2004	19	0419W47	82	86	205	207	262	278	-1	-1
2004	19	0419W48	82	86	207	211	-1	-1	-1	-1
2004	19	0419W49	82	86	205	209	262	278	-1	-1
2004	19	0419W50	82	86	207	211	262	273	-1	-1
2004	19	0419W51	82	86	205	209	262	278	-1	-1
2004	19	0419W52	88	88	205	207	262	266	282	282
2004	19	0419W53	82	88	205	207	262	266	282	282
2004	19	0419W54	82	86	207	209	262	273	290	290
2004	19	0419W55	82	86	207	209	269	273	-1	-1
2004	19	0419W56	82	88	205	207	262	266	282	282
2004	23	0423W41	82	88	213	215	266	273	-1	-1
2004	23	0423W42	82	82	213	215	273	273	-1	-1
2004	23	0423W43	82	84	209	215	263	266	285	292
2004	23	0423W44	82	82	209	211	266	273	-1	-1
2004	23	0423W45	82	82	-1	-1	273	273	-1	-1
2004	23	0423W46	82	88	213	215	266	273	286	298
2004	23	0423W47	82	88	209	211	266	273	285	292
2004	23	0423W48	82	88	209	215	266	273	-1	-1
2004	23	0423W49	82	88	205	209	-1	-1	-1	-1
2004	23	0423W50	82	88	213	215	266	273	286	298
2004	23	0423W51	82	82	209	215	263	273	286	292
2004	23	0423W52	82	84	213	215	263	266	285	286
2004	23	0423W53	82	88	213	215	266	273	-1	-1
2004	23	0423W54	82	88	211	213	266	273	-1	-1
2004	23	0423W55	82	82	209	211	266	273	286	298
2004	23	0423W56	82	88	211	213	263	266	286	286
2004	24	0424W41	82	86	207	209	262	269	272	285
2004	24	0424W42	82	84	205	215	263	273	272	290
2004	24	0424W43	82	84	205	207	263	269	272	286
2004	24	0424W44	82	84	205	213	263	273	272	298
2004	24	0424W45	82	84	205	207	263	273	272	286
2004	24	0424W46	82	90	209	215	273	278	272	298
2004	24	0424W47	82	84	205	207	263	273	-1	-1
2004	24	0424W48	82	90	209	215	263	273	-1	-1
2004	24	0424W49	82	90	209	215	273	278	284	298
2004	24	0424W50	82	90	205	215	263	273	-1	-1
2004	24	0424W51	82	84	209	213	263	269	272	298
2004	24	0424W52	82	84	205	215	263	273	284	290
2004	24	0424W53	82	84	209	213	263	269	272	298
2004	24	0424W54	82	86	205	209	269	273	284	296
2004	24	0424W55	82	84	205	215	-1	-1	272	290
2004	24	0424W56	82	90	205	215	269	278	284	298
2004	28	0428W1	78	84	205	211	273	284	290	298
2004	28	0428W2	82	84	205	211	-1	-1	290	298

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2004	28	0428W3	78	84	205	211	273	276	290	298
2004	28	0428W4	82	84	205	211	-1	-1	290	298
2004	28	0428W5	78	82	205	211	273	276	288	298
2004	28	0428W6	82	84	205	211	273	284	290	298
2004	28	0428W7	78	82	205	211	-1	-1	288	298
2004	28	0428W8	82	86	205	209	262	276	290	298
2004	28	0428W9	78	86	205	211	273	276	298	302
2004	28	0428W10	78	82	205	211	273	276	-1	-1
2004	28	0428W11	78	84	205	211	273	276	290	298
2004	28	0428W12	82	84	205	211	273	284	290	298
2004	28	0428W13	78	86	205	211	273	276	298	302
2004	28	0428W14	82	86	205	211	273	276	-1	-1
2004	28	0428W15	78	84	205	211	-1	-1	290	298
2004	28	0428W16	78	82	205	211	273	284	288	298
2004	29	0429W1	86	86	207	209	263	266	284	298
2004	29	0429W2	82	86	205	209	-1	-1	284	290
2004	29	0429W3	82	86	207	209	263	266	284	298
2004	29	0429W4	86	86	207	209	263	266	294	298
2004	29	0429W5	82	82	205	209	266	278	290	293
2004	29	0429W6	86	86	207	209	263	266	293	298
2004	29	0429W7	82	86	207	209	263	266	284	298
2004	29	0429W8	82	86	205	209	266	278	290	293
2004	29	0429W9	82	86	205	209	266	278	290	293
2004	29	0429W10	82	82	205	209	266	278	290	293
2004	29	0429W11	82	86	207	209	263	266	293	298
2004	29	0429W12	82	86	205	209	266	278	284	290
2004	29	0429W13	82	82	205	209	266	278	290	293
2004	29	0429W14	82	86	207	209	263	266	284	298
2004	29	0429W15	82	86	205	209	266	278	284	290
2004	29	0429W16	82	86	207	209	263	266	284	298
2004	43	0443W1	86	86	207	211	266	266	290	294
2004	43	0443W2	86	86	207	207	266	266	290	294
2004	43	0443W3	78	88	-1	-1	260	269	-1	-1
2004	43	0443W4	78	86	207	211	263	266	290	290
2004	43	0443W5	-1	-1	-1	-1	-1	-1	-1	-1
2004	43	0443W6	78	86	207	207	263	266	290	290
2004	43	0443W7	86	86	207	207	260	266	290	294
2004	43	0443W8	86	86	209	211	266	286	270	290
2004	43	0443W9	86	86	207	207	266	266	290	294
2004	43	0443W10	78	84	205	207	266	266	274	290
2004	43	0443W11	84	86	205	211	266	266	274	290
2004	43	0443W12	78	86	207	207	266	266	290	294
2004	43	0443W13	78	88	207	211	260	269	290	290
2004	43	0443W14	78	88	207	207	266	269	290	290
2004	43	0443W15	86	86	207	207	260	263	290	290
2004	43	0443W16	78	86	207	209	260	286	270	290
2004	45	0445W1	82	86	207	213	266	266	279	282
2004	45	0445W2	82	86	207	209	266	269	282	290
2004	45	0445W3	86	88	205	209	266	266	282	290

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2004	45	0445W4	86	88	205	209	266	273	282	290
2004	45	0445W5	82	82	207	213	269	273	282	282
2004	45	0445W6	84	86	209	213	266	273	280	290
2004	45	0445W7	82	88	205	213	266	273	282	282
2004	45	0445W8	84	86	209	209	266	273	280	290
2004	45	0445W9	84	86	209	209	266	266	280	290
2004	45	0445W10	82	88	205	209	266	273	282	282
2004	45	0445W11	82	86	207	213	266	266	279	290
2004	45	0445W12	86	88	205	209	266	266	282	290
2004	45	0445W13	84	86	209	213	266	273	280	282
2004	45	0445W14	82	86	207	209	269	273	290	312
2004	45	0445W15	86	88	205	213	266	273	282	290
2004	45	0445W16	86	88	205	213	-1	-1	282	290
2006	63	0663P1	82	82	207	207	263	276	284	290
2006	63	0663P2	82	82	207	209	271	273	290	290
2006	63	0663P6	82	88	207	207	269	271	280	290
2006	63	0663P7	82	88	207	209	276	276	278	290
2006	63	0663P8	82	88	207	207	276	278	290	294
2006	63	0663P14	82	88	-1	-1	273	276	290	290
2006	63	0663P16	86	88	205	207	263	276	272	290
2006	63	0663P17	88	88	207	207	271	278	290	294
2006	63	0663P18	82	86	205	207	263	271	272	290
2006	63	0663L18	82	88	207	207	269	271	-1	-1
2006	63	0663L19	86	88	-1	-1	263	276	272	290
2006	63	0663L20	82	88	207	207	271	278	290	294
2006	63	0663L22	82	82	207	209	271	273	-1	-1
2006	63	0663L23	82	88	207	207	269	271	280	290
2006	63	0663L24	82	88	207	209	-1	-1	-1	-1
2006	65	0665P1	78	86	205	207	269	278	280	290
2006	65	0665P2	78	84	209	209	252	276	286	286
2006	65	0665P3	82	84	209	209	269	276	286	286
2006	65	0665P4	82	84	205	207	252	278	286	298
2006	65	0665P6	78	84	207	207	252	262	286	296
2006	65	0665P7	78	84	209	209	252	276	286	286
2006	65	0665P8	78	84	209	209	252	276	286	286
2006	65	0665P10	82	84	-1	-1	-1	-1	290	298
2006	65	0665P11	78	84	205	207	269	278	286	298
2006	65	0665P13	82	86	205	207	252	278	280	290
2006	65	0665P14	78	84	207	207	252	262	286	296
2006	65	0665P21	78	86	205	207	252	278	280	286
2006	65	0665P27	78	84	207	207	262	269	290	296
2006	66	0666P1	82	88	205	207	271	274	286	292
2006	66	0666P2	82	88	213	213	278	278	288	292
2006	66	0666P3	84	86	207	211	271	278	286	292
2006	66	0666P4	82	86	205	213	271	274	292	292
2006	66	0666P5	82	88	213	213	278	278	288	292
2006	66	0666P11	86	86	207	213	263	278	286	286
2006	66	0666P12	86	88	207	207	263	278	286	286
2006	66	0666P13	82	86	-1	-1	278	278	288	292

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2006	66	0666P14	86	86	207	213	263	278	286	286
2006	66	0666P16	86	86	207	213	263	278	286	286
2006	66	0666L31	84	88	-1	-1	271	278	292	292
2006	66	0666L32	82	88	205	213	271	274	292	292
2006	66	0666L33	84	86	-1	-1	271	278	286	292
2006	67	0667P1	78	84	207	215	260	274	274	298
2006	67	0667P2	78	82	207	215	266	274	274	286
2006	67	0667P3	82	84	-1	-1	260	269	280	286
2006	67	0667P5	-1	-1	207	207	260	274	274	298
2006	67	0667P6	82	84	211	215	260	269	280	298
2006	67	0667P7	-1	-1	-1	-1	260	263	-1	-1
2006	67	0667P9	82	86	207	209	260	266	282	298
2006	67	0667P10	82	82	-1	-1	-1	-1	274	286
2006	67	0667P11	84	88	205	207	260	273	286	286
2006	67	0667P14	82	82	211	211	263	266	290	298
2006	67	0667P15	84	86	207	209	260	260	282	298
2006	67	0667P16	82	88	207	215	260	271	280	298
2006	67	0667P17	82	88	205	207	266	273	286	286
2006	67	0667P23	84	88	207	215	266	271	280	298
2006	68	0668P1	84	84	209	211	273	279	270	276
2006	68	0668P2	82	84	207	211	271	273	270	282
2006	68	0668P3	82	84	207	209	271	273	270	285
2006	68	0668P4	82	84	207	209	271	273	282	285
2006	68	0668P5	82	84	207	211	271	273	282	285
2006	68	0668P6	84	84	211	211	273	279	270	276
2006	68	0668P7	-1	-1	211	211	271	273	285	285
2006	68	0668P8	-1	-1	-1	-1	-1	-1	-1	-1
2006	68	0668P9	84	84	-1	-1	-1	-1	270	285
2006	68	0668P10	-1	-1	211	211	273	273	270	282
2006	68	0668P11	82	84	207	209	271	273	270	282
2006	68	0668P13	84	84	211	211	273	279	270	276
2006	68	0668P14	84	84	-1	-1	269	273	270	282
2006	68	0668P15	82	84	207	211	271	273	285	285
2006	69	0669P13	78	88	205	207	269	269	294	312
2006	69	0669P14	82	82	205	211	269	269	288	312
2006	69	0669P15	82	88	205	211	269	269	288	312
2006	69	0669P16	82	88	211	215	-1	-1	294	312
2006	69	0669P17	82	88	205	211	269	269	294	312
2006	69	0669P18	82	82	-1	-1	269	269	288	312
2006	69	0669P19	78	82	207	215	269	269	294	312
2006	69	0669P20	82	82	205	211	273	273	294	312
2006	69	0669P21	82	82	211	215	269	273	294	312
2006	69	0669P22	-1	-1	211	215	269	273	294	312
2006	69	0669P23	82	82	211	215	-1	-1	288	312
2006	69	0669P24	82	88	211	215	269	273	288	312
2006	69	0669P25	82	82	205	211	269	273	294	312
2006	69	0669P26	82	82	205	211	269	269	294	312
2006	70	0670P1	88	88	211	211	269	269	288	292
2006	70	0670P2	82	88	211	211	263	269	292	294

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2006	70	0670P3	82	82	211	211	263	269	292	294
2006	70	0670P4	82	82	207	213	269	269	288	292
2006	70	0670P5	88	88	207	207	263	269	272	292
2006	70	0670P6	82	82	211	213	269	269	272	288
2006	70	0670P7	82	82	207	207	269	269	280	292
2006	70	0670P8	88	88	207	207	269	284	272	272
2006	70	0670P9	-1	-1	-1	-1	269	278	272	288
2006	70	0670P10	-1	-1	-1	-1	263	278	-1	-1
2006	70	0670P11	82	88	207	211	263	278	272	294
2006	70	0670P12	88	88	-1	-1	278	278	-1	-1
2006	70	0670P13	82	82	207	211	263	278	292	294
2006	70	0670P14	82	88	211	213	269	269	288	292
2006	70	0670P15	-1	-1	-1	-1	263	278	-1	-1
2006	70	0670P16	-1	-1	211	211	278	278	288	292
2006	72	0672L11	82	84	207	207	271	279	282	312
2006	72	0672L13	82	82	207	209	262	263	272	312
2006	72	0672L14	82	84	207	207	271	279	281	282
2006	72	0672L17	82	82	205	207	263	269	293	312
2006	72	0672L19	82	88	-1	-1	262	271	272	281
2006	72	0672L20	82	88	209	211	263	271	290	312
2006	72	0672L22	82	88	205	207	263	269	293	312
2006	72	0672L24	82	88	207	207	262	271	272	281
2006	72	0672L25	82	84	207	209	263	279	282	312
2006	72	0672L27	82	88	207	207	262	271	272	281
2006	72	0672L29	82	88	207	209	262	271	272	281
2006	72	0672L32	82	82	205	207	269	271	293	312
2006	72	0672L36	82	88	207	207	262	263	272	312
2006	72	0672L38	82	88	207	211	263	263	290	312
2006	76	0676P1	88	88	211	211	278	279	284	288
2006	76	0676P2	88	88	211	211	263	278	284	288
2006	76	0676P3	82	88	-1	-1	273	279	-1	-1
2006	76	0676P4	88	88	207	207	263	279	279	284
2006	76	0676P5	84	88	207	215	269	279	282	288
2006	76	0676P6	88	88	207	207	263	279	279	284
2006	76	0676P7	88	88	211	211	263	278	284	288
2006	76	0676P8	84	88	207	215	269	279	282	288
2006	76	0676P9	88	88	207	207	263	263	279	284
2006	76	0676P10	84	88	211	215	269	279	282	288
2006	76	0676P11	88	88	211	211	278	279	284	288
2006	76	0676P12	82	88	211	215	263	269	284	288
2006	76	0676P13	84	88	211	215	263	269	282	288
2006	76	0676P14	82	82	-1	-1	263	271	-1	-1
2006	76	0676P15	82	88	207	209	263	284	280	284
2006	76	0676P16	82	88	207	209	263	266	280	284
2006	76	0676P18	-1	-1	207	215	-1	-1	282	288
2006	76	0676P19	82	88	207	215	263	273	284	302
2006	76	0676P20	86	88	211	211	263	269	284	308
2006	76	0676P24	84	88	207	215	263	269	282	288
2006	76	0676P26	84	88	207	215	263	269	282	288

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2006	76	0676P27	82	88	207	211	269	279	284	292
2006	76	0676P28	82	88	207	209	263	266	280	284
2006	76	0676P38	82	88	207	209	263	284	280	284
2006	76	0676P40	82	88	207	209	263	266	-1	-1
2006	77	0677P1	82	84	207	215	263	279	272	296
2006	77	0677P2	84	84	209	209	279	279	294	296
2006	77	0677P3	78	84	209	215	263	279	285	296
2006	77	0677P4	84	84	209	209	279	279	282	294
2006	77	0677P5	82	82	207	209	279	279	285	296
2006	77	0677P6	82	84	205	207	279	279	272	296
2006	77	0677P7	82	82	209	209	279	279	294	294
2006	77	0677P8	82	84	207	209	279	279	285	296
2006	77	0677P9	84	84	209	209	279	279	282	294
2006	77	0677P10	84	84	205	207	279	279	272	296
2006	77	0677P11	84	84	209	209	279	279	282	294
2006	77	0677P12	84	84	207	209	279	279	294	296
2006	77	0677P13	82	84	207	215	263	279	272	296
2006	77	0677P14	84	84	207	209	279	279	282	294
2006	77	0677P15	82	82	209	209	279	279	285	296
2006	77	0677P16	82	84	207	209	279	279	294	294
2006	77	0677P21	82	104	-1	-1	-1	-1	-1	-1
2006	77	0677P22	82	104	-1	-1	260	279	285	296
2006	77	0677P26	82	84	-1	-1	-1	-1	272	296
2006	77	0677P29	84	84	205	207	-1	-1	272	296
2006	77	0677P31	-1	-1	209	209	279	279	282	294
2006	77	0677P36	82	104	207	209	-1	-1	285	296
2006	77	0677P38	78	82	209	215	263	279	285	296
2006	77	0677P40	-1	-1	-1	-1	-1	-1	282	294
2006	77	0677L6	-1	-1	209	215	263	279	285	296
2006	77	0677L9	-1	-1	-1	-1	260	279	285	296
2006	77	0677L22	-1	-1	207	211	269	279	272	296
2006	77	0677L40	-1	-1	205	207	-1	-1	-1	-1
2006	79	0679P1	86	88	213	213	271	278	290	294
2006	79	0679P2	86	86	205	205	278	278	278	290
2006	79	0679P3	84	86	205	205	278	279	278	294
2006	79	0679P4	82	86	205	213	271	278	270	294
2006	79	0679P5	84	86	207	213	279	279	278	290
2006	79	0679P6	84	86	205	209	278	279	290	312
2006	79	0679P7	86	86	213	213	278	279	290	292
2006	79	0679P8	86	86	213	213	278	278	292	294
2006	79	0679P9	86	86	205	213	279	279	292	294
2006	79	0679P10	84	86	205	209	278	279	290	312
2006	79	0679P11	-1	-1	205	211	263	279	292	294
2006	79	0679P12	84	86	205	209	278	278	294	312
2006	79	0679P13	84	86	211	213	263	278	292	294
2006	79	0679P14	82	84	205	205	271	278	270	294
2006	79	0679P15	86	86	205	213	279	279	292	294
2006	79	0679P16	84	86	205	207	279	279	278	294
2017	195	17195W1	82	86	207	209	269	278	284	288

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2017	195	17195W2	82	86	207	209	269	278	284	288
2017	195	17195W3	82	86	205	209	269	278	284	288
2017	195	17195W4	86	86	205	207	269	269	274	284
2017	195	17195W5	82	86	207	207	273	278	284	288
2017	195	17195W6	82	86	205	209	269	278	284	288
2017	195	17195W7	82	88	205	211	263	278	274	284
2017	195	17195W8	82	86	205	207	273	278	284	288
2017	195	17195W9	82	86	207	207	269	273	284	288
2017	195	17195W10	82	82	207	207	269	273	284	288
2017	195	17195W11	82	82	205	205	278	278	284	284
2017	195	17195W12	82	82	205	209	269	278	284	288
2017	195	17195W13	82	82	207	209	263	278	284	312
2017	195	17195W14	82	88	207	211	263	278	274	284
2017	195	17195W15	82	82	207	209	278	278	284	288
2017	195	17195W16	82	86	207	209	263	278	284	312
2017	196	17196W1	82	82	205	215	269	274	272	274
2017	196	17196W2	82	82	207	213	266	274	285	294
2017	196	17196W3	82	82	205	213	269	274	288	294
2017	196	17196W4	82	82	205	213	269	274	288	294
2017	196	17196W5	82	82	205	213	269	274	274	294
2017	196	17196W6	82	82	205	213	269	274	272	288
2017	196	17196W7	82	82	205	215	269	274	288	294
2017	196	17196W8	82	82	207	213	266	274	272	285
2017	196	17196W9	82	82	207	215	266	274	272	285
2017	196	17196W10	82	82	207	213	266	274	272	285
2017	196	17196W11	82	82	205	213	266	273	294	-1
2017	196	17196W12	82	86	211	215	274	274	272	285
2017	196	17196W13	82	82	207	215	266	266	272	285
2017	196	17196W14	82	82	207	215	274	279	272	274
2017	196	17196W15	82	82	205	215	269	274	272	274
2017	196	17196W16	82	82	207	215	266	274	272	285
2017	201	17201W1	78	84	205	211	-1	-1	288	293
2017	201	17201W2	82	88	207	213	-1	-1	288	293
2017	201	17201W3	-1	-1	207	213	263	273	282	293
2017	201	17201W4	78	88	205	211	273	278	285	288
2017	201	17201W5	82	88	205	211	263	273	272	293
2017	201	17201W6	88	88	205	211	273	273	-1	-1
2017	201	17201W7	78	88	205	211	273	278	285	288
2017	201	17201W8	88	88	205	211	273	278	285	288
2017	201	17201W9	88	88	205	211	273	278	285	288
2017	201	17201W10	88	88	-1	-1	273	273	-1	-1
2017	201	17201W11	88	88	205	211	273	278	285	288
2017	201	17201W12	88	88	205	211	273	273	285	288
2017	201	17201W13	78	82	207	213	263	273	282	293
2017	201	17201W14	82	88	207	213	263	273	285	288
2017	201	17201W15	-1	-1	207	213	263	273	285	288
2017	201	17201W16	88	88	205	211	273	273	285	288
2017	204	17204W1	-1	-1	-1	-1	269	269	284	286
2017	204	17204W2	-1	-1	-1	-1	269	278	286	288

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2017	204	17204W3	-1	-1	-1	-1	269	278	286	288
2017	204	17204W4	-1	-1	-1	-1	269	278	286	288
2017	204	17204W5	-1	-1	-1	-1	269	269	-1	-1
2017	204	17204W6	-1	-1	-1	-1	269	269	284	284
2017	204	17204W7	-1	-1	-1	-1	269	274	270	284
2017	204	17204W8	-1	-1	-1	-1	269	274	270	284
2017	204	17204W9	-1	-1	-1	-1	269	269	270	284
2017	204	17204W10	-1	-1	-1	-1	269	269	270	284
2017	204	17204W11	-1	-1	-1	-1	269	274	270	284
2017	204	17204W12	-1	-1	-1	-1	269	274	270	284
2017	204	17204W13	-1	-1	-1	-1	269	274	-1	-1
2017	204	17204W14	-1	-1	-1	-1	269	269	284	284
2017	204	17204W15	-1	-1	-1	-1	274	278	-1	-1
2017	204	17204W16	-1	-1	-1	-1	269	278	284	288
2017	205	17205W1	82	88	205	207	271	274	274	278
2017	205	17205W2	-1	-1	205	207	266	269	274	278
2017	205	17205W3	82	84	207	215	274	276	278	288
2017	205	17205W4	82	84	207	215	266	276	278	288
2017	205	17205W5	82	84	205	207	269	274	274	278
2017	205	17205W6	82	88	205	207	266	271	274	276
2017	205	17205W7	82	88	205	207	266	271	-1	-1
2017	205	17205W8	82	84	205	207	266	269	274	278
2017	205	17205W9	82	88	205	207	266	271	274	278
2017	205	17205W10	82	88	205	207	271	274	274	278
2017	205	17205W11	82	88	205	207	266	271	274	278
2017	205	17205W12	82	84	205	207	266	269	274	278
2017	205	17205W13	82	88	205	207	266	271	274	278
2017	205	17205W14	-1	-1	205	207	271	274	274	278
2017	205	17205W15	82	84	207	215	266	276	278	288
2017	205	17205W16	82	88	205	207	266	271	274	278
2017	226	17226W1	82	82	205	211	263	269	268	282
2017	226	17226W2	82	88	205	207	263	269	268	285
2017	226	17226W3	82	84	205	207	263	276	294	296
2017	226	17226W4	82	84	205	207	263	276	268	294
2017	226	17226W5	82	84	205	207	263	276	268	294
2017	226	17226W6	82	88	205	207	263	269	285	296
2017	226	17226W7	82	84	205	207	263	276	268	294
2017	226	17226W8	82	86	205	213	263	273	268	274
2017	226	17226W9	82	82	205	211	263	269	282	296
2017	226	17226W10	84	84	205	207	263	276	294	296
2017	226	17226W11	82	86	205	213	263	273	274	296
2017	226	17226W12	82	84	205	207	263	276	268	294
2017	226	17226W15	82	88	205	205	263	271	268	279
2017	226	17226W16	82	88	205	207	263	269	268	285
2017	228	17228W1	82	84	209	211	273	276	274	296
2017	228	17228W2	82	84	209	211	263	273	278	296
2017	228	17228W3	84	88	209	211	263	273	278	296
2017	228	17228W4	82	82	209	209	273	276	274	282
2017	228	17228W5	82	86	205	209	262	263	274	278

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2017	228	17228W6	-1	-1	209	209	-1	-1	278	282
2017	228	17228W7	86	88	205	209	262	276	274	274
2017	228	17228W8	82	84	209	211	263	273	274	296
2017	228	17228W9	82	82	209	209	263	273	278	282
2017	228	17228W10	82	86	205	209	262	276	274	274
2017	228	17228W11	82	86	205	209	262	263	274	274
2017	228	17228W12	82	84	209	211	263	273	274	296
2017	228	17228W13	82	82	209	209	273	276	278	282
2017	228	17228W14	82	84	209	211	273	276	278	296
2017	228	17228W15	82	84	209	211	263	273	278	296
2017	228	17228W16	82	86	205	209	262	276	274	278
2017	229	17229W1	78	82	199	207	263	263	288	296
2017	229	17229W2	78	88	207	207	266	278	279	296
2017	229	17229W3	82	82	207	207	263	263	288	296
2017	229	17229W4	78	84	199	207	278	284	282	-1
2017	229	17229W5	-1	-1	-1	-1	-1	-1	-1	-1
2017	229	17229W6	82	82	-1	-1	263	278	-1	-1
2017	229	17229W7	78	82	199	207	266	278	294	296
2017	229	17229W8	82	82	199	207	266	278	294	296
2017	229	17229W9	78	82	199	207	263	278	282	288
2017	229	17229W10	78	82	207	207	263	278	282	288
2017	229	17229W11	82	82	199	207	263	263	288	296
2017	229	17229W12	82	88	207	207	266	278	279	282
2017	229	17229W13	82	84	207	207	263	284	296	-1
2017	229	17229W14	78	84	207	207	278	284	296	-1
2017	229	17229W15	78	82	207	207	263	278	288	296
2017	229	17229W16	78	88	199	207	263	266	279	282
2017	230	17230W1	82	84	205	209	263	269	278	312
2017	230	17230W2	82	86	205	211	-1	-1	286	288
2017	230	17230W3	82	82	205	209	269	278	298	312
2017	230	17230W4	82	82	205	209	269	278	288	298
2017	230	17230W5	82	82	205	205	269	278	298	312
2017	230	17230W6	82	82	205	205	269	278	288	298
2017	230	17230W8	-1	-1	205	205	-1	-1	294	312
2017	230	17230W9	84	84	-1	-1	269	279	-1	-1
2017	230	17230W10	82	82	205	209	278	279	298	312
2017	230	17230W11	82	82	205	209	269	271	288	294
2017	230	17230W12	82	84	209	209	263	269	278	312
2017	230	17230W13	82	84	205	209	271	279	294	312
2017	230	17230W14	84	84	205	209	263	279	278	312
2017	230	17230W15	82	84	205	215	278	279	285	288
2017	230	17230W16	84	84	209	209	263	269	278	312
2017	193	17193W1	82	88	207	209	266	273	288	288
2017	193	17193W2	82	86	209	211	263	266	270	270
2017	193	17193W3	82	88	207	209	266	273	270	288
2017	193	17193W4	82	86	205	209	-1	-1	268	288
2017	193	17193W5	82	86	209	211	263	263	268	270
2017	193	17193W6	82	86	205	209	263	269	270	288
2017	193	17193W7	82	86	207	211	263	263	270	270

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2017	193	17193W8	82	86	207	211	263	266	270	270
2017	193	17193W9	82	86	205	207	263	269	270	288
2017	193	17193W10	82	88	207	209	263	273	288	288
2017	193	17193W11	82	86	205	207	263	269	270	288
2017	193	17193W12	82	86	207	211	263	263	268	270
2017	193	17193W13	-1	-1	205	209	-1	-1	-1	-1
2017	193	17193W14	82	86	205	209	263	269	270	288
2017	193	17193W15	82	86	205	209	263	269	270	288
2017	193	17193W16	82	86	205	209	266	273	268	288
2017	198	17198W1	82	84	207	209	269	273	294	308
2017	198	17198W2	82	82	207	209	269	273	-1	-1
2017	198	17198W3	82	84	207	207	266	276	274	286
2017	198	17198W4	82	84	207	209	266	269	294	308
2017	198	17198W5	82	88	199	207	266	269	286	296
2017	198	17198W6	82	84	207	209	266	269	294	308
2017	198	17198W7	82	82	207	209	266	269	294	308
2017	198	17198W8	78	82	207	207	266	274	274	308
2017	198	17198W9	84	88	199	207	269	273	286	296
2017	198	17198W10	82	84	207	207	266	276	274	286
2017	198	17198W11	82	84	207	209	266	269	286	294
2017	198	17198W12	82	88	199	207	266	269	286	296
2017	198	17198W13	82	82	207	207	266	276	274	286
2017	198	17198W14	82	84	207	209	269	273	286	294
2017	198	17198W15	82	88	199	207	269	273	286	296
2017	198	17198W16	82	82	207	207	273	274	274	286
2017	199	17199W1	82	84	-1	-1	269	278	-1	-1
2017	199	17199W2	82	82	-1	-1	278	278	268	270
2017	199	17199W3	82	84	207	213	269	271	-1	-1
2017	199	17199W4	82	84	209	213	271	278	274	298
2017	199	17199W5	82	82	209	209	278	278	268	298
2017	199	17199W6	82	82	209	209	278	278	268	270
2017	199	17199W7	82	88	207	209	260	278	270	-1
2017	199	17199W8	82	84	209	213	271	278	270	274
2017	199	17199W9	82	82	209	209	278	278	268	270
2017	199	17199W10	82	82	209	209	278	278	268	298
2017	199	17199W11	82	88	207	209	-1	-1	-1	-1
2017	199	17199W12	82	84	209	213	271	278	270	274
2017	199	17199W13	82	88	207	209	260	278	270	-1
2017	199	17199W14	82	84	209	209	269	278	270	298
2017	199	17199W15	82	88	209	211	271	278	268	270
2017	199	17199W16	82	84	209	209	271	278	298	302
2017	200	17200W1	78	84	-1	-1	273	278	282	294
2017	200	17200W2	84	86	209	215	266	273	288	294
2017	200	17200W3	82	84	207	207	273	276	274	302
2017	200	17200W4	84	86	209	215	266	274	285	294
2017	200	17200W5	84	86	209	215	266	266	288	302
2017	200	17200W6	84	84	215	215	260	266	288	294
2017	200	17200W7	82	84	207	207	266	276	274	302
2017	200	17200W8	-1	-1	211	215	266	279	286	294

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2017	200	17200W9	82	84	209	215	269	273	288	302
2017	200	17200W10	82	84	207	209	266	269	282	302
2017	200	17200W11	84	84	207	213	-1	-1	294	302
2017	200	17200W12	84	86	209	215	266	274	285	294
2017	200	17200W13	84	84	207	211	266	279	-1	-1
2017	200	17200W14	84	84	213	215	269	273	294	302
2017	200	17200W15	82	84	207	207	273	276	274	294
2017	200	17200W16	84	86	207	209	-1	-1	285	302
2017	203	17203W1	82	82	205	213	263	278	274	274
2017	203	17203W2	82	82	199	207	263	269	274	274
2017	203	17203W3	82	82	199	213	263	278	274	274
2017	203	17203W4	82	82	199	213	263	269	274	274
2017	203	17203W5	82	82	205	213	263	278	274	274
2017	203	17203W6	82	82	205	213	263	278	274	274
2017	203	17203W7	82	82	205	213	263	269	274	274
2017	203	17203W8	82	82	199	213	263	278	274	274
2017	203	17203W9	82	82	205	213	263	269	274	282
2017	203	17203W10	82	82	199	207	263	269	274	282
2017	203	17203W11	-1	-1	199	207	278	279	274	294
2017	203	17203W12	78	82	205	207	278	279	274	294
2017	203	17203W13	78	82	199	207	278	279	282	294
2017	203	17203W14	-1	-1	205	215	-1	-1	278	282
2017	203	17203W15	78	82	205	207	278	279	282	294
2017	203	17203W16	82	82	205	207	263	269	274	274
2017	209	17209W2	82	82	-1	-1	-1	-1	-1	-1
2017	209	17209W3	-1	-1	-1	-1	-1	-1	-1	-1
2017	209	17209W4	82	86	207	207	266	271	288	298
2017	209	17209W5	82	82	207	209	271	271	278	288
2017	209	17209W6	-1	-1	-1	-1	-1	-1	-1	-1
2017	209	17209W7	82	82	207	207	266	271	272	278
2017	209	17209W8	82	84	207	207	271	278	282	298
2017	209	17209W9	84	86	205	207	271	278	282	298
2017	209	17209W10	82	86	-1	-1	266	279	278	298
2017	209	17209W11	84	86	205	205	266	279	288	298
2017	209	17209W12	82	86	207	209	271	279	288	298
2017	209	17209W13	82	84	207	207	271	279	278	284
2017	209	17209W14	82	82	207	207	266	271	272	298
2017	209	17209W15	84	86	205	205	278	279	278	284
2017	209	17209W16	86	86	207	207	266	279	278	288
2017	210	17210W1	82	84	207	211	262	266	285	288
2017	210	17210W2	82	84	205	211	-1	-1	288	294
2017	210	17210W3	82	84	205	211	266	271	288	294
2017	210	17210W4	82	84	205	211	266	271	285	288
2017	210	17210W5	84	86	205	209	262	278	285	288
2017	210	17210W6	82	84	205	211	266	271	-1	-1
2017	210	17210W7	82	86	205	211	262	266	288	294
2017	210	17210W8	86	86	207	209	-1	-1	285	288
2017	210	17210W9	86	86	205	209	271	278	285	288
2017	210	17210W10	82	86	205	211	262	266	288	294

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2017	210	17210W11	82	84	207	211	262	266	288	294
2017	210	17210W12	82	84	207	211	271	276	285	288
2017	210	17210W13	82	86	207	211	262	266	288	294
2017	210	17210W14	82	84	205	211	262	266	285	288
2017	210	17210W15	82	86	207	211	262	266	285	288
2017	210	17210W16	82	84	207	211	271	276	285	288
2017	215	17215W1	82	84	207	209	276	276	282	288
2017	215	17215W2	82	84	207	209	276	-1	282	288
2017	215	17215W3	82	86	207	215	266	-1	282	288
2017	215	17215W4	86	86	205	215	266	269	288	294
2017	215	17215W5	82	86	205	209	266	273	288	294
2017	215	17215W6	84	86	207	215	269	276	282	288
2017	215	17215W7	84	86	207	215	269	276	282	288
2017	215	17215W8	82	86	205	209	266	273	288	294
2017	215	17215W9	84	84	207	209	266	266	285	294
2017	215	17215W10	86	86	205	215	262	266	285	294
2017	215	17215W11	84	86	-1	-1	260	266	270	294
2017	215	17215W12	82	86	209	215	266	269	294	294
2017	215	17215W13	84	86	205	209	262	276	285	288
2017	215	17215W14	84	84	207	209	260	276	270	294
2017	215	17215W15	84	84	207	215	266	266	285	294
2017	215	17215W16	82	84	207	209	276	-1	282	294
2017	217	17217W1	82	84	205	213	274	278	288	294
2017	217	17217W2	82	84	199	205	269	274	284	294
2017	217	17217W3	82	84	205	213	269	274	284	294
2017	217	17217W4	82	84	199	205	274	274	294	298
2017	217	17217W5	82	84	199	205	276	278	288	294
2017	217	17217W6	82	84	205	213	276	278	288	294
2017	217	17217W7	82	84	205	213	269	276	284	294
2017	217	17217W8	82	84	205	213	269	276	284	294
2017	217	17217W9	82	84	205	213	269	274	284	294
2017	217	17217W10	82	82	199	205	269	274	284	294
2017	217	17217W11	82	82	205	213	276	278	288	294
2017	217	17217W12	82	104	199	205	276	278	288	294
2017	217	17217W13	82	84	199	205	269	276	284	294
2017	217	17217W14	82	104	205	213	274	278	288	294
2017	217	17217W15	82	104	199	205	276	278	288	294
2017	217	17217W16	82	82	199	205	276	278	288	294
2017	221	17221W1	82	84	205	207	-1	-1	-1	-1
2017	221	17221W2	82	82	205	211	260	262	288	288
2017	221	17221W3	82	84	207	207	266	279	274	288
2017	221	17221W4	82	84	205	205	260	262	-1	-1
2017	221	17221W5	82	82	205	211	262	279	288	288
2017	221	17221W6	82	88	205	205	263	279	284	288
2017	221	17221W7	82	82	207	207	269	279	274	274
2017	221	17221W8	84	86	205	207	260	273	288	288
2017	221	17221W9	82	84	207	207	269	279	274	288
2017	221	17221W10	82	86	205	207	273	279	288	288
2017	221	17221W11	82	82	205	205	262	279	288	288

Table A-1 continued

Year	Colony	Individual	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2017	221	17221W12	82	84	207	207	260	266	288	288
2017	221	17221W13	82	82	207	207	260	269	-1	-1
2017	221	17221W14	82	86	207	207	260	273	288	288
2017	221	17221W15	84	86	205	207	260	273	288	288
2017	221	17221W16	82	82	207	207	260	266	274	288
2017	227	17227W1	84	84	209	215	263	278	296	296
2017	227	17227W2	84	84	207	209	263	278	285	296
2017	227	17227W3	82	82	215	215	263	266	294	296
2017	227	17227W4	82	84	215	215	263	266	294	296
2017	227	17227W5	84	86	207	215	263	-1	294	296
2017	227	17227W6	-1	-1	207	211	-1	-1	285	296
2017	227	17227W7	82	84	209	215	263	278	296	296
2017	227	17227W8	82	82	215	215	263	266	294	296
2017	227	17227W9	84	86	207	207	263	-1	285	294
2017	227	17227W10	84	86	207	207	263	-1	285	294
2017	227	17227W11	82	84	209	215	263	278	285	296
2017	227	17227W12	82	86	207	215	263	-1	294	296
2017	227	17227W13	82	82	211	215	263	266	285	285
2017	227	17227W14	82	84	215	215	263	266	285	294
2017	227	17227W15	82	82	207	211	263	266	285	296

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2004	8	0408W41	-1	-1	175	178	208	220	153	168
2004	8	0408W42	-1	-1	178	181	197	208	168	174
2004	8	0408W43	-1	-1	181	181	208	220	168	174
2004	8	0408W44	-1	-1	175	181	208	220	153	168
2004	8	0408W45	-1	-1	178	178	-1	-1	153	156
2004	8	0408W46	-1	-1	178	181	-1	-1	-1	-1
2004	8	0408W47	-1	-1	175	178	197	208	153	168
2004	8	0408W48	-1	-1	178	181	208	220	156	174
2004	8	0408W49	250	262	178	178	-1	-1	153	156
2004	8	0408W50	236	250	175	178	208	220	168	174
2004	8	0408W51	240	242	181	181	197	208	168	174
2004	8	0408W52	250	262	181	184	197	220	153	174
2004	8	0408W53	236	242	175	181	197	208	153	168
2004	8	0408W54	236	250	175	178	197	208	153	168
2004	8	0408W55	250	258	178	181	-1	-1	153	165
2004	8	0408W56	250	258	178	181	-1	-1	153	165
2004	10	0410W41	250	256	172	184	197	204	146	150
2004	10	0410W42	242	260	172	172	197	204	149	159
2004	10	0410W43	242	256	184	184	197	200	146	150
2004	10	0410W44	242	260	172	184	197	204	146	149
2004	10	0410W45	242	256	172	184	197	204	150	159
2004	10	0410W46	-1	-1	-1	-1	197	204	146	149
2004	10	0410W47	250	260	172	172	197	204	149	159
2004	10	0410W48	242	256	-1	-1	197	200	146	153
2004	10	0410W49	242	256	184	184	197	200	146	153
2004	10	0410W50	250	260	172	172	200	204	149	159
2004	10	0410W51	246	250	-1	-1	197	200	159	174
2004	10	0410W52	250	256	-1	-1	197	200	153	159
2004	10	0410W53	-1	-1	172	184	-1	-1	150	159
2004	10	0410W54	250	262	184	184	197	204	159	168
2004	10	0410W55	242	262	172	184	197	204	159	168
2004	10	0410W56	246	250	172	175	197	204	146	174
2004	13	0413W41	250	250	166	184	200	208	146	174
2004	13	0413W42	250	258	166	178	-1	-1	153	156
2004	13	0413W43	250	262	166	178	192	200	153	156
2004	13	0413W44	250	262	166	178	192	200	153	156
2004	13	0413W45	266	262	166	178	192	200	153	174
2004	13	0413W46	250	262	166	178	192	208	153	156
2004	13	0413W47	250	250	-1	-1	-1	-1	153	174
2004	13	0413W48	250	262	175	178	192	200	-1	-1
2004	13	0413W49	250	258	-1	-1	-1	-1	153	156
2004	13	0413W50	250	250	-1	-1	197	200	153	153
2004	13	0413W51	250	262	-1	-1	192	200	153	156
2004	13	0413W52	250	262	175	178	200	208	-1	-1
2004	13	0413W53	-1	-1	166	166	-1	-1	153	156
2004	13	0413W54	-1	-1	166	178	208	208	153	156
2004	13	0413W55	-1	-1	-1	-1	-1	-1	153	156
2004	13	0413W56	250	262	-1	-1	-1	-1	153	156

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2004	15	0415W1	-1	-1	-1	-1	204	204	-1	-1
2004	15	0415W2	242	250	163	169	204	210	-1	-1
2004	15	0415W3	242	268	163	190	204	210	-1	-1
2004	15	0415W4	-1	-1	163	169	210	210	-1	-1
2004	15	0415W5	242	250	172	190	204	210	-1	-1
2004	15	0415W6	-1	-1	163	190	204	204	-1	-1
2004	15	0415W7	250	258	163	172	-1	-1	-1	-1
2004	15	0415W8	258	270	163	163	204	204	-1	-1
2004	15	0415W9	242	250	163	169	204	210	-1	-1
2004	15	0415W10	250	258	172	190	-1	-1	-1	-1
2004	15	0415W11	250	258	163	169	210	210	-1	-1
2004	15	0415W12	242	250	163	169	204	210	-1	-1
2004	15	0415W13	250	258	163	172	204	210	-1	-1
2004	15	0415W14	250	258	169	190	204	210	-1	-1
2004	15	0415W15	250	258	163	169	204	210	-1	-1
2004	15	0415W16	250	258	163	169	204	210	-1	-1
2004	16	0416W41	-1	-1	172	175	192	200	156	156
2004	16	0416W42	242	250	181	187	200	208	156	-1
2004	16	0416W43	250	258	178	181	192	200	146	156
2004	16	0416W44	242	250	172	181	192	208	156	162
2004	16	0416W45	250	250	181	181	192	200	156	159
2004	16	0416W46	250	258	-1	-1	192	200	156	159
2004	16	0416W47	242	250	172	175	-1	-1	156	156
2004	16	0416W48	242	250	172	175	192	200	156	156
2004	16	0416W49	250	256	166	181	192	200	156	159
2004	16	0416W50	242	256	172	181	192	192	153	156
2004	16	0416W51	242	250	172	181	192	192	153	156
2004	16	0416W52	242	250	172	181	192	208	156	162
2004	16	0416W53	242	250	172	181	192	192	153	156
2004	16	0416W54	250	256	166	172	200	200	156	159
2004	16	0416W55	250	256	166	181	192	200	156	159
2004	16	0416W56	242	250	172	187	200	208	-1	-1
2004	18	0418W41	250	250	-1	-1	-1	-1	153	159
2004	18	0418W42	250	264	-1	-1	200	204	153	162
2004	18	0418W43	-1	-1	-1	-1	204	204	-1	-1
2004	18	0418W44	242	260	172	193	192	200	153	156
2004	18	0418W45	250	260	193	193	204	208	153	165
2004	18	0418W46	-1	-1	-1	-1	204	204	153	162
2004	18	0418W47	250	250	-1	-1	200	208	153	165
2004	18	0418W48	250	250	-1	-1	200	210	153	159
2004	18	0418W49	-1	-1	175	193	204	204	153	162
2004	18	0418W50	-1	-1	169	175	-1	-1	153	162
2004	18	0418W51	250	264	169	175	204	204	153	162
2004	18	0418W52	250	250	172	193	200	210	153	159
2004	18	0418W53	250	250	169	193	200	208	153	165
2004	18	0418W54	242	250	169	172	192	200	153	156
2004	18	0418W55	250	250	169	172	204	210	153	159
2004	18	0418W56	250	260	169	172	200	210	153	159
2004	19	0419W41	-1	-1	-1	-1	-1	-1	156	156

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2004	19	0419W42	250	250	166	184	204	204	156	159
2004	19	0419W43	-1	-1	172	184	192	200	159	162
2004	19	0419W44	-1	-1	-1	-1	204	204	156	156
2004	19	0419W45	250	250	172	181	200	204	156	162
2004	19	0419W46	250	250	169	181	192	204	149	156
2004	19	0419W47	250	250	166	184	204	204	156	156
2004	19	0419W48	-1	-1	175	184	192	204	146	156
2004	19	0419W49	-1	-1	166	181	204	204	156	156
2004	19	0419W50	-1	-1	172	181	192	200	156	165
2004	19	0419W51	-1	-1	166	184	192	204	156	159
2004	19	0419W52	250	250	172	184	200	204	156	162
2004	19	0419W53	-1	-1	172	181	200	204	156	162
2004	19	0419W54	244	244	181	187	200	204	159	165
2004	19	0419W55	-1	-1	181	187	200	204	156	165
2004	19	0419W56	250	250	172	184	192	200	156	162
2004	23	0423W41	-1	-1	169	172	192	210	159	165
2004	23	0423W42	-1	-1	163	178	192	208	153	165
2004	23	0423W43	-1	-1	163	178	204	208	159	178
2004	23	0423W44	-1	-1	169	178	192	200	153	165
2004	23	0423W45	266	274	163	178	192	208	153	165
2004	23	0423W46	266	-1	169	172	208	210	159	165
2004	23	0423W47	236	266	172	178	192	208	146	153
2004	23	0423W48	250	-1	169	172	208	210	159	165
2004	23	0423W49	250	256	169	178	208	210	159	159
2004	23	0423W50	266	-1	169	172	208	210	159	165
2004	23	0423W51	250	274	163	169	192	208	153	159
2004	23	0423W52	250	266	163	178	204	208	159	178
2004	23	0423W53	250	-1	169	172	208	210	159	165
2004	23	0423W54	-1	-1	169	172	208	208	146	153
2004	23	0423W55	250	266	-1	-1	192	200	153	165
2004	23	0423W56	236	250	172	178	192	208	146	153
2004	24	0424W41	242	250	178	193	197	216	159	162
2004	24	0424W42	250	256	178	178	197	208	153	171
2004	24	0424W43	250	262	175	178	197	200	153	159
2004	24	0424W44	-1	-1	172	178	-1	-1	153	153
2004	24	0424W45	250	262	175	178	197	200	159	159
2004	24	0424W46	250	258	178	178	184	210	153	159
2004	24	0424W47	250	262	175	178	200	210	159	159
2004	24	0424W48	242	250	178	178	197	210	153	153
2004	24	0424W49	250	258	178	178	184	210	153	153
2004	24	0424W50	242	250	178	178	197	210	153	153
2004	24	0424W51	250	262	172	178	208	210	153	153
2004	24	0424W52	250	256	178	178	208	210	153	171
2004	24	0424W53	250	262	172	178	197	208	153	153
2004	24	0424W54	250	258	178	178	192	197	153	159
2004	24	0424W55	250	256	178	178	197	208	159	171
2004	24	0424W56	236	258	178	178	184	197	153	159
2004	28	0428W1	250	252	178	184	200	204	-1	-1
2004	28	0428W2	238	252	178	184	197	208	-1	-1

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2004	28	0428W3	250	252	178	184	200	204	-1	-1
2004	28	0428W4	238	252	169	184	197	208	-1	-1
2004	28	0428W5	238	252	178	178	192	208	-1	-1
2004	28	0428W6	250	252	178	184	200	204	-1	-1
2004	28	0428W7	250	252	178	178	192	204	-1	-1
2004	28	0428W8	238	258	169	184	204	208	-1	-1
2004	28	0428W9	238	252	175	178	192	208	-1	-1
2004	28	0428W10	250	252	169	178	192	208	-1	-1
2004	28	0428W11	250	252	178	184	200	204	-1	-1
2004	28	0428W12	238	252	178	184	200	204	-1	-1
2004	28	0428W13	250	252	169	175	192	204	-1	-1
2004	28	0428W14	238	252	175	178	192	208	-1	-1
2004	28	0428W15	-1	-1	178	184	200	204	-1	-1
2004	28	0428W16	238	250	169	178	192	208	-1	-1
2004	29	0429W1	242	250	184	184	210	216	-1	-1
2004	29	0429W2	250	258	175	178	-1	-1	-1	-1
2004	29	0429W3	242	250	184	184	204	216	-1	-1
2004	29	0429W4	242	250	178	184	204	216	-1	-1
2004	29	0429W5	248	258	175	178	204	216	-1	-1
2004	29	0429W6	242	248	184	184	210	216	-1	-1
2004	29	0429W7	242	250	184	184	204	216	-1	-1
2004	29	0429W8	-1	-1	175	184	204	216	-1	-1
2004	29	0429W9	-1	-1	175	178	204	204	156	159
2004	29	0429W10	250	258	175	178	204	216	156	159
2004	29	0429W11	242	250	184	184	210	216	146	156
2004	29	0429W12	248	258	175	178	204	204	156	159
2004	29	0429W13	248	258	175	178	204	216	156	159
2004	29	0429W14	242	248	184	184	204	216	146	156
2004	29	0429W15	250	258	175	184	204	204	156	159
2004	29	0429W16	242	250	184	184	204	216	146	156
2004	43	0443W1	242	242	178	181	200	200	149	159
2004	43	0443W2	242	260	178	181	197	200	149	159
2004	43	0443W3	238	260	178	190	197	210	159	171
2004	43	0443W4	242	260	184	184	200	208	156	159
2004	43	0443W5	236	260	181	184	-1	-1	153	159
2004	43	0443W6	260	260	178	184	197	208	153	156
2004	43	0443W7	-1	-1	178	181	197	200	149	153
2004	43	0443W8	252	260	178	184	197	200	153	159
2004	43	0443W9	242	260	178	181	200	200	149	153
2004	43	0443W10	236	260	178	181	197	216	159	159
2004	43	0443W11	236	260	181	184	197	216	159	159
2004	43	0443W12	242	242	178	181	197	200	149	159
2004	43	0443W13	238	242	184	190	200	210	159	171
2004	43	0443W14	238	242	178	190	197	210	153	171
2004	43	0443W15	260	260	184	184	197	208	156	159
2004	43	0443W16	252	260	184	184	197	200	153	159
2004	45	0445W1	240	252	172	187	200	200	153	156
2004	45	0445W2	250	258	172	184	200	210	156	165
2004	45	0445W3	-1	-1	169	172	204	208	147	153

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2004	45	0445W4	250	258	169	172	200	208	147	153
2004	45	0445W5	250	258	172	184	204	210	156	165
2004	45	0445W6	240	250	178	184	200	204	153	153
2004	45	0445W7	250	258	169	172	200	208	147	156
2004	45	0445W8	-1	-1	178	184	200	200	153	156
2004	45	0445W9	240	250	178	184	200	204	153	156
2004	45	0445W10	240	258	169	172	204	208	147	156
2004	45	0445W11	-1	-1	178	187	200	204	153	153
2004	45	0445W12	250	258	169	172	204	208	147	156
2004	45	0445W13	240	250	178	184	200	204	153	156
2004	45	0445W14	250	258	169	172	204	210	156	165
2004	45	0445W15	250	258	169	172	204	208	147	153
2004	45	0445W16	250	258	169	172	200	208	147	153
2006	63	0663P1	250	266	169	187	197	208	149	159
2006	63	0663P2	242	250	169	190	192	204	153	159
2006	63	0663P6	250	266	169	175	197	208	156	156
2006	63	0663P7	250	250	169	184	208	208	147	159
2006	63	0663P8	250	270	178	187	208	210	147	159
2006	63	0663P14	242	266	187	190	204	208	153	156
2006	63	0663P16	236	250	187	196	208	208	153	159
2006	63	0663P17	250	272	178	187	192	210	147	156
2006	63	0663P18	236	250	187	196	192	208	153	156
2006	63	0663L18	250	266	169	175	197	208	156	156
2006	63	0663L19	236	250	187	196	192	208	153	159
2006	63	0663L20	250	270	169	178	208	210	147	156
2006	63	0663L22	242	266	169	190	204	208	153	156
2006	63	0663L23	250	266	175	187	192	197	156	156
2006	63	0663L24	242	266	169	-1	204	208	153	156
2006	65	0665P1	250	268	178	181	192	204	153	156
2006	65	0665P2	238	268	178	187	204	204	149	153
2006	65	0665P3	238	268	178	187	204	204	149	153
2006	65	0665P4	250	268	178	187	204	208	153	159
2006	65	0665P6	250	268	178	181	204	204	153	159
2006	65	0665P7	238	242	178	187	204	204	149	153
2006	65	0665P8	238	242	178	187	204	204	149	153
2006	65	0665P10	242	250	178	187	204	208	153	159
2006	65	0665P11	242	250	178	187	204	208	153	159
2006	65	0665P13	242	250	178	181	192	204	153	156
2006	65	0665P14	242	250	181	184	204	204	153	159
2006	65	0665P21	242	250	181	184	192	204	153	156
2006	65	0665P27	242	250	178	181	204	204	153	159
2006	66	0666P1	-1	-1	199	199	180	204	153	159
2006	66	0666P2	250	270	199	199	180	204	150	153
2006	66	0666P3	250	254	199	199	208	210	153	168
2006	66	0666P4	256	270	199	199	180	204	153	159
2006	66	0666P5	250	270	199	199	180	204	150	156
2006	66	0666P11	250	270	175	199	180	208	153	162
2006	66	0666P12	270	270	175	184	208	208	156	162
2006	66	0666P13	250	250	199	199	204	208	150	153

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2006	66	0666P14	270	270	175	184	180	208	156	162
2006	66	0666P16	250	270	175	184	180	208	156	162
2006	66	0666L31	-1	-1	184	199	-1	-1	153	168
2006	66	0666L32	-1	-1	-1	-1	180	204	153	159
2006	66	0666L33	-1	-1	184	199	180	210	156	168
2006	67	0667P1	254	268	172	172	192	200	156	162
2006	67	0667P2	254	268	172	172	-1	-1	156	162
2006	67	0667P3	232	240	184	190	192	208	156	156
2006	67	0667P5	-1	-1	172	172	208	210	149	159
2006	67	0667P6	240	268	184	190	208	208	156	159
2006	67	0667P7	248	268	172	178	-1	-1	-1	-1
2006	67	0667P9	232	268	172	175	208	208	-1	-1
2006	67	0667P10	232	248	172	193	-1	-1	-1	-1
2006	67	0667P11	232	256	172	184	208	210	146	156
2006	67	0667P14	232	248	172	178	192	204	149	159
2006	67	0667P15	232	232	175	184	-1	-1	156	159
2006	67	0667P16	232	248	175	184	192	208	149	159
2006	67	0667P17	232	256	184	184	-1	-1	146	156
2006	67	0667P23	232	248	175	184	192	208	149	159
2006	68	0668P1	254	264	178	181	-1	-1	156	162
2006	68	0668P2	264	268	178	178	204	216	156	156
2006	68	0668P3	268	268	178	190	-1	-1	153	156
2006	68	0668P4	264	264	178	178	204	204	-1	-1
2006	68	0668P5	264	268	178	178	204	204	153	156
2006	68	0668P6	254	268	178	181	204	208	156	162
2006	68	0668P7	268	268	178	178	204	216	153	156
2006	68	0668P8	264	278	-1	-1	-1	-1	153	153
2006	68	0668P9	264	268	178	190	-1	-1	153	156
2006	68	0668P10	264	278	-1	-1	192	204	153	153
2006	68	0668P11	264	264	178	178	204	204	156	156
2006	68	0668P13	254	264	178	181	208	216	153	162
2006	68	0668P14	254	264	178	184	200	216	156	159
2006	68	0668P15	268	268	-1	-1	-1	-1	153	156
2006	69	0669P13	236	250	172	178	200	220	149	153
2006	69	0669P14	236	252	178	181	200	220	149	168
2006	69	0669P15	252	252	172	181	200	204	149	153
2006	69	0669P16	236	252	172	178	200	220	149	168
2006	69	0669P17	-1	-1	172	178	200	204	149	168
2006	69	0669P18	236	236	172	178	200	220	149	153
2006	69	0669P19	236	250	172	178	200	220	149	153
2006	69	0669P20	236	252	172	190	200	220	149	168
2006	69	0669P21	-1	-1	172	181	200	220	149	153
2006	69	0669P22	236	252	172	178	-1	-1	149	153
2006	69	0669P23	252	252	172	181	200	204	-1	-1
2006	69	0669P24	236	252	172	181	200	204	149	168
2006	69	0669P25	252	252	172	190	200	220	-1	-1
2006	69	0669P26	236	252	172	178	200	220	-1	-1
2006	70	0670P1	256	256	184	199	204	204	146	153
2006	70	0670P2	250	250	184	184	204	204	146	156

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2006	70	0670P3	250	256	-1	-1	204	204	146	156
2006	70	0670P4	256	256	172	172	204	210	162	178
2006	70	0670P5	-1	-1	-1	-1	204	204	162	178
2006	70	0670P6	256	256	172	172	210	210	-1	-1
2006	70	0670P7	256	256	184	184	204	204	-1	-1
2006	70	0670P8	-1	-1	-1	-1	204	204	-1	-1
2006	70	0670P9	-1	-1	-1	-1	-1	-1	-1	-1
2006	70	0670P10	-1	-1	-1	-1	-1	-1	162	-1
2006	70	0670P11	250	256	172	184	204	210	146	156
2006	70	0670P12	-1	-1	-1	-1	-1	-1	146	162
2006	70	0670P13	250	256	-1	-1	204	204	146	156
2006	70	0670P14	256	256	172	172	210	210	-1	-1
2006	70	0670P15	256	256	-1	-1	-1	-1	-1	-1
2006	70	0670P16	-1	-1	-1	-1	204	216	-1	-1
2006	72	0672L11	250	250	181	181	-1	-1	-1	-1
2006	72	0672L13	250	250	-1	-1	-1	-1	-1	-1
2006	72	0672L14	250	274	181	181	-1	-1	-1	-1
2006	72	0672L17	250	274	178	178	204	204	-1	-1
2006	72	0672L19	250	250	178	178	-1	-1	-1	-1
2006	72	0672L20	-1	-1	181	181	-1	-1	-1	-1
2006	72	0672L22	-1	-1	178	178	-1	-1	-1	-1
2006	72	0672L24	250	250	178	178	208	208	-1	-1
2006	72	0672L25	250	274	181	181	204	204	-1	-1
2006	72	0672L27	250	274	178	178	204	208	-1	-1
2006	72	0672L29	250	250	178	181	204	208	-1	-1
2006	72	0672L32	250	250	178	178	-1	-1	-1	-1
2006	72	0672L36	250	250	178	178	-1	-1	-1	-1
2006	72	0672L38	250	274	178	181	-1	-1	-1	-1
2006	76	0676P1	250	256	187	187	192	200	153	156
2006	76	0676P2	250	250	187	187	192	192	153	156
2006	76	0676P3	-1	-1	-1	-1	200	200	153	153
2006	76	0676P4	250	250	187	187	200	200	153	159
2006	76	0676P5	-1	-1	187	193	192	204	153	171
2006	76	0676P6	250	250	187	193	200	200	153	159
2006	76	0676P7	250	250	187	193	192	192	153	156
2006	76	0676P8	242	256	187	193	200	204	153	171
2006	76	0676P9	250	256	187	187	200	200	153	159
2006	76	0676P10	242	256	187	187	200	204	153	171
2006	76	0676P11	250	250	187	187	192	192	153	156
2006	76	0676P12	-1	-1	178	193	200	216	153	159
2006	76	0676P13	242	256	187	193	200	204	-1	-1
2006	76	0676P14	-1	-1	-1	-1	200	216	153	153
2006	76	0676P15	250	278	184	187	192	204	153	159
2006	76	0676P16	256	270	178	193	192	210	153	159
2006	76	0676P18	242	256	187	193	200	204	-1	-1
2006	76	0676P19	250	250	175	193	192	200	-1	-1
2006	76	0676P20	250	276	-1	-1	200	208	-1	-1
2006	76	0676P24	242	256	187	187	192	204	-1	-1
2006	76	0676P26	242	256	187	187	200	204	-1	-1

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2006	76	0676P27	256	256	178	193	200	216	-1	-1
2006	76	0676P28	250	270	178	193	192	210	-1	-1
2006	76	0676P38	256	278	184	193	192	204	-1	-1
2006	76	0676P40	-1	-1	178	187	200	210	-1	-1
2006	77	0677P1	-1	-1	184	199	192	210	156	156
2006	77	0677P2	250	250	178	178	200	200	153	156
2006	77	0677P3	250	274	178	178	184	192	156	156
2006	77	0677P4	274	274	184	199	200	200	156	159
2006	77	0677P5	274	274	178	184	192	200	159	159
2006	77	0677P6	274	274	178	190	192	192	156	156
2006	77	0677P7	-1	-1	178	178	192	192	159	159
2006	77	0677P8	274	274	178	178	200	200	159	159
2006	77	0677P9	250	274	178	199	192	200	156	159
2006	77	0677P10	274	274	184	190	192	200	156	156
2006	77	0677P11	274	274	178	199	200	200	156	156
2006	77	0677P12	250	250	178	184	200	200	153	159
2006	77	0677P13	-1	-1	178	199	192	210	156	159
2006	77	0677P14	250	274	184	199	200	200	156	156
2006	77	0677P15	274	274	178	184	200	200	156	159
2006	77	0677P16	250	250	178	178	192	192	-1	-1
2006	77	0677P21	-1	-1	178	184	192	204	-1	-1
2006	77	0677P22	-1	-1	178	184	192	204	-1	-1
2006	77	0677P26	250	274	184	190	-1	-1	-1	-1
2006	77	0677P29	274	274	184	190	-1	-1	-1	-1
2006	77	0677P31	-1	-1	184	199	-1	-1	-1	-1
2006	77	0677P36	262	274	178	184	200	204	-1	-1
2006	77	0677P38	274	274	178	178	-1	-1	-1	-1
2006	77	0677P40	-1	-1	178	199	192	200	-1	-1
2006	77	0677L6	274	274	178	178	184	200	-1	-1
2006	77	0677L9	-1	-1	178	184	-1	-1	-1	-1
2006	77	0677L22	-1	-1	175	184	200	200	-1	-1
2006	77	0677L40	-1	-1	178	184	200	208	-1	-1
2006	79	0679P1	260	260	178	181	204	210	149	153
2006	79	0679P2	-1	-1	178	178	204	210	149	153
2006	79	0679P3	-1	-1	178	178	204	204	149	153
2006	79	0679P4	232	250	175	178	188	210	153	174
2006	79	0679P5	250	250	178	178	204	210	153	174
2006	79	0679P6	-1	-1	178	184	192	204	153	174
2006	79	0679P7	250	250	178	178	204	210	149	165
2006	79	0679P8	-1	-1	178	178	204	204	165	174
2006	79	0679P9	-1	-1	178	178	204	210	149	156
2006	79	0679P10	250	250	178	184	192	204	153	174
2006	79	0679P11	250	256	178	178	204	210	149	153
2006	79	0679P12	250	250	178	184	192	210	153	174
2006	79	0679P13	250	256	178	178	204	204	149	-1
2006	79	0679P14	232	260	175	178	210 ?		153	174
2006	79	0679P15	-1	-1	178	178	204	210	156	174
2006	79	0679P16	-1	-1	178	178	204	210	-1	-1
2017	195	17195W1	252	256	178	178	197	204	156	171

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2017	195	17195W2	252	256	178	178	197	204	153	171
2017	195	17195W3	250	252	178	178	197	204	-1	-1
2017	195	17195W4	250	256	178	-1	197	197	156	156
2017	195	17195W5	250	254	178	-1	197	210	153	153
2017	195	17195W6	-1	-1	178	178	197	204	156	171
2017	195	17195W7	-1	-1	178	190	197	204	147	153
2017	195	17195W8	250	254	178	-1	197	210	153	153
2017	195	17195W9	254	256	-1	-1	197	210	153	156
2017	195	17195W10	250	254	178	-1	197	210	153	153
2017	195	17195W11	250	250	178	178	197	197	153	153
2017	195	17195W12	252	256	178	178	197	204	156	171
2017	195	17195W13	250	252	178	178	197	197	156	159
2017	195	17195W14	252	256	178	190	197	204	147	153
2017	195	17195W15	250	252	178	178	197	204	156	171
2017	195	17195W16	252	256	178	-1	197	197	153	159
2017	196	17196W1	236	238	-1	-1	204	210	153	159
2017	196	17196W2	232	258	-1	-1	200	210	156	156
2017	196	17196W3	236	258	-1	-1	197	204	156	178
2017	196	17196W4	236	258	-1	-1	197	204	156	178
2017	196	17196W5	236	238	-1	-1	204	210	153	159
2017	196	17196W6	236	238	-1	-1	204	210	156	178
2017	196	17196W7	236	258	-1	-1	204	210	156	178
2017	196	17196W8	232	238	-1	-1	200	210	156	156
2017	196	17196W9	232	258	-1	-1	197	200	156	156
2017	196	17196W10	232	238	-1	-1	197	200	156	156
2017	196	17196W11	-1	-1	-1	-1	204	210	156	178
2017	196	17196W12	236	238	-1	-1	204	210	153	159
2017	196	17196W13	232	258	-1	-1	200	210	156	156
2017	196	17196W14	-1	-1	-1	-1	210	210	156	165
2017	196	17196W15	236	238	-1	-1	204	210	153	159
2017	196	17196W16	232	258	-1	-1	200	210	156	156
2017	201	17201W1	246	272	178	178	197	200	174	174
2017	201	17201W2	242	250	178	184	200	210	-1	-1
2017	201	17201W3	242	250	178	178	197	204	153	174
2017	201	17201W4	-1	-1	178	178	-1	-1	146	156
2017	201	17201W5	246	272	175	-1	200	204	146	153
2017	201	17201W6	246	256	178	178	200	204	156	174
2017	201	17201W7	246	256	175	175	204	204	156	174
2017	201	17201W8	246	256	178	178	200	204	156	174
2017	201	17201W9	246	256	175	175	204	204	146	156
2017	201	17201W10	246	256	178	178	200	204	156	174
2017	201	17201W11	246	256	175	178	204	204	156	174
2017	201	17201W12	246	256	178	178	200	204	146	156
2017	201	17201W13	242	250	175	178	197	200	146	153
2017	201	17201W14	242	250	178	178	197	204	153	174
2017	201	17201W15	242	250	178	178	197	204	146	153
2017	201	17201W16	246	256	178	178	200	204	146	156
2017	204	17204W1	246	252	169	172	192	197	146	159
2017	204	17204W2	-1	-1	178	178	180	180	146	-1

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2017	204	17204W3	264	272	178	178	180	197	146	-1
2017	204	17204W4	264	272	-1	-1	180	197	146	-1
2017	204	17204W5	246	250	-1	-1	180	180	-1	-1
2017	204	17204W6	248	272	-1	-1	180	192	146	159
2017	204	17204W7	-1	-1	-1	-1	180	197	-1	-1
2017	204	17204W8	246	250	-1	-1	180	188	-1	-1
2017	204	17204W9	250	272	-1	-1	180	180	-1	-1
2017	204	17204W10	250	272	-1	-1	180	180	-1	-1
2017	204	17204W11	246	250	-1	-1	180	180	-1	-1
2017	204	17204W12	246	250	-1	-1	180	180	-1	-1
2017	204	17204W13	-1	-1	169	172	180	197	146	-1
2017	204	17204W14	248	272	-1	-1	192	197	-1	-1
2017	204	17204W15	264	272	-1	-1	180	197	-1	-1
2017	204	17204W16	264	272	-1	-1	180	197	146	-1
2017	205	17205W1	250	270	169	169	192	204	159	159
2017	205	17205W2	256	-1	184	184	204	216	159	168
2017	205	17205W3	246	250	169	184	192	192	159	165
2017	205	17205W4	246	256	169	184	192	204	159	159
2017	205	17205W5	250	-1	184	184	192	216	165	168
2017	205	17205W6	250	270	169	169	192	204	159	165
2017	205	17205W7	256	270	169	184	192	204	-1	-1
2017	205	17205W8	250	-1	184	184	204	216	165	168
2017	205	17205W9	256	270	172	184	192	192	159	165
2017	205	17205W10	250	270	169	169	192	204	159	165
2017	205	17205W11	256	270	172	184	192	192	159	165
2017	205	17205W12	256	-1	169	184	204	216	159	168
2017	205	17205W13	256	270	169	184	192	204	159	165
2017	205	17205W14	250	270	169	172	192	192	159	165
2017	205	17205W15	246	250	169	169	192	192	159	165
2017	205	17205W16	250	270	169	184	192	204	159	165
2017	226	17226W1	238	258	169	184	197	200	153	162
2017	226	17226W2	238	278	172	175	200	204	153	159
2017	226	17226W3	242	258	172	184	197	200	-1	-1
2017	226	17226W4	242	258	172	172	197	200	153	153
2017	226	17226W5	238	242	172	184	197	200	-1	-1
2017	226	17226W6	238	278	175	184	197	204	159	159
2017	226	17226W7	238	242	172	172	197	200	-1	-1
2017	226	17226W8	242	258	184	190	197	197	153	159
2017	226	17226W9	258	258	169	184	200	200	159	162
2017	226	17226W10	242	258	172	172	197	200	153	153
2017	226	17226W11	242	258	184	190	197	197	153	159
2017	226	17226W12	238	242	172	172	-1	-1	153	153
2017	226	17226W15	238	258	184	190	200	200	150	159
2017	226	17226W16	258	278	175	184	200	204	153	159
2017	228	17228W1	242	248	178	178	197	210	153	162
2017	228	17228W2	242	248	178	178	192	210	153	162
2017	228	17228W3	242	248	178	178	197	210	153	162
2017	228	17228W4	248	248	178	178	192	210	153	162
2017	228	17228W5	248	250	178	178	192	210	162	174

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2017	228	17228W6	248	248	178	178	192	210	153	162
2017	228	17228W7	248	250	178	178	192	210	162	174
2017	228	17228W8	242	248	178	178	197	210	162	174
2017	228	17228W9	248	248	178	178	192	210	153	162
2017	228	17228W10	248	250	178	178	-1	-1	162	174
2017	228	17228W11	248	250	178	178	192	210	153	162
2017	228	17228W12	242	242	178	178	197	210	162	174
2017	228	17228W13	248	248	178	178	197	210	153	162
2017	228	17228W14	242	248	178	178	197	210	153	162
2017	228	17228W15	242	242	178	178	192	210	153	162
2017	228	17228W16	248	250	178	178	192	210	162	174
2017	229	17229W1	250	250	181	184	192	204	159	162
2017	229	17229W2	250	250	178	190	192	204	149	159
2017	229	17229W3	250	250	181	184	204	210	159	162
2017	229	17229W4	250	250	178	178	204	210	156	159
2017	229	17229W5	252	252	178	-1	192	192	147	159
2017	229	17229W6	250	258	178	184	-1	-1	159	178
2017	229	17229W7	252	258	178	181	-1	-1	159	178
2017	229	17229W8	250	258	178	181	197	210	159	178
2017	229	17229W9	250	250	178	184	204	210	159	162
2017	229	17229W10	250	250	181	184	192	204	159	162
2017	229	17229W11	250	252	181	184	192	204	159	162
2017	229	17229W12	252	258	178	178	192	210	159	159
2017	229	17229W13	-1	-1	178	181	192	204	156	159
2017	229	17229W14	-1	-1	178	181	192	204	156	159
2017	229	17229W15	250	252	181	184	204	210	159	162
2017	229	17229W16	250	250	178	190	192	204	149	159
2017	230	17230W1	270	270	178	184	208	216	159	159
2017	230	17230W2	250	250	178	184	-1	-1	159	159
2017	230	17230W3	250	270	172	178	200	216	159	159
2017	230	17230W4	250	250	172	184	200	216	159	159
2017	230	17230W5	250	250	172	178	200	216	156	159
2017	230	17230W6	250	270	172	184	200	216	156	159
2017	230	17230W8	256	270	178	187	200	204	156	159
2017	230	17230W9	-1	-1	184	187	200	204	159	159
2017	230	17230W10	250	270	172	178	208	216	156	159
2017	230	17230W11	256	270	178	187	204	208	156	156
2017	230	17230W12	250	250	178	184	208	216	159	159
2017	230	17230W13	256	270	184	187	204	208	156	156
2017	230	17230W14	250	250	184	184	208	216	156	159
2017	230	17230W15	236	250	184	184	208	208	156	168
2017	230	17230W16	250	270	178	184	200	216	156	159
2017	193	17193W1	252	258	169	187	192	192	147	168
2017	193	17193W2	250	252	187	190	192	204	147	162
2017	193	17193W3	252	258	169	190	192	192	147	168
2017	193	17193W4	252	252	178	187	192	200	147	156
2017	193	17193W5	250	252	187	190	192	204	147	162
2017	193	17193W6	242	252	178	190	192	197	147	153
2017	193	17193W7	250	252	187	187	204	210	147	162

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2017	193	17193W8	250	252	187	187	192	204	147	162
2017	193	17193W9	242	252	178	187	197	210	147	153
2017	193	17193W10	252	258	169	187	192	210	147	168
2017	193	17193W11	242	252	178	190	192	197	147	153
2017	193	17193W12	250	252	187	190	204	210	147	162
2017	193	17193W13	242	252	-1	-1	-1	-1	147	153
2017	193	17193W14	242	252	178	187	197	210	147	153
2017	193	17193W15	242	252	178	187	192	197	147	153
2017	193	17193W16	252	252	178	187	192	200	147	156
2017	198	17198W1	242	250	169	169	200	208	149	159
2017	198	17198W2	242	250	169	169	200	208	149	159
2017	198	17198W3	232	250	-1	-1	200	204	153	159
2017	198	17198W4	242	250	169	169	200	208	149	159
2017	198	17198W5	250	258	178	190	200	204	153	159
2017	198	17198W6	242	250	169	169	200	208	149	159
2017	198	17198W7	242	250	169	169	200	208	149	159
2017	198	17198W8	250	250	169	169	197	200	156	159
2017	198	17198W9	250	258	169	178	200	204	153	159
2017	198	17198W10	232	250	178	190	200	204	-1	-1
2017	198	17198W11	242	250	169	190	200	208	149	159
2017	198	17198W12	250	258	178	190	204	204	153	159
2017	198	17198W13	232	250	178	190	200	204	153	159
2017	198	17198W14	242	250	-1	-1	200	208	-1	-1
2017	198	17198W15	250	258	178	190	200	204	153	159
2017	198	17198W16	232	250	178	190	204	204	153	159
2017	199	17199W1	250	262	178	181	208	216	-1	-1
2017	199	17199W2	254	262	178	199	197	208	-1	-1
2017	199	17199W3	-1	-1	-1	-1	208	216	-1	-1
2017	199	17199W4	254	262	178	-1	192	210	-1	-1
2017	199	17199W5	250	262	178	181	208	210	-1	-1
2017	199	17199W6	250	254	178	199	197	208	-1	-1
2017	199	17199W7	-1	-1	178	190	208	210	-1	-1
2017	199	17199W8	254	262	178	-1	192	208	-1	-1
2017	199	17199W9	250	262	178	181	208	210	-1	-1
2017	199	17199W10	254	262	178	199	197	208	-1	-1
2017	199	17199W11	-1	-1	178	190	208	210	-1	-1
2017	199	17199W12	-1	-1	178	-1	192	208	-1	-1
2017	199	17199W13	252	262	178	190	208	210	-1	-1
2017	199	17199W14	-1	-1	178	181	208	216	-1	-1
2017	199	17199W15	262	262	178	178	192	210	-1	-1
2017	199	17199W16	244	262	-1	-1	204	208	-1	-1
2017	200	17200W1	244	250	181	199	-1	-1	153	159
2017	200	17200W2	264	264	178	190	200	204	153	-1
2017	200	17200W3	250	250	178	178	197	204	156	-1
2017	200	17200W4	244	250	178	190	197	204	153	-1
2017	200	17200W5	264	264	178	190	197	204	153	159
2017	200	17200W6	250	250	178	190	192	197	156	-1
2017	200	17200W7	250	250	178	178	197	204	156	159
2017	200	17200W8	250	250	178	190	197	210	156	159

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2017	200	17200W9	250	250	181	181	197	210	-1	-1
2017	200	17200W10	244	250	178	190	197	204	149	-1
2017	200	17200W11	244	250	-1	-1	197	210	147	159
2017	200	17200W12	244	250	178	190	200	204	153	159
2017	200	17200W13	-1	-1	178	190	-1	-1	156	-1
2017	200	17200W14	244	250	178	190	200	210	147	159
2017	200	17200W15	250	250	-1	-1	200	204	156	159
2017	200	17200W16	244	250	178	190	197	204	153	159
2017	203	17203W1	240	252	169	178	192	210	149	162
2017	203	17203W2	252	256	169	190	192	192	149	153
2017	203	17203W3	240	252	169	178	192	210	153	162
2017	203	17203W4	240	252	169	178	192	210	149	162
2017	203	17203W5	240	250	169	178	192	192	149	162
2017	203	17203W6	240	252	169	178	192	192	149	162
2017	203	17203W7	240	250	169	178	192	210	153	162
2017	203	17203W8	240	252	169	178	192	210	149	162
2017	203	17203W9	240	252	169	178	192	192	153	162
2017	203	17203W10	250	256	169	190	192	210	149	149
2017	203	17203W11	252	252	169	181	192	200	149	156
2017	203	17203W12	-1	-1	169	181	-1	-1	149	156
2017	203	17203W13	252	252	169	181	200	210	153	156
2017	203	17203W14	250	252	169	178	192	204	149	159
2017	203	17203W15	250	252	169	181	192	200	153	156
2017	203	17203W16	252	256	169	190	192	210	149	149
2017	209	17209W2	-1	-1	184	190	-1	-1	147	156
2017	209	17209W3	262	262	184	190	-1	-1	156	178
2017	209	17209W4	250	250	175	-1	208	216	147	147
2017	209	17209W5	250	250	-1	-1	200	208	147	171
2017	209	17209W6	-1	-1	178	-1	-1	-1	159	178
2017	209	17209W7	246	262	184	190	200	200	162	178
2017	209	17209W8	250	258	178	190	197	200	159	178
2017	209	17209W9	250	258	178	190	197	200	147	159
2017	209	17209W10	250	274	184	-1	200	200	174	178
2017	209	17209W11	262	-1	178	-1	200	200	-1	-1
2017	209	17209W12	-1	-1	190	-1	200	208	171	178
2017	209	17209W13	250	274	178	190	197	216	162	178
2017	209	17209W14	246	250	184	-1	-1	-1	162	178
2017	209	17209W15	250	258	178	190	197	216	147	178
2017	209	17209W16	-1	-1	-1	-1	200	208	-1	-1
2017	210	17210W1	268	276	178	184	210	220	159	174
2017	210	17210W2	256	268	178	184	-1	-1	156	159
2017	210	17210W3	256	268	178	184	216	220	159	174
2017	210	17210W4	256	268	178	184	216	220	159	174
2017	210	17210W5	250	256	178	181	197	210	156	174
2017	210	17210W6	268	276	178	178	216	220	156	159
2017	210	17210W7	256	268	178	178	210	220	156	159
2017	210	17210W8	250	256	178	181	-1	-1	156	174
2017	210	17210W9	250	256	181	184	-1	-1	156	156
2017	210	17210W10	268	276	178	178	216	220	159	174

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2017	210	17210W11	256	268	178	184	216	220	156	159
2017	210	17210W12	256	258	178	178	204	216	147	156
2017	210	17210W13	268	276	178	184	216	220	156	159
2017	210	17210W14	268	276	178	178	210	220	159	174
2017	210	17210W15	268	276	178	178	210	220	156	159
2017	210	17210W16	256	258	178	184	204	210	147	174
2017	215	17215W1	236	256	172	172	-1	-1	153	168
2017	215	17215W2	236	256	172	172	200	210	153	174
2017	215	17215W3	256	-1	172	172	200	210	153	168
2017	215	17215W4	236	278	172	181	200	204	147	174
2017	215	17215W5	236	236	169	181	204	210	150	174
2017	215	17215W6	236	252	172	181	204	204	147	168
2017	215	17215W7	236	252	172	181	204	204	147	174
2017	215	17215W8	236	236	169	172	204	204	150	168
2017	215	17215W9	278	-1	172	181	204	210	159	168
2017	215	17215W10	236	242	172	181	-1	-1	159	174
2017	215	17215W11	236	252	172	172	208	210	150	174
2017	215	17215W12	242	-1	169	172	204	204	153	168
2017	215	17215W13	236	242	172	181	-1	-1	159	174
2017	215	17215W14	236	252	172	172	204	208	150	168
2017	215	17215W15	278	-1	172	181	204	210	159	168
2017	215	17215W16	236	256	172	172	200	210	153	174
2017	217	17217W1	238	-1	169	172	204	208	153	156
2017	217	17217W2	238	252	169	190	200	204	156	156
2017	217	17217W3	238	252	169	190	200	204	156	159
2017	217	17217W4	238	262	172	178	200	204	153	159
2017	217	17217W5	238	-1	169	172	208	208	156	159
2017	217	17217W6	-1	-1	172	178	208	208	153	156
2017	217	17217W7	262	-1	169	190	200	208	156	156
2017	217	17217W8	238	252	178	190	200	208	156	156
2017	217	17217W9	238	262	178	190	200	208	156	156
2017	217	17217W10	238	252	169	190	200	208	156	159
2017	217	17217W11	238	-1	169	172	204	208	153	159
2017	217	17217W12	238	-1	169	172	204	204	156	156
2017	217	17217W13	238	252	178	190	200	208	-1	-1
2017	217	17217W14	-1	-1	172	178	204	204	-1	-1
2017	217	17217W15	-1	-1	169	172	204	208	156	159
2017	217	17217W16	-1	-1	169	172	204	208	153	156
2017	221	17221W1	242	258	172	190	192	210	150	156
2017	221	17221W2	250	258	172	190	192	204	153	174
2017	221	17221W3	242	258	172	178	192	210	150	156
2017	221	17221W4	242	258	181	190	192	204	156	156
2017	221	17221W5	250	258	172	190	192	204	156	174
2017	221	17221W6	242	242	181	190	192	192	156	162
2017	221	17221W7	250	258	178	178	192	200	153	159
2017	221	17221W8	250	250	172	190	192	192	156	-1
2017	221	17221W9	242	258	178	178	192	200	156	159
2017	221	17221W10	250	250	172	178	192	192	153	-1
2017	221	17221W11	242	258	181	190	200	204	156	156

Table A-1 continued

Year	Colony	Individual	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2017	221	17221W12	242	258	172	190	192	210	150	153
2017	221	17221W13	250	258	178	190	192	192	156	159
2017	221	17221W14	250	250	172	190	192	200	156	-1
2017	221	17221W15	250	250	172	190	192	192	156	-1
2017	221	17221W16	242	258	172	178	200	210	150	156
2017	227	17227W1	252	252	178	187	197	208	156	168
2017	227	17227W2	252	252	172	187	204	208	156	168
2017	227	17227W3	252	254	178	-1	197	208	162	-1
2017	227	17227W4	-1	-1	172	-1	197	208	156	162
2017	227	17227W5	252	254	172	178	197	204	156	178
2017	227	17227W6	250	254	172	-1	-1	-1	156	159
2017	227	17227W7	252	254	178	187	197	208	156	168
2017	227	17227W8	252	254	172	-1	197	208	156	162
2017	227	17227W9	252	254	178	178	197	204	156	178
2017	227	17227W10	252	254	178	178	197	204	156	178
2017	227	17227W11	252	252	178	187	204	208	156	168
2017	227	17227W12	252	254	178	178	204	204	178	-1
2017	227	17227W13	250	252	178	-1	204	204	159	-1
2017	227	17227W14	252	254	172	-1	197	208	162	-1
2017	227	17227W15	250	252	172	-1	204	204	159	-1

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2004	8	0408W41	152	154	151	151	183	191	201	207
2004	8	0408W42	152	156	141	151	183	191	203	207
2004	8	0408W43	152	156	141	151	183	191	203	207
2004	8	0408W44	152	154	151	151	183	191	201	207
2004	8	0408W45	152	154	145	147	189	191	201	201
2004	8	0408W46	152	154	145	151	189	191	201	207
2004	8	0408W47	152	154	151	151	183	183	201	207
2004	8	0408W48	152	154	145	151	189	191	201	207
2004	8	0408W49	152	154	145	147	183	189	201	207
2004	8	0408W50	152	154	151	151	183	183	207	207
2004	8	0408W51	152	156	141	151	183	191	203	207
2004	8	0408W52	148	152	147	157	191	195	201	207
2004	8	0408W53	152	154	151	151	183	183	207	207
2004	8	0408W54	152	154	151	151	183	183	207	207
2004	8	0408W55	152	156	147	147	191	193	185	207
2004	8	0408W56	152	156	147	147	183	193	185	207
2004	10	0410W41	150	156	147	149	173	191	185	203
2004	10	0410W42	156	158	145	149	173	191	203	203
2004	10	0410W43	150	150	147	149	173	191	185	206
2004	10	0410W44	150	158	145	149	173	191	-1	-1
2004	10	0410W45	150	156	147	147	173	191	185	203
2004	10	0410W46	-1	-1	145	149	173	191	203	203
2004	10	0410W47	156	158	145	149	173	191	203	203
2004	10	0410W48	150	158	147	149	187	191	203	206
2004	10	0410W49	150	158	147	149	187	191	206	206
2004	10	0410W50	156	158	145	149	173	191	203	203
2004	10	0410W51	-1	-1	149	153	191	193	203	207
2004	10	0410W52	150	158	147	149	187	191	203	206
2004	10	0410W53	150	156	147	147	173	191	185	203
2004	10	0410W54	150	152	147	151	181	191	206	206
2004	10	0410W55	150	152	149	151	181	191	203	206
2004	10	0410W56	150	158	149	153	191	193	206	207
2004	13	0413W41	150	154	151	153	185	193	201	203
2004	13	0413W42	152	156	141	147	193	193	203	203
2004	13	0413W43	154	156	151	151	193	195	201	203
2004	13	0413W44	156	156	151	151	193	195	201	203
2004	13	0413W45	156	156	151	151	193	195	201	203
2004	13	0413W46	154	156	147	151	179	195	201	201
2004	13	0413W47	154	156	141	151	-1	-1	203	207
2004	13	0413W48	156	156	151	151	-1	-1	-1	-1
2004	13	0413W49	-1	-1	141	147	193	193	-1	-1
2004	13	0413W50	-1	-1	147	149	193	195	-1	-1
2004	13	0413W51	-1	-1	147	151	193	195	201	203
2004	13	0413W52	154	156	147	151	193	195	-1	-1
2004	13	0413W53	150	154	141	151	193	193	201	203
2004	13	0413W54	152	154	141	151	193	193	203	203
2004	13	0413W55	-1	-1	141	151	-1	-1	-1	-1
2004	13	0413W56	-1	-1	147	151	-1	-1	201	203

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2004	15	0415W1	-1	-1	151	155	173	195	201	207
2004	15	0415W2	154	158	149	151	173	193	206	207
2004	15	0415W3	158	160	147	151	173	173	207	213
2004	15	0415W4	154	158	149	153	173	193	206	207
2004	15	0415W5	158	-1	153	157	193	-1	207	213
2004	15	0415W6	158	160	147	151	173	173	207	213
2004	15	0415W7	158	-1	151	157	193	-1	207	207
2004	15	0415W8	148	158	151	155	173	195	201	207
2004	15	0415W9	154	158	149	153	193	-1	207	207
2004	15	0415W10	158	-1	153	157	193	-1	207	207
2004	15	0415W11	154	158	149	153	193	-1	207	207
2004	15	0415W12	154	158	149	153	173	193	206	207
2004	15	0415W13	158	-1	153	157	193	-1	207	213
2004	15	0415W14	154	158	153	157	173	193	206	213
2004	15	0415W15	154	158	151	157	193	193	206	213
2004	15	0415W16	154	158	153	157	173	193	206	213
2004	16	0416W41	154	154	-1	-1	187	191	206	207
2004	16	0416W42	152	156	141	155	189	193	206	207
2004	16	0416W43	152	156	145	155	191	195	207	207
2004	16	0416W44	150	152	141	147	187	191	203	207
2004	16	0416W45	146	152	141	141	191	199	203	203
2004	16	0416W46	150	152	141	147	-1	-1	203	207
2004	16	0416W47	-1	-1	141	147	187	191	206	207
2004	16	0416W48	154	154	145	147	187	191	206	207
2004	16	0416W49	154	158	147	149	191	191	201	207
2004	16	0416W50	152	154	141	141	187	191	207	207
2004	16	0416W51	154	154	141	145	187	193	207	207
2004	16	0416W52	150	154	141	147	187	191	203	207
2004	16	0416W53	154	154	141	141	187	191	207	207
2004	16	0416W54	152	158	141	149	191	191	201	207
2004	16	0416W55	152	158	141	149	191	191	201	207
2004	16	0416W56	154	156	141	155	189	191	-1	-1
2004	18	0418W41	-1	-1	141	149	189	193	203	207
2004	18	0418W42	148	156	147	153	-1	-1	202	203
2004	18	0418W43	-1	-1	147	153	-1	-1	-1	-1
2004	18	0418W44	154	158	147	153	185	193	206	208
2004	18	0418W45	154	154	149	153	185	185	202	208
2004	18	0418W46	148	156	147	147	185	193	207	208
2004	18	0418W47	154	154	149	153	185	193	202	208
2004	18	0418W48	154	158	141	147	185	189	207	208
2004	18	0418W49	154	156	147	153	185	195	202	203
2004	18	0418W50	-1	-1	149	153	193	195	202	208
2004	18	0418W51	148	156	149	153	185	195	202	208
2004	18	0418W52	148	158	141	149	-1	-1	207	208
2004	18	0418W53	154	154	-1	-1	185	185	202	203
2004	18	0418W54	148	158	149	153	185	193	206	208
2004	18	0418W55	148	158	141	147	185	189	207	208
2004	18	0418W56	154	158	141	147	189	193	207	208
2004	19	0419W41	150	156	147	149	185	191	203	207

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2004	19	0419W42	148	154	145	149	191	197	201	203
2004	19	0419W43	148	156	149	155	191	197	201	207
2004	19	0419W44	150	154	145	149	193	197	201	207
2004	19	0419W45	150	156	153	155	193	197	201	203
2004	19	0419W46	150	154	149	149	191	195	193	203
2004	19	0419W47	150	154	145	149	-1	-1	-1	-1
2004	19	0419W48	150	154	-1	-1	181	191	203	203
2004	19	0419W49	-1	-1	145	149	-1	-1	-1	-1
2004	19	0419W50	150	-1	149	153	187	191	203	207
2004	19	0419W51	-1	-1	145	153	191	197	201	203
2004	19	0419W52	148	156	149	155	193	197	201	203
2004	19	0419W53	150	156	149	155	193	197	201	203
2004	19	0419W54	148	148	153	155	187	193	203	207
2004	19	0419W55	148	148	149	155	187	191	203	207
2004	19	0419W56	148	156	149	155	193	197	201	207
2004	23	0423W41	150	156	145	147	191	197	193	207
2004	23	0423W42	154	154	147	151	183	191	203	203
2004	23	0423W43	150	154	147	149	181	193	193	207
2004	23	0423W44	150	154	147	151	183	193	193	203
2004	23	0423W45	154	154	147	151	183	191	203	203
2004	23	0423W46	150	156	145	147	191	197	193	207
2004	23	0423W47	150	154	141	147	185	193	201	207
2004	23	0423W48	150	156	145	147	191	197	193	207
2004	23	0423W49	154	156	147	147	-1	-1	-1	-1
2004	23	0423W50	150	156	145	147	191	197	193	207
2004	23	0423W51	154	154	147	151	183	191	203	203
2004	23	0423W52	150	154	147	149	181	193	193	207
2004	23	0423W53	150	156	145	147	191	197	193	207
2004	23	0423W54	150	156	141	147	185	191	201	207
2004	23	0423W55	150	154	147	151	183	193	193	203
2004	23	0423W56	150	156	141	147	185	193	201	207
2004	24	0424W41	152	154	141	149	191	193	201	203
2004	24	0424W42	150	150	141	147	189	193	203	204
2004	24	0424W43	150	156	149	149	173	193	203	206
2004	24	0424W44	152	154	147	149	179	191	203	207
2004	24	0424W45	150	156	147	149	173	179	203	206
2004	24	0424W46	152	152	149	149	193	195	203	207
2004	24	0424W47	152	156	147	149	173	193	203	206
2004	24	0424W48	150	150	147	149	193	195	206	207
2004	24	0424W49	152	152	149	149	193	195	206	207
2004	24	0424W50	150	152	149	149	193	195	206	207
2004	24	0424W51	150	154	149	149	191	193	206	207
2004	24	0424W52	150	152	141	147	179	189	203	204
2004	24	0424W53	150	154	147	149	179	191	203	207
2004	24	0424W54	152	156	149	149	193	193	203	203
2004	24	0424W55	150	152	141	147	189	193	203	204
2004	24	0424W56	150	152	147	149	179	195	206	207
2004	28	0428W1	160	162	149	-1	181	195	189	207
2004	28	0428W2	160	162	149	-1	181	181	189	207

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2004	28	0428W3	160	162	149	-1	191	195	189	203
2004	28	0428W4	160	162	149	-1	181	191	189	207
2004	28	0428W5	158	162	149	149	191	195	203	207
2004	28	0428W6	160	162	149	-1	181	195	189	207
2004	28	0428W7	158	162	149	149	181	195	203	203
2004	28	0428W8	158	162	145	149	181	187	201	203
2004	28	0428W9	158	162	147	149	187	191	189	203
2004	28	0428W10	158	162	149	149	191	195	203	207
2004	28	0428W11	160	162	149	-1	181	195	189	207
2004	28	0428W12	160	162	149	-1	181	195	189	207
2004	28	0428W13	158	162	147	149	187	191	189	203
2004	28	0428W14	158	162	147	149	181	191	189	203
2004	28	0428W15	160	162	149	-1	-1	-1	189	207
2004	28	0428W16	158	162	149	149	181	195	203	207
2004	29	0429W1	148	158	141	149	185	187	203	207
2004	29	0429W2	146	158	147	149	179	191	203	206
2004	29	0429W3	148	158	141	149	185	187	203	207
2004	29	0429W4	148	158	141	149	185	187	203	207
2004	29	0429W5	146	158	147	149	179	191	203	206
2004	29	0429W6	148	158	141	149	185	187	203	203
2004	29	0429W7	148	158	141	149	185	187	203	203
2004	29	0429W8	146	156	147	149	179	191	206	207
2004	29	0429W9	152	156	141	149	191	191	203	207
2004	29	0429W10	146	156	147	149	179	191	203	206
2004	29	0429W11	148	156	141	149	185	187	203	203
2004	29	0429W12	152	156	147	149	191	191	203	207
2004	29	0429W13	146	156	147	149	179	191	203	206
2004	29	0429W14	148	156	141	149	185	187	203	207
2004	29	0429W15	152	156	147	149	191	191	207	207
2004	29	0429W16	148	156	141	149	185	187	203	203
2004	43	0443W1	148	148	149	153	191	195	203	207
2004	43	0443W2	148	148	149	153	195	195	203	207
2004	43	0443W3	148	156	149	-1	195	195	207	207
2004	43	0443W4	156	156	149	155	183	191	203	207
2004	43	0443W5	148	154	-1	-1	191	191	206	207
2004	43	0443W6	148	156	149	155	183	191	203	207
2004	43	0443W7	148	156	149	153	191	195	203	207
2004	43	0443W8	156	156	141	149	191	195	203	207
2004	43	0443W9	148	156	149	153	191	195	203	207
2004	43	0443W10	154	156	149	149	191	191	206	207
2004	43	0443W11	154	156	149	149	191	191	206	207
2004	43	0443W12	148	156	149	153	195	195	203	207
2004	43	0443W13	156	156	149	-1	195	195	207	207
2004	43	0443W14	156	156	149	-1	191	195	207	207
2004	43	0443W15	148	156	149	155	183	191	203	207
2004	43	0443W16	156	156	141	149	195	195	203	207
2004	45	0445W1	150	154	141	147	191	195	203	207
2004	45	0445W2	158	158	145	147	195	-1	203	207
2004	45	0445W3	158	158	141	149	195	195	203	207

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2004	45	0445W4	158	158	141	149	195	195	203	207
2004	45	0445W5	158	158	145	147	195	-1	207	207
2004	45	0445W6	156	158	147	159	187	195	206	207
2004	45	0445W7	158	158	141	149	195	195	203	207
2004	45	0445W8	156	158	141	159	187	195	206	207
2004	45	0445W9	150	156	147	159	187	195	206	207
2004	45	0445W10	150	158	141	149	195	195	203	207
2004	45	0445W11	154	158	147	147	191	195	203	207
2004	45	0445W12	158	158	147	149	195	195	203	207
2004	45	0445W13	150	156	141	159	187	195	206	207
2004	45	0445W14	150	158	147	147	195	195	203	203
2004	45	0445W15	150	158	147	149	195	195	203	203
2004	45	0445W16	158	158	147	149	195	195	203	207
2006	63	0663P1	154	154	-1	-1	193	199	203	203
2006	63	0663P2	152	156	147	147	179	193	203	205
2006	63	0663P6	158	154	147	159	185	191	201	205
2006	63	0663P7	150	156	147	147	187	193	205	205
2006	63	0663P8	156	162	155	159	-1	-1	205	211
2006	63	0663P14	152	154	147	147	179	191	205	205
2006	63	0663P16	154	158	-1	-1	189	191	203	205
2006	63	0663P17	156	162	155	159	191	191	203	211
2006	63	0663P18	156	158	141	147	189	191	203	203
2006	63	0663L18	154	158	-1	-1	185	193	201	205
2006	63	0663L19	156	158	141	147	189	193	203	203
2006	63	0663L20	154	162	147	155	191	193	203	211
2006	63	0663L22	152	154	147	159	179	191	205	205
2006	63	0663L23	154	156	147	159	185	193	201	203
2006	63	0663L24	-1	-1	147	147	179	193	205	205
2006	65	0665P1	150	154	147	147	189	193	205	207
2006	65	0665P2	146	150	149	149	193	193	189	205
2006	65	0665P3	146	148	149	149	193	193	189	207
2006	65	0665P4	148	154	147	147	193	193	207	207
2006	65	0665P6	148	156	149	149	193	193	201	207
2006	65	0665P7	146	150	149	149	193	193	189	205
2006	65	0665P8	146	148	149	149	193	193	189	207
2006	65	0665P10	150	154	147	149	193	193	207	207
2006	65	0665P11	148	154	147	147	193	193	205	207
2006	65	0665P13	148	154	147	147	189	193	207	207
2006	65	0665P14	148	156	147	149	193	193	201	205
2006	65	0665P21	148	154	147	147	189	193	207	207
2006	65	0665P27	148	156	147	149	193	193	201	207
2006	66	0666P1	152	156	141	153	193	193	202	207
2006	66	0666P2	156	158	147	149	187	193	205	205
2006	66	0666P3	148	152	141	153	193	193	205	207
2006	66	0666P4	152	152	141	153	193	193	205	207
2006	66	0666P5	152	158	147	149	187	193	205	205
2006	66	0666P11	150	152	149	153	173	193	205	205
2006	66	0666P12	150	152	149	153	173	193	205	205
2006	66	0666P13	152	158	147	149	-1	-1	202	205

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2006	66	0666P14	150	156	149	153	173	193	205	205
2006	66	0666P16	150	156	149	153	173	193	205	205
2006	66	0666L31	148	152	-1	-1	193	193	205	207
2006	66	0666L32	152	156	141	153	193	193	202	207
2006	66	0666L33	148	152	141	153	-1	-1	202	207
2006	67	0667P1	148	152	-1	-1	179	193	205	205
2006	67	0667P2	148	152	147	153	-1	-1	205	207
2006	67	0667P3	-1	-1	149	149	173	179	201	207
2006	67	0667P5	154	154	141	149	187	193	201	207
2006	67	0667P6	152	152	149	153	173	179	201	207
2006	67	0667P7	-1	-1	149	149	179	187	-1	-1
2006	67	0667P9	152	154	149	153	187	179	-1	-1
2006	67	0667P10	148	154	-1	-1	179	193	-1	-1
2006	67	0667P11	152	154	149	153	187	193	207	207
2006	67	0667P14	154	158	149	153	179	187	205	207
2006	67	0667P15	152	154	149	153	179	179	205	205
2006	67	0667P16	154	158	149	149	-1	-1	201	205
2006	67	0667P17	152	154	149	149	179	193	205	207
2006	67	0667P23	154	158	149	149	185	187	201	207
2006	68	0668P1	154	156	147	147	191	193	189	207
2006	68	0668P2	154	156	-1	-1	179	185	189	207
2006	68	0668P3	152	154	147	151	179	189	189	202
2006	68	0668P4	152	154	147	147	185	191	-1	-1
2006	68	0668P5	-1	-1	147	149	185	191	189	207
2006	68	0668P6	154	156	147	149	-1	-1	205	207
2006	68	0668P7	152	156	147	149	-1	-1	189	207
2006	68	0668P8	-1	-1	149	149	-1	-1	-1	-1
2006	68	0668P9	-1	-1	-1	-1	189	191	189	205
2006	68	0668P10	-1	-1	149	149	191	193	202	205
2006	68	0668P11	152	154	147	149	179	185	189	207
2006	68	0668P13	154	156	147	147	191	193	-1	-1
2006	68	0668P14	-1	-1	141	147	191	193	189	205
2006	68	0668P15	152	154	149	151	179	189	202	205
2006	69	0669P13	150	152	149	151	193	193	201	203
2006	69	0669P14	150	152	141	149	193	193	203	205
2006	69	0669P15	150	158	141	147	189	193	203	205
2006	69	0669P16	150	152	141	145	193	193	201	203
2006	69	0669P17	152	156	141	145	193	193	203	205
2006	69	0669P18	152	156	141	145	189	193	203	205
2006	69	0669P19	152	156	149	151	189	193	203	205
2006	69	0669P20	150	158	141	149	193	193	201	203
2006	69	0669P21	150	158	141	147	189	193	203	205
2006	69	0669P22	150	152	141	145	193	193	201	203
2006	69	0669P23	150	158	141	147	193	193	201	203
2006	69	0669P24	150	152	141	145	-1	-1	-1	-1
2006	69	0669P25	150	152	141	147	189	193	201	203
2006	69	0669P26	150	152	141	145	-1	-1	-1	-1
2006	70	0670P1	152	152	147	147	173	193	206	213
2006	70	0670P2	152	152	147	147	185	185	206	206

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2006	70	0670P3	152	152	147	147	185	193	206	206
2006	70	0670P4	152	152	147	147	193	193	203	206
2006	70	0670P5	152	152	141	157	193	193	206	206
2006	70	0670P6	152	152	147	147	193	193	-1	-1
2006	70	0670P7	152	152	147	147	185	185	189	206
2006	70	0670P8	152	152	147	147	173	185	206	213
2006	70	0670P9	-1	-1	147	147	-1	-1	-1	-1
2006	70	0670P10	152	152	-1	-1	-1	-1	-1	-1
2006	70	0670P11	152	152	147	147	185	193	206	213
2006	70	0670P12	152	152	141	141	-1	-1	-1	-1
2006	70	0670P13	152	152	141	147	185	193	206	206
2006	70	0670P14	152	152	147	147	193	193	203	213
2006	70	0670P15	-1	-1	-1	-1	-1	-1	-1	-1
2006	70	0670P16	152	152	147	147	193	193	-1	-1
2006	72	0672L11	150	156	149	149	173	193	193	206
2006	72	0672L13	-1	-1	147	147	187	193	203	206
2006	72	0672L14	150	156	147	149	173	193	193	206
2006	72	0672L17	152	154	147	149	179	193	206	206
2006	72	0672L19	154	154	147	149	187	193	203	209
2006	72	0672L20	150	154	147	149	187	193	185	206
2006	72	0672L22	152	154	147	149	179	193	206	206
2006	72	0672L24	154	154	147	149	193	193	203	206
2006	72	0672L25	150	156	147	149	173	187	193	209
2006	72	0672L27	154	156	147	149	187	193	203	209
2006	72	0672L29	154	156	147	149	187	193	203	206
2006	72	0672L32	152	154	147	149	179	187	206	206
2006	72	0672L36	154	156	147	149	193	-1	203	209
2006	72	0672L38	150	156	147	149	187	193	185	209
2006	76	0676P1	152	152	151	157	-1	-1	-1	-1
2006	76	0676P2	152	152	149	151	193	193	206	206
2006	76	0676P3	152	152	145	157	185	191	201	201
2006	76	0676P4	148	148	149	151	193	193	201	203
2006	76	0676P5	152	156	149	149	191	191	203	206
2006	76	0676P6	148	148	149	151	193	193	203	206
2006	76	0676P7	148	152	151	157	193	193	201	206
2006	76	0676P8	148	156	149	149	191	193	203	206
2006	76	0676P9	148	148	151	157	191	193	201	203
2006	76	0676P10	148	156	149	149	191	193	203	206
2006	76	0676P11	152	152	149	151	191	193	206	206
2006	76	0676P12	148	154	149	157	191	191	206	208
2006	76	0676P13	152	156	149	149	191	191	201	203
2006	76	0676P14	152	152	147	149	193	193	206	208
2006	76	0676P15	148	152	149	149	191	193	201	206
2006	76	0676P16	148	156	149	151	191	193	-1	-1
2006	76	0676P18	148	156	-1	-1	191	191	201	203
2006	76	0676P19	152	152	145	149	185	191	201	201
2006	76	0676P20	150	152	149	149	191	191	203	206
2006	76	0676P24	148	156	149	149	191	191	201	203
2006	76	0676P26	148	156	149	149	191	191	203	206

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2006	76	0676P27	152	154	141	149	191	193	-1	-1
2006	76	0676P28	148	154	149	151	191	193	206	208
2006	76	0676P38	148	148	149	149	191	193	201	206
2006	76	0676P40	152	154	149	151	191	193	201	208
2006	77	0677P1	154	154	145	149	193	193	207	207
2006	77	0677P2	154	154	147	147	185	185	207	207
2006	77	0677P3	152	156	147	153	185	185	201	207
2006	77	0677P4	154	156	145	147	185	193	203	203
2006	77	0677P5	156	156	147	147	185	185	203	203
2006	77	0677P6	154	156	147	149	187	193	207	207
2006	77	0677P7	154	154	145	145	193	193	203	207
2006	77	0677P8	154	156	145	147	185	185	203	207
2006	77	0677P9	156	156	147	147	193	193	203	207
2006	77	0677P10	154	154	147	149	187	193	207	207
2006	77	0677P11	156	156	145	147	185	193	203	207
2006	77	0677P12	154	154	145	147	185	185	207	207
2006	77	0677P13	154	156	145	149	193	193	207	207
2006	77	0677P14	156	156	145	147	193	193	203	203
2006	77	0677P15	156	156	147	147	185	185	203	203
2006	77	0677P16	154	156	145	145	185	193	203	203
2006	77	0677P21	152	156	141	145	191	193	207	209
2006	77	0677P22	152	154	141	145	185	191	207	209
2006	77	0677P26	154	156	147	149	187	193	203	207
2006	77	0677P29	-1	-1	147	149	185	187	-1	-1
2006	77	0677P31	154	156	145	147	193	193	203	207
2006	77	0677P36	152	156	141	147	191	193	203	209
2006	77	0677P38	152	154	145	153	185	193	201	203
2006	77	0677P40	154	156	145	147	185	193	203	207
2006	77	0677L6	152	154	145	153	185	193	201	207
2006	77	0677L9	152	156	141	145	191	193	207	209
2006	77	0677L22	154	156	147	147	185	191	205	207
2006	77	0677L40	154	154	-1	-1	189	193	201	207
2006	79	0679P1	148	154	147	147	181	189	201	203
2006	79	0679P2	154	154	147	147	189	189	203	203
2006	79	0679P3	154	154	147	147	189	189	203	203
2006	79	0679P4	148	152	147	149	189	191	201	206
2006	79	0679P5	152	152	147	147	189	189	201	204
2006	79	0679P6	154	156	147	147	181	191	201	203
2006	79	0679P7	154	154	147	147	181	189	203	203
2006	79	0679P8	154	154	147	147	181	181	203	203
2006	79	0679P9	152	152	147	147	181	189	201	206
2006	79	0679P10	152	156	147	147	181	191	201	203
2006	79	0679P11	154	156	147	149	181	193	185	201
2006	79	0679P12	152	156	147	147	181	191	201	203
2006	79	0679P13	152	156	147	149	181	193	185	201
2006	79	0679P14	148	154	147	149	189	191	203	206
2006	79	0679P15	152	152	147	147	181	181	201	206
2006	79	0679P16	152	154	147	147	189	189	201	204
2017	195	17195W1	150	152	149	149	-1	-1	207	207

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2017	195	17195W2	152	154	149	149	173	191	207	207
2017	195	17195W3	152	154	149	149	-1	-1	-1	-1
2017	195	17195W4	148	150	147	149	185	191	209	209
2017	195	17195W5	154	154	149	149	189	191	209	211
2017	195	17195W6	150	152	149	149	173	193	207	207
2017	195	17195W7	150	158	147	147	191	193	209	209
2017	195	17195W8	154	154	149	149	189	193	209	211
2017	195	17195W9	154	154	149	149	189	191	207	211
2017	195	17195W10	150	154	149	149	189	191	207	211
2017	195	17195W11	154	154	147	147	191	191	209	209
2017	195	17195W12	150	152	149	149	173	193	207	207
2017	195	17195W13	154	154	141	149	191	193	205	209
2017	195	17195W14	154	158	147	149	193	193	209	209
2017	195	17195W15	150	152	147	149	173	191	207	209
2017	195	17195W16	150	154	141	147	191	193	205	209
2017	196	17196W1	156	156	147	151	179	193	201	203
2017	196	17196W2	146	156	149	151	191	191	201	209
2017	196	17196W3	148	156	147	151	189	193	205	206
2017	196	17196W4	148	156	147	159	189	193	205	206
2017	196	17196W5	156	156	147	159	179	193	203	205
2017	196	17196W6	148	156	147	159	189	191	201	205
2017	196	17196W7	148	156	147	159	189	191	201	205
2017	196	17196W8	146	156	149	151	191	191	201	209
2017	196	17196W9	146	156	149	159	191	191	201	209
2017	196	17196W10	146	156	149	151	191	193	205	209
2017	196	17196W11	156	156	145	159	191	191	201	205
2017	196	17196W12	148	156	147	151	191	199	205	211
2017	196	17196W13	146	156	149	151	191	193	205	209
2017	196	17196W14	150	156	147	151	179	193	205	206
2017	196	17196W15	156	156	147	159	179	193	205	209
2017	196	17196W16	146	156	149	151	191	193	205	209
2017	201	17201W1	154	154	-1	-1	193	193	211	211
2017	201	17201W2	152	154	-1	-1	191	193	211	211
2017	201	17201W3	154	156	151	153	181	193	201	206
2017	201	17201W4	154	156	147	147	181	193	209	211
2017	201	17201W5	150	154	147	153	179	193	201	206
2017	201	17201W6	154	156	147	153	181	193	209	211
2017	201	17201W7	152	154	147	153	181	193	206	209
2017	201	17201W8	154	156	147	147	181	193	206	209
2017	201	17201W9	152	154	147	153	181	193	209	211
2017	201	17201W10	154	156	147	153	173	181	209	211
2017	201	17201W11	154	156	147	147	173	181	206	209
2017	201	17201W12	154	156	147	153	173	181	206	209
2017	201	17201W13	154	156	151	153	181	193	201	206
2017	201	17201W14	-1	-1	151	153	173	181	201	206
2017	201	17201W15	154	156	151	153	173	181	201	206
2017	201	17201W16	154	156	147	153	181	193	206	209
2017	204	17204W1	162	-1	145	155	193	187	-1	-1
2017	204	17204W2	178	-1	147	155	193	193	-1	-1

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2017	204	17204W3	178	-1	141	147	-1	-1	-1	-1
2017	204	17204W4	178	-1	141	147	-1	-1	-1	-1
2017	204	17204W5	-1	-1	149	155	193	193	-1	-1
2017	204	17204W6	178	-1	145	155	187	187	-1	-1
2017	204	17204W7	178	-1	149	155	187	193	-1	-1
2017	204	17204W8	-1	-1	149	155	187	191	-1	-1
2017	204	17204W9	178	-1	149	155	187	193	-1	-1
2017	204	17204W10	-1	-1	149	155	187	193	-1	-1
2017	204	17204W11	-1	-1	141	149	-1	-1	-1	-1
2017	204	17204W12	-1	-1	149	155	-1	-1	-1	-1
2017	204	17204W13	178	-1	149	155	187	-1	-1	-1
2017	204	17204W14	-1	-1	141	145	187	187	-1	-1
2017	204	17204W15	178	-1	141	147	187	193	-1	-1
2017	204	17204W16	178	-1	141	147	193	193	-1	-1
2017	205	17205W1	152	152	157	157	173	185	205	208
2017	205	17205W2	150	162	147	147	173	191	203	205
2017	205	17205W3	-1	-1	149	157	191	191	203	205
2017	205	17205W4	150	152	147	149	191	191	203	205
2017	205	17205W5	152	162	147	147	191	191	-1	-1
2017	205	17205W6	150	152	147	157	185	191	205	208
2017	205	17205W7	150	152	149	157	173	185	-1	-1
2017	205	17205W8	152	162	147	147	191	191	203	205
2017	205	17205W9	152	152	147	149	173	185	205	208
2017	205	17205W10	-1	-1	147	157	185	191	205	208
2017	205	17205W11	152	152	147	149	185	191	205	208
2017	205	17205W12	150	162	147	147	191	191	203	205
2017	205	17205W13	152	152	149	157	185	191	205	208
2017	205	17205W14	152	152	149	157	185	191	205	208
2017	205	17205W15	152	152	147	149	173	191	203	205
2017	205	17205W16	150	152	149	157	185	191	205	208
2017	226	17226W1	154	158	147	147	179	191	203	207
2017	226	17226W2	150	154	147	147	185	193	207	209
2017	226	17226W3	152	154	151	151	-1	-1	-1	-1
2017	226	17226W4	152	154	151	151	179	187	205	207
2017	226	17226W5	152	154	151	151	-1	-1	-1	-1
2017	226	17226W6	150	152	147	151	185	193	205	209
2017	226	17226W7	154	154	151	147	-1	-1	-1	-1
2017	226	17226W8	154	154	149	151	179	191	207	207
2017	226	17226W9	154	158	147	151	179	191	203	205
2017	226	17226W10	154	154	151	151	179	187	205	207
2017	226	17226W11	152	154	147	149	179	191	207	207
2017	226	17226W12	154	154	151	151	179	187	205	207
2017	226	17226W15	152	154	147	147	185	193	193	205
2017	226	17226W16	150	152	147	151	185	193	205	209
2017	228	17228W1	154	158	147	151	193	193	205	211
2017	228	17228W2	154	158	147	151	193	193	205	206
2017	228	17228W3	154	158	149	151	193	193	205	211
2017	228	17228W4	152	158	149	157	185	193	205	211
2017	228	17228W5	152	158	141	147	191	191	206	209

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2017	228	17228W6	152	158	-1	-1	185	193	205	206
2017	228	17228W7	152	158	141	147	191	193	209	211
2017	228	17228W8	154	158	149	151	191	193	205	211
2017	228	17228W9	152	158	149	157	185	193	205	211
2017	228	17228W10	154	158	141	149	191	191	209	211
2017	228	17228W11	152	158	141	147	191	191	209	211
2017	228	17228W12	154	158	147	151	193	193	205	211
2017	228	17228W13	152	158	147	157	185	193	205	206
2017	228	17228W14	154	158	147	151	191	193	205	206
2017	228	17228W15	154	158	149	151	193	193	205	206
2017	228	17228W16	152	158	141	149	191	193	206	209
2017	229	17229W1	148	152	147	153	185	193	209	209
2017	229	17229W2	154	154	147	149	173	193	203	205
2017	229	17229W3	148	152	147	147	185	193	203	209
2017	229	17229W4	152	152	141	153	181	193	203	209
2017	229	17229W5	152	152	-1	-1	191	193	209	209
2017	229	17229W6	148	152	147	147	-1	-1	-1	-1
2017	229	17229W7	154	154	147	153	173	189	209	213
2017	229	17229W8	152	154	147	153	173	189	209	213
2017	229	17229W9	148	152	147	153	185	193	209	209
2017	229	17229W10	148	152	147	153	173	185	209	209
2017	229	17229W11	148	152	147	153	173	185	203	209
2017	229	17229W12	152	154	147	149	173	193	209	209
2017	229	17229W13	152	152	141	153	173	181	203	203
2017	229	17229W14	152	152	141	147	173	181	203	203
2017	229	17229W15	148	152	147	147	173	185	209	209
2017	229	17229W16	154	154	149	153	173	193	205	209
2017	230	17230W1	152	154	147	159	179	181	205	213
2017	230	17230W2	152	154	147	147	179	193	205	213
2017	230	17230W3	154	158	149	149	179	193	203	213
2017	230	17230W4	154	158	149	149	179	193	203	213
2017	230	17230W5	154	158	149	149	179	193	203	213
2017	230	17230W6	154	158	149	149	193	193	203	213
2017	230	17230W8	154	154	-1	-1	193	193	206	213
2017	230	17230W9	154	158	149	151	189	193	185	213
2017	230	17230W10	154	158	149	149	179	193	203	213
2017	230	17230W11	154	154	149	155	179	193	206	213
2017	230	17230W12	154	154	147	159	179	181	205	213
2017	230	17230W13	154	154	149	155	193	193	206	213
2017	230	17230W14	152	154	147	159	179	181	205	213
2017	230	17230W15	154	158	149	151	179	193	185	213
2017	230	17230W16	152	154	147	159	179	181	205	213
2017	193	17193W1	154	156	153	153	185	191	207	207
2017	193	17193W2	152	152	147	153	191	193	207	207
2017	193	17193W3	152	154	147	153	185	191	207	207
2017	193	17193W4	148	152	147	153	173	187	207	209
2017	193	17193W5	152	156	147	153	173	193	207	207
2017	193	17193W6	152	154	149	153	173	191	207	209
2017	193	17193W7	152	152	147	153	173	193	207	207

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2017	193	17193W8	152	156	147	153	173	193	207	207
2017	193	17193W9	152	154	149	153	173	191	209	209
2017	193	17193W10	154	156	147	153	185	191	207	207
2017	193	17193W11	152	154	149	153	191	191	209	209
2017	193	17193W12	152	152	147	153	173	193	207	207
2017	193	17193W13	154	156	-1	-1	173	191	207	209
2017	193	17193W14	152	154	149	153	173	191	207	209
2017	193	17193W15	154	156	149	153	173	191	209	209
2017	193	17193W16	148	156	147	153	173	187	209	209
2017	198	17198W1	154	156	147	151	185	193	205	207
2017	198	17198W2	154	156	147	147	185	193	205	207
2017	198	17198W3	-1	-1	141	151	173	193	205	207
2017	198	17198W4	154	154	147	151	185	193	205	207
2017	198	17198W5	156	156	151	153	189	191	207	207
2017	198	17198W6	154	156	147	151	185	189	205	207
2017	198	17198W7	154	156	147	151	185	193	205	207
2017	198	17198W8	156	158	149	151	189	191	205	207
2017	198	17198W9	154	156	147	153	189	191	207	207
2017	198	17198W10	152	154	141	147	173	189	205	207
2017	198	17198W11	154	156	147	151	185	189	205	207
2017	198	17198W12	156	156	147	153	189	191	207	207
2017	198	17198W13	152	154	141	147	173	189	205	207
2017	198	17198W14	-1	-1	147	147	185	189	205	207
2017	198	17198W15	154	156	151	153	191	193	207	207
2017	198	17198W16	152	154	141	151	173	193	205	207
2017	199	17199W1	154	156	147	151	179	191	193	207
2017	199	17199W2	152	154	151	151	191	193	207	209
2017	199	17199W3	154	154	147	151	179	185	193	207
2017	199	17199W4	154	154	151	-1	185	189	205	207
2017	199	17199W5	150	156	151	151	185	193	207	209
2017	199	17199W6	152	156	151	151	185	193	209	209
2017	199	17199W7	150	156	149	151	189	191	205	207
2017	199	17199W8	154	154	151	-1	185	189	205	207
2017	199	17199W9	150	154	151	151	191	193	207	209
2017	199	17199W10	152	154	151	151	185	193	209	209
2017	199	17199W11	-1	-1	149	151	185	189	205	207
2017	199	17199W12	154	154	151	-1	185	189	205	207
2017	199	17199W13	150	156	149	151	189	191	205	207
2017	199	17199W14	154	156	147	151	179	191	193	209
2017	199	17199W15	154	154	151	151	185	193	205	209
2017	199	17199W16	154	154	151	151	185	189	207	207
2017	200	17200W1	152	154	151	153	179	193	205	211
2017	200	17200W2	154	154	141	147	187	189	203	208
2017	200	17200W3	150	154	141	151	189	189	203	208
2017	200	17200W4	150	154	141	147	179	193	211	211
2017	200	17200W5	154	154	141	147	187	189	203	208
2017	200	17200W6	146	152	149	151	179	189	203	208
2017	200	17200W7	150	152	141	151	179	189	208	211
2017	200	17200W8	152	154	147	151	173	179	-1	-1

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2017	200	17200W9	152	154	147	151	179	193	208	211
2017	200	17200W10	152	154	151	151	187	189	203	211
2017	200	17200W11	154	154	141	147	179	189	203	205
2017	200	17200W12	150	154	141	147	179	193	211	211
2017	200	17200W13	152	154	141	147	173	189	211	211
2017	200	17200W14	154	154	147	151	189	189	203	205
2017	200	17200W15	-1	-1	141	141	179	189	189	211
2017	200	17200W16	150	152	141	147	179	193	203	211
2017	203	17203W1	156	156	-1	-1	191	193	209	211
2017	203	17203W2	-1	-1	-1	-1	181	193	202	209
2017	203	17203W3	152	156	-1	-1	191	193	209	211
2017	203	17203W4	152	156	-1	-1	191	193	209	211
2017	203	17203W5	152	156	-1	-1	191	193	209	211
2017	203	17203W6	156	156	-1	-1	191	193	209	211
2017	203	17203W7	152	156	-1	-1	191	193	209	211
2017	203	17203W8	152	156	-1	-1	191	193	209	211
2017	203	17203W9	152	156	-1	-1	191	193	209	211
2017	203	17203W10	156	156	-1	-1	181	193	202	209
2017	203	17203W11	156	156	-1	-1	193	193	209	211
2017	203	17203W12	-1	-1	-1	-1	193	193	209	211
2017	203	17203W13	156	156	-1	-1	193	193	209	211
2017	203	17203W14	152	152	-1	-1	193	193	205	209
2017	203	17203W15	152	152	-1	-1	193	193	209	211
2017	203	17203W16	156	156	-1	-1	181	193	202	209
2017	209	17209W2	152	158	-1	-1	181	185	-1	-1
2017	209	17209W3	152	158	-1	-1	181	185	206	211
2017	209	17209W4	152	154	149	159	181	193	206	211
2017	209	17209W5	154	156	149	151	173	181	206	211
2017	209	17209W6	150	154	-1	-1	181	189	202	206
2017	209	17209W7	154	154	141	149	181	181	209	211
2017	209	17209W8	150	154	147	149	181	189	202	206
2017	209	17209W9	150	154	147	149	181	189	202	206
2017	209	17209W10	154	158	149	159	179	181	209	211
2017	209	17209W11	154	156	149	149	191	193	189	202
2017	209	17209W12	154	156	149	151	173	191	202	206
2017	209	17209W13	150	154	149	159	181	189	202	209
2017	209	17209W14	154	154	141	149	181	181	209	211
2017	209	17209W15	150	154	149	149	181	189	189	202
2017	209	17209W16	152	154	149	159	181	193	206	211
2017	210	17210W1	158	162	149	153	179	179	209	209
2017	210	17210W2	156	162	147	149	-1	-1	209	209
2017	210	17210W3	156	162	147	149	179	179	209	209
2017	210	17210W4	156	162	147	149	179	179	209	211
2017	210	17210W5	156	158	151	153	189	193	205	209
2017	210	17210W6	156	162	147	149	179	179	209	211
2017	210	17210W7	158	162	149	153	179	179	209	209
2017	210	17210W8	156	158	147	151	189	193	205	211
2017	210	17210W9	156	158	151	153	189	193	205	211
2017	210	17210W10	158	162	149	153	179	179	209	209

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2017	210	17210W11	158	162	147	149	179	179	209	209
2017	210	17210W12	156	158	149	153	179	191	189	211
2017	210	17210W13	158	162	147	149	179	179	209	211
2017	210	17210W14	158	162	149	153	179	193	209	209
2017	210	17210W15	158	162	149	153	179	193	209	209
2017	210	17210W16	156	158	147	149	179	191	189	211
2017	215	17215W1	152	152	147	149	191	191	189	211
2017	215	17215W2	152	152	147	149	191	191	204	211
2017	215	17215W3	152	156	147	147	191	191	189	211
2017	215	17215W4	148	158	147	147	173	191	204	209
2017	215	17215W5	152	158	149	153	185	191	204	203
2017	215	17215W6	152	152	149	153	191	191	189	204
2017	215	17215W7	152	152	149	153	191	191	189	204
2017	215	17215W8	152	152	149	153	185	191	189	203
2017	215	17215W9	156	158	147	149	191	193	189	193
2017	215	17215W10	152	152	149	149	191	191	189	209
2017	215	17215W11	150	158	149	149	185	191	204	211
2017	215	17215W12	150	158	149	153	187	191	204	207
2017	215	17215W13	152	152	149	149	191	191	189	209
2017	215	17215W14	150	158	149	149	185	191	204	211
2017	215	17215W15	156	158	147	147	191	193	189	193
2017	215	17215W16	152	158	147	147	191	191	189	211
2017	217	17217W1	148	158	147	149	191	191	203	211
2017	217	17217W2	152	154	147	147	191	193	203	211
2017	217	17217W3	154	158	147	147	191	193	189	203
2017	217	17217W4	158	158	147	149	191	191	189	203
2017	217	17217W5	148	152	149	153	189	191	203	211
2017	217	17217W6	148	152	147	149	189	191	203	211
2017	217	17217W7	152	154	147	153	191	193	189	203
2017	217	17217W8	152	154	147	147	191	193	189	203
2017	217	17217W9	154	158	147	153	191	193	189	203
2017	217	17217W10	154	158	147	147	191	193	189	203
2017	217	17217W11	148	152	147	149	189	191	203	211
2017	217	17217W12	148	158	147	149	189	193	189	203
2017	217	17217W13	152	154	147	147	191	193	189	203
2017	217	17217W14	148	152	147	149	-1	-1	-1	-1
2017	217	17217W15	148	152	147	149	-1	-1	-1	-1
2017	217	17217W16	148	158	147	149	189	191	203	211
2017	221	17221W1	148	158	149	149	191	193	205	213
2017	221	17221W2	148	154	147	149	191	193	205	205
2017	221	17221W3	148	158	149	149	191	193	205	213
2017	221	17221W4	154	154	141	151	179	191	207	209
2017	221	17221W5	148	154	147	149	191	193	205	205
2017	221	17221W6	148	158	147	149	191	199	205	213
2017	221	17221W7	148	154	141	147	191	199	205	213
2017	221	17221W8	148	154	141	149	191	193	207	209
2017	221	17221W9	148	154	141	147	191	199	205	213
2017	221	17221W10	148	154	141	149	191	193	207	209
2017	221	17221W11	154	154	141	151	179	191	205	209

Table A-1 continued

Year	Colony	Individual	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2017	221	17221W12	148	158	141	149	191	193	205	213
2017	221	17221W13	148	154	141	147	-1	-1	205	213
2017	221	17221W14	148	154	141	149	191	193	207	209
2017	221	17221W15	148	154	141	149	191	193	207	209
2017	221	17221W16	148	158	149	149	191	193	205	213
2017	227	17227W1	148	152	141	149	193	193	205	209
2017	227	17227W2	148	152	141	149	193	193	205	209
2017	227	17227W3	148	156	149	149	181	193	209	209
2017	227	17227W4	148	156	149	149	173	181	209	209
2017	227	17227W5	148	152	149	159	193	193	209	209
2017	227	17227W6	148	156	-1	-1	173	191	203	209
2017	227	17227W7	148	152	141	149	193	193	205	209
2017	227	17227W8	148	156	149	149	181	193	209	209
2017	227	17227W9	148	152	149	159	173	193	209	209
2017	227	17227W10	148	152	149	159	193	193	209	209
2017	227	17227W11	148	152	141	149	173	193	205	209
2017	227	17227W12	148	152	149	159	173	193	209	209
2017	227	17227W13	148	156	149	159	191	193	203	209
2017	227	17227W14	148	156	149	149	181	193	209	209
2017	227	17227W15	148	156	149	159	173	191	203	209

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2004	8	0408W41	164	168	160	164	139	148	-1	-1
2004	8	0408W42	166	168	154	160	139	148	232	238
2004	8	0408W43	166	168	154	160	139	148	232	238
2004	8	0408W44	164	168	160	164	139	148	234	252
2004	8	0408W45	160	166	154	160	148	148	252	-1
2004	8	0408W46	166	168	154	160	139	148	238	-1
2004	8	0408W47	164	168	160	164	139	148	-1	-1
2004	8	0408W48	166	168	154	160	139	148	238	-1
2004	8	0408W49	166	168	154	160	148	148	252	-1
2004	8	0408W50	164	168	160	164	148	148	234	252
2004	8	0408W51	166	168	154	160	148	148	232	252
2004	8	0408W52	166	168	154	160	148	152	242	252
2004	8	0408W53	164	168	164	164	148	148	234	252
2004	8	0408W54	164	168	160	164	148	148	234	252
2004	8	0408W55	168	170	158	160	148	148	234	252
2004	8	0408W56	168	170	158	160	148	148	234	238
2004	10	0410W41	168	170	154	166	139	148	236	238
2004	10	0410W42	168	170	158	158	139	148	236	252
2004	10	0410W43	-1	-1	158	166	139	148	236	238
2004	10	0410W44	168	170	154	158	139	148	236	252
2004	10	0410W45	168	170	158	166	139	148	236	238
2004	10	0410W46	168	170	154	158	-1	-1	236	252
2004	10	0410W47	168	170	158	158	139	148	236	252
2004	10	0410W48	168	168	158	158	148	152	236	252
2004	10	0410W49	168	168	158	158	139	148	236	252
2004	10	0410W50	168	170	154	158	139	148	236	252
2004	10	0410W51	164	168	154	172	139	148	234	238
2004	10	0410W52	168	168	158	158	139	148	236	252
2004	10	0410W53	168	170	154	166	139	148	236	238
2004	10	0410W54	164	168	154	158	139	148	236	252
2004	10	0410W55	164	168	158	158	139	148	236	252
2004	10	0410W56	164	168	158	172	139	148	234	238
2004	13	0413W41	164	168	154	176	139	148	236	246
2004	13	0413W42	166	168	154	160	139	148	236	244
2004	13	0413W43	166	166	154	166	139	148	-1	-1
2004	13	0413W44	166	168	154	166	139	148	236	246
2004	13	0413W45	166	168	154	166	139	148	236	246
2004	13	0413W46	166	168	154	166	139	148	236	246
2004	13	0413W47	164	168	154	158	139	139	-1	-1
2004	13	0413W48	166	168	154	166	139	148	-1	-1
2004	13	0413W49	166	166	154	160	139	148	-1	-1
2004	13	0413W50	166	166	154	154	139	148	-1	-1
2004	13	0413W51	166	166	158	166	139	148	236	246
2004	13	0413W52	166	168	154	166	139	148	236	246
2004	13	0413W53	164	168	154	158	139	148	-1	-1
2004	13	0413W54	166	168	154	160	139	148	-1	-1
2004	13	0413W55	164	168	154	158	139	148	252	252
2004	13	0413W56	166	168	154	166	139	148	236	246

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2004	15	0415W1	160	166	-1	-1	-1	-1	238	-1
2004	15	0415W2	160	168	154	166	137	148	234	-1
2004	15	0415W3	164	166	154	166	-1	148	-1	-1
2004	15	0415W4	160	168	154	166	137	148	234	-1
2004	15	0415W5	166	168	154	166	144	148	234	238
2004	15	0415W6	164	166	158	166	148	148	236	238
2004	15	0415W7	168	168	154	166	139	144	234	-1
2004	15	0415W8	160	166	154	154	139	139	238	238
2004	15	0415W9	164	168	154	154	137	148	-1	-1
2004	15	0415W10	166	168	158	166	139	144	234	238
2004	15	0415W11	164	168	154	158	137	148	234	-1
2004	15	0415W12	160	168	154	166	137	148	234	-1
2004	15	0415W13	166	168	154	166	139	144	234	238
2004	15	0415W14	160	166	154	166	137	139	234	-1
2004	15	0415W15	160	166	158	166	137	148	-1	-1
2004	15	0415W16	160	166	158	166	137	139	234	-1
2004	16	0416W41	166	168	154	158	139	148	234	238
2004	16	0416W42	168	168	158	166	135	139	234	246
2004	16	0416W43	166	168	154	166	139	148	236	238
2004	16	0416W44	166	168	154	166	139	148	234	248
2004	16	0416W45	168	168	158	164	139	148	234	238
2004	16	0416W46	166	168	154	166	139	148	234	234
2004	16	0416W47	166	168	154	158	139	148	234	238
2004	16	0416W48	166	168	154	158	139	148	234	238
2004	16	0416W49	166	168	158	158	139	148	236	250
2004	16	0416W50	168	168	154	166	139	148	234	238
2004	16	0416W51	168	168	154	166	139	148	234	238
2004	16	0416W52	166	168	154	166	148	152	234	248
2004	16	0416W53	168	168	154	166	139	148	234	238
2004	16	0416W54	166	168	158	158	139	148	236	250
2004	16	0416W55	166	168	158	158	139	148	236	250
2004	16	0416W56	168	168	158	166	135	139	236	246
2004	18	0418W41	166	166	158	160	139	148	-1	-1
2004	18	0418W42	170	170	154	158	139	148	-1	-1
2004	18	0418W43	170	170	154	158	139	148	-1	-1
2004	18	0418W44	166	170	-1	-1	139	152	226	236
2004	18	0418W45	166	168	158	166	139	148	236	244
2004	18	0418W46	166	170	158	160	139	148	236	236
2004	18	0418W47	168	170	158	166	139	148	236	244
2004	18	0418W48	166	166	158	160	139	148	236	234
2004	18	0418W49	170	170	154	158	139	148	238	246
2004	18	0418W50	170	170	154	158	139	148	236	246
2004	18	0418W51	166	170	154	158	139	148	238	246
2004	18	0418W52	166	166	158	160	139	148	-1	-1
2004	18	0418W53	166	168	158	166	139	148	238	244
2004	18	0418W54	166	166	154	158	139	152	226	238
2004	18	0418W55	166	170	158	160	139	148	234	236
2004	18	0418W56	166	166	158	160	139	148	234	236
2004	19	0419W41	168	168	154	158	148	152	232	236

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2004	19	0419W42	164	168	154	166	135	152	236	236
2004	19	0419W43	164	168	158	158	144	152	232	236
2004	19	0419W44	164	168	154	166	135	152	236	246
2004	19	0419W45	164	168	158	158	144	152	232	236
2004	19	0419W46	168	168	158	158	148	152	232	236
2004	19	0419W47	164	168	154	166	135	148	-1	-1
2004	19	0419W48	164	168	154	158	148	152	232	236
2004	19	0419W49	164	168	154	166	135	148	236	246
2004	19	0419W50	168	168	154	154	148	152	246	246
2004	19	0419W51	164	168	158	166	135	152	236	246
2004	19	0419W52	164	168	154	158	144	152	232	236
2004	19	0419W53	164	168	154	158	144	152	232	236
2004	19	0419W54	168	168	158	166	148	152	234	236
2004	19	0419W55	168	168	154	166	148	152	234	246
2004	19	0419W56	164	168	154	158	144	152	232	246
2004	23	0423W41	168	170	158	160	139	148	244	244
2004	23	0423W42	166	170	154	154	148	152	236	236
2004	23	0423W43	166	170	154	160	139	148	236	246
2004	23	0423W44	168	170	158	160	139	152	236	236
2004	23	0423W45	168	170	154	158	139	152	236	236
2004	23	0423W46	166	170	154	160	139	139	244	244
2004	23	0423W47	168	170	154	158	148	148	232	236
2004	23	0423W48	166	170	154	160	139	148	244	244
2004	23	0423W49	166	168	154	158	148	148	236	244
2004	23	0423W50	166	170	154	160	139	148	244	244
2004	23	0423W51	166	170	154	154	139	152	236	236
2004	23	0423W52	166	170	154	160	139	148	244	246
2004	23	0423W53	168	170	154	160	139	148	236	244
2004	23	0423W54	166	170	154	158	148	148	232	244
2004	23	0423W55	168	170	154	160	139	152	236	244
2004	23	0423W56	166	170	154	158	148	148	232	236
2004	24	0424W41	166	168	154	154	139	148	232	234
2004	24	0424W42	164	168	154	158	148	148	240	246
2004	24	0424W43	164	168	158	160	148	148	-1	-1
2004	24	0424W44	164	168	154	158	148	148	234	236
2004	24	0424W45	164	168	154	160	148	148	234	242
2004	24	0424W46	168	170	154	154	139	148	234	244
2004	24	0424W47	164	168	158	160	148	148	234	242
2004	24	0424W48	168	174	154	154	139	148	234	246
2004	24	0424W49	166	170	154	154	139	148	234	244
2004	24	0424W50	168	174	154	158	139	148	234	246
2004	24	0424W51	168	168	154	154	148	148	234	236
2004	24	0424W52	164	168	154	154	148	148	234	240
2004	24	0424W53	168	168	154	154	148	148	234	236
2004	24	0424W54	164	168	154	154	148	148	234	246
2004	24	0424W55	164	168	154	154	148	148	234	240
2004	24	0424W56	166	170	154	154	139	148	234	244
2004	28	0428W1	160	168	154	168	139	152	234	248
2004	28	0428W2	160	166	154	168	139	148	238	248

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2004	28	0428W3	160	168	154	168	139	152	234	248
2004	28	0428W4	160	166	154	168	139	152	238	248
2004	28	0428W5	160	168	154	154	148	152	-1	-1
2004	28	0428W6	160	166	154	168	139	152	248	248
2004	28	0428W7	160	168	154	154	148	152	234	248
2004	28	0428W8	160	166	154	158	139	148	234	248
2004	28	0428W9	162	168	154	158	139	152	234	248
2004	28	0428W10	160	168	154	154	152	152	238	248
2004	28	0428W11	160	166	154	168	139	148	-1	-1
2004	28	0428W12	160	166	154	168	139	148	248	248
2004	28	0428W13	162	166	154	158	139	148	234	248
2004	28	0428W14	162	166	154	158	139	148	248	248
2004	28	0428W15	160	166	154	168	139	148	248	248
2004	28	0428W16	160	168	154	154	-1	-1	234	238
2004	29	0429W1	166	166	154	164	139	148	-1	-1
2004	29	0429W2	166	168	154	154	139	139	236	236
2004	29	0429W3	166	166	154	164	139	148	246	246
2004	29	0429W4	166	166	154	164	139	148	246	246
2004	29	0429W5	166	168	154	154	139	139	236	246
2004	29	0429W6	166	166	156	164	139	148	246	246
2004	29	0429W7	166	166	154	164	139	148	-1	-1
2004	29	0429W8	166	168	154	154	139	139	236	246
2004	29	0429W9	166	166	154	154	139	139	236	238
2004	29	0429W10	166	168	154	154	139	139	236	246
2004	29	0429W11	166	166	156	164	139	148	246	246
2004	29	0429W12	166	166	154	156	139	139	236	238
2004	29	0429W13	166	168	154	154	139	139	236	246
2004	29	0429W14	166	166	154	164	139	148	246	246
2004	29	0429W15	166	166	154	156	139	139	236	238
2004	29	0429W16	166	166	154	164	139	148	246	246
2004	43	0443W1	166	166	154	156	148	152	236	238
2004	43	0443W2	166	166	154	156	148	152	236	238
2004	43	0443W3	166	168	-1	-1	148	148	234	246
2004	43	0443W4	166	166	154	154	139	148	226	238
2004	43	0443W5	-1	-1	-1	-1	148	152	234	246
2004	43	0443W6	166	168	154	154	139	148	226	238
2004	43	0443W7	166	166	154	156	148	152	236	246
2004	43	0443W8	166	168	154	154	148	148	238	-1
2004	43	0443W9	166	166	154	156	148	152	236	238
2004	43	0443W10	168	168	154	164	148	152	234	246
2004	43	0443W11	166	168	154	164	148	152	234	238
2004	43	0443W12	166	168	154	156	148	152	236	238
2004	43	0443W13	166	168	154	162	148	148	234	238
2004	43	0443W14	166	168	154	162	148	148	234	238
2004	43	0443W15	166	168	154	154	139	148	226	246
2004	43	0443W16	168	168	154	154	148	148	246	-1
2004	45	0445W1	166	170	158	176	139	148	234	244
2004	45	0445W2	166	168	154	158	139	139	234	252
2004	45	0445W3	166	168	154	158	139	139	234	240

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2004	45	0445W4	168	168	154	154	139	139	232	240
2004	45	0445W5	166	166	158	158	139	139	234	252
2004	45	0445W6	166	168	154	158	139	148	232	244
2004	45	0445W7	168	168	154	154	139	139	232	240
2004	45	0445W8	166	168	154	158	139	148	232	244
2004	45	0445W9	166	168	154	158	139	148	232	244
2004	45	0445W10	166	168	154	154	139	139	232	240
2004	45	0445W11	166	170	158	176	139	148	234	244
2004	45	0445W12	168	168	154	158	139	139	232	240
2004	45	0445W13	166	168	154	158	139	148	232	244
2004	45	0445W14	166	166	154	154	139	139	234	234
2004	45	0445W15	166	168	154	154	139	139	232	240
2004	45	0445W16	166	168	154	158	139	139	232	240
2006	63	0663P1	166	170	158	158	139	139	236	238
2006	63	0663P2	164	168	158	158	135	139	232	234
2006	63	0663P6	164	166	158	164	139	152	238	238
2006	63	0663P7	164	168	158	158	139	148	232	-1
2006	63	0663P8	166	168	158	158	139	139	232	238
2006	63	0663P14	166	168	158	158	135	139	232	234
2006	63	0663P16	164	168	154	154	139	139	232	238
2006	63	0663P17	-1	-1	158	158	139	139	238	238
2006	63	0663P18	164	168	154	158	139	139	232	232
2006	63	0663L18	166	166	158	164	139	152	232	238
2006	63	0663L19	166	168	154	154	139	139	232	238
2006	63	0663L20	166	168	158	158	139	139	238	238
2006	63	0663L22	164	168	158	158	135	139	232	234
2006	63	0663L23	166	166	158	164	139	152	232	238
2006	63	0663L24	166	168	158	158	135	139	232	234
2006	65	0665P1	166	168	158	164	139	139	240	246
2006	65	0665P2	168	168	158	162	139	148	232	246
2006	65	0665P3	168	168	158	162	139	148	232	246
2006	65	0665P4	164	168	158	160	139	148	236	246
2006	65	0665P6	168	168	158	158	148	148	238	246
2006	65	0665P7	168	168	158	162	139	148	232	246
2006	65	0665P8	168	168	158	162	139	148	232	246
2006	65	0665P10	164	168	158	160	148	148	236	246
2006	65	0665P11	164	168	158	160	148	148	236	246
2006	65	0665P13	166	168	158	164	139	139	240	246
2006	65	0665P14	168	168	158	158	139	148	238	246
2006	65	0665P21	166	168	158	164	139	139	240	246
2006	65	0665P27	168	168	158	158	139	148	238	246
2006	66	0666P1	166	168	158	164	139	139	236	246
2006	66	0666P2	166	168	158	176	148	148	236	252
2006	66	0666P3	166	166	154	166	139	148	236	236
2006	66	0666P4	164	164	154	164	139	148	244	246
2006	66	0666P5	166	168	158	176	139	148	244	252
2006	66	0666P11	166	166	154	158	139	148	238	244
2006	66	0666P12	160	164	154	158	139	148	238	244
2006	66	0666P13	166	168	158	176	139	148	236	252

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2006	66	0666P14	160	166	154	158	139	148	238	244
2006	66	0666P16	160	166	154	158	139	139	238	244
2006	66	0666L31	160	166	158	166	139	148	-1	-1
2006	66	0666L32	166	168	158	164	139	139	244	246
2006	66	0666L33	-1	-1	154	166	139	139	236	236
2006	67	0667P1	160	166	154	168	139	139	-1	-1
2006	67	0667P2	-1	-1	154	154	139	139	-1	-1
2006	67	0667P3	166	166	154	158	139	152	238	252
2006	67	0667P5	160	168	154	158	139	139	232	234
2006	67	0667P6	160	166	158	168	139	152	232	238
2006	67	0667P7	-1	-1	160	168	139	139	-1	-1
2006	67	0667P9	166	166	164	168	139	144	234	252
2006	67	0667P10	-1	-1	154	154	139	139	-1	-1
2006	67	0667P11	160	168	154	166	139	139	232	234
2006	67	0667P14	160	164	154	160	139	139	238	252
2006	67	0667P15	-1	-1	154	164	139	144	-1	-1
2006	67	0667P16	160	164	154	168	139	152	232	234
2006	67	0667P17	-1	-1	154	166	139	139	234	252
2006	67	0667P23	160	164	154	154	139	152	232	234
2006	68	0668P1	-1	-1	162	162	139	144	-1	-1
2006	68	0668P2	166	166	154	158	139	148	244	246
2006	68	0668P3	-1	-1	154	154	148	152	-1	-1
2006	68	0668P4	166	168	-1	-1	139	139	240	246
2006	68	0668P5	166	168	154	158	139	148	240	246
2006	68	0668P6	166	168	154	162	139	144	238	240
2006	68	0668P7	166	168	154	158	139	144	244	246
2006	68	0668P8	166	166	-1	-1	-1	-1	-1	-1
2006	68	0668P9	-1	-1	154	154	139	152	-1	-1
2006	68	0668P10	166	168	154	158	135	139	236	240
2006	68	0668P11	166	168	-1	-1	139	139	240	246
2006	68	0668P13	166	166	162	162	139	144	238	244
2006	68	0668P14	166	168	154	154	139	148	244	248
2006	68	0668P15	-1	-1	154	154	139	152	-1	-1
2006	69	0669P13	164	168	158	164	148	152	226	248
2006	69	0669P14	162	168	154	164	148	148	246	252
2006	69	0669P15	162	166	154	158	148	148	248	252
2006	69	0669P16	162	168	154	164	148	148	248	252
2006	69	0669P17	162	166	154	164	148	148	248	252
2006	69	0669P18	162	166	154	164	148	148	248	252
2006	69	0669P19	164	168	158	164	148	152	226	248
2006	69	0669P20	162	166	154	158	148	155	246	252
2006	69	0669P21	162	166	154	158	148	148	246	252
2006	69	0669P22	-1	-1	154	164	148	148	248	252
2006	69	0669P23	162	166	154	154	148	148	248	252
2006	69	0669P24	162	166	154	164	148	148	-1	-1
2006	69	0669P25	162	168	154	158	148	152	246	252
2006	69	0669P26	162	168	154	164	148	148	-1	-1
2006	70	0670P1	166	166	154	154	139	152	236	246
2006	70	0670P2	166	166	-1	-1	139	139	226	234

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2006	70	0670P3	166	166	164	164	139	139	234	236
2006	70	0670P4	166	166	154	154	139	148	236	236
2006	70	0670P5	168	168	154	154	139	139	-1	-1
2006	70	0670P6	166	166	154	154	139	148	226	236
2006	70	0670P7	166	166	164	164	139	148	226	234
2006	70	0670P8	166	166	154	154	139	152	236	246
2006	70	0670P9	-1	-1	154	154	-1	-1	-1	-1
2006	70	0670P10	-1	-1	154	154	-1	-1	-1	-1
2006	70	0670P11	166	168	154	164	139	152	234	236
2006	70	0670P12	164	166	154	166	-1	-1	-1	-1
2006	70	0670P13	166	166	-1	-1	139	139	226	234
2006	70	0670P14	166	166	154	154	139	148	226	236
2006	70	0670P15	166	168	-1	-1	139	139	-1	-1
2006	70	0670P16	164	168	154	168	-1	-1	236	236
2006	72	0672L11	164	166	154	164	139	152	238	248
2006	72	0672L13	168	170	154	156	139	148	238	248
2006	72	0672L14	-1	-1	154	164	139	152	226	248
2006	72	0672L17	-1	-1	154	158	144	152	226	234
2006	72	0672L19	168	170	154	156	148	152	226	248
2006	72	0672L20	168	168	154	156	139	152	238	252
2006	72	0672L22	-1	-1	154	158	-1	-1	226	234
2006	72	0672L24	-1	-1	154	156	148	152	238	248
2006	72	0672L25	166	168	154	164	139	152	226	248
2006	72	0672L27	164	170	154	156	148	152	238	248
2006	72	0672L29	164	170	154	156	148	152	226	248
2006	72	0672L32	166	168	158	158	144	152	226	234
2006	72	0672L36	168	170	154	156	148	152	226	248
2006	72	0672L38	168	168	154	156	139	139	238	252
2006	76	0676P1	164	166	158	158	148	152	-1	-1
2006	76	0676P2	166	166	158	158	139	152	238	240
2006	76	0676P3	166	168	-1	-1	139	139	-1	-1
2006	76	0676P4	164	164	154	158	148	148	-1	-1
2006	76	0676P5	166	166	154	158	139	148	236	240
2006	76	0676P6	164	164	154	154	139	148	240	250
2006	76	0676P7	166	166	158	158	139	152	238	240
2006	76	0676P8	164	166	154	154	139	148	236	240
2006	76	0676P9	164	166	154	154	139	148	240	250
2006	76	0676P10	166	166	154	154	-1	-1	232	236
2006	76	0676P11	166	166	158	158	148	152	238	240
2006	76	0676P12	166	168	154	154	148	152	232	236
2006	76	0676P13	164	166	154	154	148	148	236	240
2006	76	0676P14	166	166	154	160	-1	-1	-1	-1
2006	76	0676P15	164	168	158	164	139	148	240	242
2006	76	0676P16	166	170	154	154	139	148	232	236
2006	76	0676P18	-1	-1	154	154	148	148	232	236
2006	76	0676P19	166	168	154	158	139	148	232	240
2006	76	0676P20	-1	-1	158	158	148	152	232	240
2006	76	0676P24	164	166	154	158	148	148	236	240
2006	76	0676P26	166	166	154	154	139	148	236	240

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2006	76	0676P27	166	168	154	158	139	152	232	236
2006	76	0676P28	166	170	154	158	139	148	232	236
2006	76	0676P38	164	168	158	164	139	148	240	242
2006	76	0676P40	166	170	154	158	-1	-1	232	236
2006	77	0677P1	164	168	154	154	148	148	232	244
2006	77	0677P2	168	168	154	154	148	148	244	246
2006	77	0677P3	166	168	158	160	139	148	236	238
2006	77	0677P4	168	168	154	160	137	139	232	244
2006	77	0677P5	168	168	154	160	148	148	236	236
2006	77	0677P6	168	168	158	160	137	139	236	236
2006	77	0677P7	168	168	160	160	148	148	232	244
2006	77	0677P8	168	168	160	160	148	148	236	236
2006	77	0677P9	168	168	154	154	137	139	232	244
2006	77	0677P10	168	168	154	158	137	139	236	236
2006	77	0677P11	168	168	154	154	137	139	232	244
2006	77	0677P12	168	168	154	154	148	148	244	246
2006	77	0677P13	164	168	154	160	137	148	232	244
2006	77	0677P14	168	168	154	154	137	139	232	244
2006	77	0677P15	168	168	160	160	148	148	236	236
2006	77	0677P16	168	168	160	160	148	148	244	244
2006	77	0677P21	166	168	154	158	148	152	236	244
2006	77	0677P22	168	168	154	158	148	152	236	244
2006	77	0677P26	166	168	158	160	137	139	236	236
2006	77	0677P29	168	168	154	158	137	139	236	236
2006	77	0677P31	168	168	154	160	137	139	232	236
2006	77	0677P36	166	168	154	158	148	152	236	244
2006	77	0677P38	166	166	158	160	139	148	236	238
2006	77	0677P40	166	168	154	160	139	148	232	236
2006	77	0677L6	166	168	158	160	139	148	236	238
2006	77	0677L9	168	168	154	158	148	152	236	244
2006	77	0677L22	168	168	154	160	148	152	238	244
2006	77	0677L40	164	168	154	168	-1	-1	232	236
2006	79	0679P1	164	166	158	158	135	153	232	-1
2006	79	0679P2	166	166	158	158	135	139	234	236
2006	79	0679P3	166	168	158	158	139	139	234	236
2006	79	0679P4	166	168	-1	-1	135	139	232	-1
2006	79	0679P5	166	168	172	172	135	139	234	236
2006	79	0679P6	166	168	158	172	139	152	234	246
2006	79	0679P7	168	168	172	172	135	152	232	236
2006	79	0679P8	168	168	158	172	135	152	234	236
2006	79	0679P9	166	166	172	172	139	139	232	234
2006	79	0679P10	166	168	172	172	135	152	232	246
2006	79	0679P11	166	166	158	158	139	144	232	252
2006	79	0679P12	166	166	172	172	139	152	232	246
2006	79	0679P13	166	168	158	158	135	144	232	252
2006	79	0679P14	166	168	154	172	139	139	232	236
2006	79	0679P15	166	166	172	172	139	139	234	246
2006	79	0679P16	166	166	172	172	135	139	234	236
2017	195	17195W1	164	166	158	166	144	148	-1	-1

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2017	195	17195W2	164	164	158	166	144	148	242	246
2017	195	17195W3	164	166	158	166	144	148	-1	-1
2017	195	17195W4	166	166	160	162	139	144	236	246
2017	195	17195W5	164	166	158	158	139	144	234	246
2017	195	17195W6	164	164	160	166	144	148	234	242
2017	195	17195W7	164	166	154	158	144	148	234	244
2017	195	17195W8	164	164	158	158	139	139	246	246
2017	195	17195W9	164	166	-1	-1	139	139	246	246
2017	195	17195W10	164	164	158	160	139	144	246	246
2017	195	17195W11	166	166	158	158	144	144	234	234
2017	195	17195W12	164	166	158	166	144	148	234	242
2017	195	17195W13	164	166	158	164	144	152	234	238
2017	195	17195W14	166	166	154	158	144	148	244	246
2017	195	17195W15	164	166	-1	-1	144	148	242	246
2017	195	17195W16	166	166	160	164	139	152	234	238
2017	196	17196W1	160	168	154	156	139	148	234	244
2017	196	17196W2	166	168	154	172	139	148	234	234
2017	196	17196W3	166	168	154	164	139	148	234	244
2017	196	17196W4	160	166	154	164	139	148	234	244
2017	196	17196W5	160	160	156	164	139	148	234	244
2017	196	17196W6	166	168	154	164	139	144	234	244
2017	196	17196W7	166	168	154	164	139	144	234	244
2017	196	17196W8	160	166	154	172	139	148	234	234
2017	196	17196W9	160	166	154	172	139	144	234	234
2017	196	17196W10	160	166	154	172	139	148	234	234
2017	196	17196W11	160	166	162	164	139	148	234	244
2017	196	17196W12	160	168	154	156	144	148	234	236
2017	196	17196W13	166	168	154	172	139	144	234	234
2017	196	17196W14	160	168	154	158	148	152	234	238
2017	196	17196W15	160	160	154	156	139	144	234	244
2017	196	17196W16	166	168	154	172	139	148	234	234
2017	201	17201W1	160	164	154	160	139	144	234	252
2017	201	17201W2	160	166	158	160	148	148	248	252
2017	201	17201W3	160	166	154	160	148	148	236	252
2017	201	17201W4	-1	-1	156	160	139	148	236	252
2017	201	17201W5	160	168	158	160	135	148	244	252
2017	201	17201W6	160	168	156	160	139	148	236	252
2017	201	17201W7	160	168	156	166	139	153	236	252
2017	201	17201W8	160	168	156	160	139	148	236	252
2017	201	17201W9	160	168	156	166	139	153	236	238
2017	201	17201W10	160	168	156	160	139	139	236	238
2017	201	17201W11	160	168	156	160	139	148	236	252
2017	201	17201W12	160	168	156	160	139	148	236	252
2017	201	17201W13	160	166	154	160	148	148	236	252
2017	201	17201W14	160	166	154	160	-1	-1	236	252
2017	201	17201W15	160	166	154	156	139	148	236	238
2017	201	17201W16	160	168	156	160	139	148	236	238
2017	204	17204W1	166	166	154	158	-1	-1	240	240
2017	204	17204W2	164	166	154	158	-1	-1	-1	-1

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2017	204	17204W3	166	166	154	158	-1	-1	-1	-1
2017	204	17204W4	166	166	-1	-1	-1	-1	-1	-1
2017	204	17204W5	-1	-1	154	158	-1	-1	238	238
2017	204	17204W6	166	166	154	158	-1	-1	-1	-1
2017	204	17204W7	164	164	-1	-1	-1	-1	-1	-1
2017	204	17204W8	164	164	158	162	-1	-1	-1	-1
2017	204	17204W9	164	164	154	162	-1	-1	-1	-1
2017	204	17204W10	164	166	154	162	-1	-1	-1	-1
2017	204	17204W11	164	164	162	162	-1	-1	-1	-1
2017	204	17204W12	164	164	-1	-1	-1	-1	-1	-1
2017	204	17204W13	164	166	154	158	-1	-1	-1	-1
2017	204	17204W14	164	166	-1	-1	-1	-1	-1	-1
2017	204	17204W15	164	166	154	162	-1	-1	-1	-1
2017	204	17204W16	166	166	-1	-1	-1	-1	-1	-1
2017	205	17205W1	166	168	154	154	139	139	234	236
2017	205	17205W2	166	168	154	162	139	148	234	234
2017	205	17205W3	166	166	158	158	-1	-1	234	244
2017	205	17205W4	166	166	158	158	139	152	236	244
2017	205	17205W5	166	168	158	162	139	148	234	236
2017	205	17205W6	166	168	154	158	139	139	234	234
2017	205	17205W7	168	168	154	154	139	152	236	236
2017	205	17205W8	168	168	158	162	139	148	234	248
2017	205	17205W9	168	168	154	154	139	139	234	236
2017	205	17205W10	166	168	154	154	-1	-1	234	236
2017	205	17205W11	168	168	154	158	139	139	234	236
2017	205	17205W12	168	168	154	162	148	152	234	248
2017	205	17205W13	166	168	154	158	139	152	234	236
2017	205	17205W14	166	168	154	154	139	152	234	236
2017	205	17205W15	166	168	158	158	152	152	234	244
2017	205	17205W16	168	168	154	158	139	152	236	236
2017	226	17226W1	162	166	154	160	152	152	236	244
2017	226	17226W2	166	168	154	162	152	152	234	236
2017	226	17226W3	164	166	154	154	148	152	-1	-1
2017	226	17226W4	164	166	154	154	148	152	236	248
2017	226	17226W5	164	166	154	154	148	152	-1	-1
2017	226	17226W6	166	168	154	162	152	152	236	-1
2017	226	17226W7	164	166	154	154	148	152	-1	-1
2017	226	17226W8	166	168	154	154	139	152	234	236
2017	226	17226W9	162	166	154	160	152	152	236	244
2017	226	17226W10	166	166	154	154	148	152	234	234
2017	226	17226W11	164	168	154	154	139	-1	236	236
2017	226	17226W12	166	166	154	154	-1	-1	234	248
2017	226	17226W15	164	164	154	166	-1	-1	232	236
2017	226	17226W16	166	168	154	162	152	-1	234	236
2017	228	17228W1	166	166	158	160	-1	-1	238	266
2017	228	17228W2	166	168	158	160	-1	-1	238	266
2017	228	17228W3	166	166	154	158	-1	-1	238	266
2017	228	17228W4	166	168	160	162	-1	-1	226	244
2017	228	17228W5	164	166	154	164	-1	-1	238	266

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2017	228	17228W6	166	168	160	162	-1	-1	226	244
2017	228	17228W7	164	168	154	164	-1	-1	226	238
2017	228	17228W8	166	168	154	158	-1	-1	226	238
2017	228	17228W9	166	166	154	162	-1	-1	226	244
2017	228	17228W10	164	168	160	164	-1	-1	238	266
2017	228	17228W11	164	168	154	164	-1	-1	238	266
2017	228	17228W12	166	168	154	158	-1	-1	238	266
2017	228	17228W13	166	168	154	162	-1	-1	226	244
2017	228	17228W14	166	166	154	158	-1	-1	238	266
2017	228	17228W15	166	168	154	158	-1	-1	226	238
2017	228	17228W16	164	168	154	164	-1	-1	238	266
2017	229	17229W1	162	166	154	158	139	139	238	238
2017	229	17229W2	166	168	154	154	139	148	232	238
2017	229	17229W3	166	168	154	158	139	139	238	238
2017	229	17229W4	166	168	158	164	139	148	236	238
2017	229	17229W5	162	166	-1	-1	-1	-1	232	238
2017	229	17229W6	-1	-1	-1	-1	-1	-1	-1	-1
2017	229	17229W7	166	168	158	164	139	148	232	232
2017	229	17229W8	166	168	158	164	139	148	232	232
2017	229	17229W9	162	166	158	158	139	148	232	238
2017	229	17229W10	162	166	154	158	139	148	238	238
2017	229	17229W11	162	166	154	158	139	139	232	238
2017	229	17229W12	166	168	154	158	139	148	238	238
2017	229	17229W13	166	168	158	164	139	148	236	238
2017	229	17229W14	162	166	154	164	139	148	236	238
2017	229	17229W15	166	168	154	158	139	148	238	238
2017	229	17229W16	166	168	154	158	139	148	232	238
2017	230	17230W1	168	168	158	160	139	152	236	248
2017	230	17230W2	-1	-1	158	160	139	148	236	248
2017	230	17230W3	164	-1	154	160	139	152	234	248
2017	230	17230W4	164	-1	154	160	139	152	234	248
2017	230	17230W5	164	168	154	160	139	152	234	248
2017	230	17230W6	164	168	154	160	139	152	234	248
2017	230	17230W8	164	168	158	160	139	155	244	248
2017	230	17230W9	166	-1	158	160	139	148	234	248
2017	230	17230W10	164	168	154	160	139	152	234	248
2017	230	17230W11	164	168	158	160	139	155	244	248
2017	230	17230W12	168	-1	158	160	139	152	236	248
2017	230	17230W13	164	168	158	160	139	155	244	248
2017	230	17230W14	168	168	158	160	-1	-1	236	248
2017	230	17230W15	164	-1	160	160	139	139	234	248
2017	230	17230W16	168	168	158	160	139	152	236	248
2017	193	17193W1	164	168	154	154	139	139	234	238
2017	193	17193W2	166	168	-1	-1	148	148	226	232
2017	193	17193W3	164	168	-1	-1	139	148	232	238
2017	193	17193W4	164	168	154	160	148	-1	232	238
2017	193	17193W5	166	168	154	154	139	148	226	232
2017	193	17193W6	166	168	154	160	139	148	-1	-1
2017	193	17193W7	166	168	154	154	139	148	226	234

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2017	193	17193W8	166	168	-1	-1	148	148	226	234
2017	193	17193W9	166	168	160	164	139	148	234	236
2017	193	17193W10	164	168	154	154	139	148	232	238
2017	193	17193W11	168	168	154	160	148	148	232	236
2017	193	17193W12	166	168	154	154	139	148	226	232
2017	193	17193W13	-1	-1	154	160	139	148	234	236
2017	193	17193W14	166	168	-1	-1	139	148	234	236
2017	193	17193W15	166	168	160	164	139	148	234	236
2017	193	17193W16	164	168	154	160	139	-1	232	238
2017	198	17198W1	166	166	158	166	139	139	238	246
2017	198	17198W2	166	166	158	164	135	139	234	246
2017	198	17198W3	166	166	164	164	139	152	234	236
2017	198	17198W4	166	166	158	164	135	139	238	246
2017	198	17198W5	166	168	164	164	135	139	234	238
2017	198	17198W6	166	166	158	166	135	139	238	246
2017	198	17198W7	166	166	158	164	135	139	234	246
2017	198	17198W8	166	168	160	164	135	139	234	236
2017	198	17198W9	166	168	164	164	135	139	234	234
2017	198	17198W10	166	166	164	166	135	152	234	236
2017	198	17198W11	166	166	158	164	139	139	238	246
2017	198	17198W12	166	168	164	166	135	139	234	234
2017	198	17198W13	166	166	164	164	139	152	234	236
2017	198	17198W14	166	166	158	164	-1	-1	238	246
2017	198	17198W15	166	168	164	166	135	139	234	234
2017	198	17198W16	166	166	164	166	139	152	236	238
2017	199	17199W1	164	164	154	162	139	148	234	246
2017	199	17199W2	166	166	-1	-1	139	148	238	238
2017	199	17199W3	164	166	154	162	139	148	234	246
2017	199	17199W4	164	166	158	166	148	148	232	238
2017	199	17199W5	164	166	154	166	148	148	238	246
2017	199	17199W6	166	166	154	166	139	148	238	238
2017	199	17199W7	164	166	154	154	139	148	238	246
2017	199	17199W8	166	166	158	166	148	148	232	238
2017	199	17199W9	166	166	154	166	139	148	238	246
2017	199	17199W10	166	166	154	166	139	148	238	238
2017	199	17199W11	164	166	154	154	139	148	246	246
2017	199	17199W12	164	166	154	166	148	148	232	238
2017	199	17199W13	164	166	154	154	139	148	246	246
2017	199	17199W14	164	164	158	162	139	148	234	246
2017	199	17199W15	164	166	158	162	139	148	238	238
2017	199	17199W16	166	168	158	158	148	148	238	246
2017	200	17200W1	160	168	158	162	139	148	234	246
2017	200	17200W2	166	166	154	158	148	152	240	246
2017	200	17200W3	160	168	158	164	139	148	234	246
2017	200	17200W4	160	164	154	158	148	152	246	252
2017	200	17200W5	160	166	158	158	139	148	232	240
2017	200	17200W6	160	170	154	154	139	139	232	236
2017	200	17200W7	166	168	154	164	148	152	232	234
2017	200	17200W8	160	160	158	158	139	148	232	232

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2017	200	17200W9	160	168	158	158	139	148	232	232
2017	200	17200W10	166	168	154	158	139	152	234	246
2017	200	17200W11	160	168	154	156	139	148	238	246
2017	200	17200W12	160	164	158	158	148	152	246	252
2017	200	17200W13	160	166	154	158	139	148	232	232
2017	200	17200W14	160	168	154	156	152	148	-1	-1
2017	200	17200W15	160	168	158	164	148	152	232	234
2017	200	17200W16	160	164	154	158	148	152	246	252
2017	203	17203W1	160	168	154	158	135	139	240	244
2017	203	17203W2	164	166	154	158	-1	-1	240	246
2017	203	17203W3	160	168	158	158	135	139	240	244
2017	203	17203W4	160	168	154	158	137	139	240	244
2017	203	17203W5	160	166	154	158	137	139	-1	-1
2017	203	17203W6	160	168	158	158	137	139	240	244
2017	203	17203W7	160	166	154	158	137	139	240	244
2017	203	17203W8	160	168	154	158	137	139	240	244
2017	203	17203W9	160	168	158	158	137	139	240	244
2017	203	17203W10	164	168	154	154	135	148	240	246
2017	203	17203W11	166	168	158	158	137	139	236	240
2017	203	17203W12	-1	-1	158	158	-1	-1	236	240
2017	203	17203W13	168	168	158	158	137	139	236	240
2017	203	17203W14	160	168	154	158	135	139	234	240
2017	203	17203W15	166	168	158	158	135	139	236	240
2017	203	17203W16	164	168	154	158	137	148	240	246
2017	209	17209W2	-1	-1	-1	-1	-1	-1	232	238
2017	209	17209W3	-1	-1	-1	-1	-1	-1	232	236
2017	209	17209W4	166	166	158	166	139	144	234	238
2017	209	17209W5	164	166	158	166	-1	-1	234	238
2017	209	17209W6	164	166	-1	-1	139	152	236	248
2017	209	17209W7	166	166	158	162	139	144	236	238
2017	209	17209W8	164	166	158	168	139	152	238	248
2017	209	17209W9	164	166	158	168	139	152	236	248
2017	209	17209W10	166	168	-1	-1	139	152	238	248
2017	209	17209W11	164	166	158	160	139	148	238	-1
2017	209	17209W12	164	166	158	166	148	148	-1	-1
2017	209	17209W13	164	166	158	160	139	148	238	-1
2017	209	17209W14	166	166	158	162	144	148	236	238
2017	209	17209W15	164	166	158	162	139	148	238	-1
2017	209	17209W16	166	166	158	166	-1	-1	234	236
2017	210	17210W1	162	168	154	158	135	148	236	246
2017	210	17210W2	-1	-1	154	158	135	148	-1	-1
2017	210	17210W3	162	166	154	158	135	139	236	246
2017	210	17210W4	162	168	154	158	135	148	236	248
2017	210	17210W5	160	168	164	168	139	148	236	248
2017	210	17210W6	162	168	158	164	135	148	236	246
2017	210	17210W7	162	168	154	158	135	148	236	248
2017	210	17210W8	-1	-1	154	168	139	148	236	248
2017	210	17210W9	-1	-1	164	168	139	148	-1	-1
2017	210	17210W10	162	168	154	158	135	148	236	246

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2017	210	17210W11	162	168	158	164	135	148	236	246
2017	210	17210W12	166	168	154	158	139	148	246	246
2017	210	17210W13	162	168	158	164	135	148	236	246
2017	210	17210W14	162	166	154	158	135	148	236	246
2017	210	17210W15	162	166	154	158	135	148	236	246
2017	210	17210W16	166	168	154	158	139	148	246	248
2017	215	17215W1	-1	-1	158	168	148	152	232	234
2017	215	17215W2	168	168	158	168	139	148	232	234
2017	215	17215W3	168	168	158	168	148	152	234	246
2017	215	17215W4	160	166	154	158	152	152	246	246
2017	215	17215W5	160	166	158	168	139	148	232	250
2017	215	17215W6	164	168	154	158	152	152	232	242
2017	215	17215W7	164	168	154	158	152	152	242	246
2017	215	17215W8	160	168	154	168	139	148	246	250
2017	215	17215W9	168	170	154	160	139	148	232	244
2017	215	17215W10	-1	-1	158	162	148	152	232	246
2017	215	17215W11	166	168	154	164	139	148	232	238
2017	215	17215W12	166	168	154	158	152	152	236	246
2017	215	17215W13	160	166	158	162	148	152	232	246
2017	215	17215W14	166	168	158	164	139	148	238	246
2017	215	17215W15	168	170	158	160	139	148	244	246
2017	215	17215W16	168	168	158	168	148	152	232	234
2017	217	17217W1	164	166	154	164	148	148	236	246
2017	217	17217W2	166	168	160	168	148	148	236	246
2017	217	17217W3	166	168	160	168	148	148	236	246
2017	217	17217W4	-1	-1	154	158	148	148	236	246
2017	217	17217W5	164	166	154	164	148	148	236	244
2017	217	17217W6	164	166	154	164	148	148	236	244
2017	217	17217W7	166	168	154	168	148	148	236	246
2017	217	17217W8	166	168	-1	-1	148	148	236	246
2017	217	17217W9	166	168	160	168	148	148	236	246
2017	217	17217W10	166	168	154	168	148	148	236	246
2017	217	17217W11	164	166	160	164	148	148	236	244
2017	217	17217W12	164	166	160	164	148	148	236	246
2017	217	17217W13	166	168	160	168	148	148	236	246
2017	217	17217W14	164	166	160	164	148	148	-1	-1
2017	217	17217W15	164	166	154	164	148	148	-1	-1
2017	217	17217W16	164	166	154	164	148	148	236	244
2017	221	17221W1	166	166	158	164	148	152	236	246
2017	221	17221W2	166	166	154	166	148	152	234	236
2017	221	17221W3	166	166	158	164	139	152	234	236
2017	221	17221W4	166	168	158	158	148	148	244	246
2017	221	17221W5	166	166	154	158	139	152	236	246
2017	221	17221W6	166	170	158	160	148	148	232	234
2017	221	17221W7	164	170	166	166	139	139	234	246
2017	221	17221W8	166	170	158	166	139	139	234	250
2017	221	17221W9	164	170	158	166	139	148	246	246
2017	221	17221W10	166	170	158	158	139	148	234	250
2017	221	17221W11	168	170	158	158	139	148	234	244

Table A-1 continued

Year	Colony	Individual	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2017	221	17221W12	166	166	158	164	148	152	236	246
2017	221	17221W13	164	170	166	166	139	139	-1	-1
2017	221	17221W14	166	166	158	158	139	148	246	250
2017	221	17221W15	166	170	158	166	139	139	234	250
2017	221	17221W16	166	166	158	164	148	152	234	236
2017	227	17227W1	166	168	158	160	144	152	234	252
2017	227	17227W2	166	168	158	158	144	152	234	252
2017	227	17227W3	168	168	158	158	139	144	234	248
2017	227	17227W4	168	168	158	158	-1	-1	234	248
2017	227	17227W5	168	170	154	160	-1	-1	236	236
2017	227	17227W6	-1	-1	158	164	-1	-1	234	252
2017	227	17227W7	166	168	158	158	-1	-1	234	252
2017	227	17227W8	168	168	158	158	-1	-1	234	248
2017	227	17227W9	166	170	154	158	-1	-1	236	236
2017	227	17227W10	166	170	154	158	-1	-1	234	236
2017	227	17227W11	166	168	158	160	-1	-1	234	252
2017	227	17227W12	168	170	154	160	-1	-1	236	236
2017	227	17227W13	166	166	158	164	-1	-1	234	252
2017	227	17227W14	168	168	158	160	-1	-1	234	248
2017	227	17227W15	166	166	158	164	-1	-1	234	252

Table A-2. Inferred genotypes of diploid queens and their haploid male mates heading Vespula maculifrons colonies at 16 polymorphic DNA microsatellite loci. Inferred genotypes are based on empirically obtained worker genotypes. Year = year collected. Colony = colony number. Q = queen. M = male. -1 = missing data.

Year	Caste	Colony	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2004	Q	8	82	82	205	211	263	269	290	296
2004	M	8		86		207		282		302
2004	M	8		86		205		278		298
2004	M	8		86		207		276		298
2004	M	8		88		213		273		294
2004	M	8		84		211		273		286
2004	Q	10	82	84	207	207	263	273	281	284
2004	M	10		84		213		266		282
2004	M	10		82		213		266		274
2004	M	10		84		207		266		312
2004	M	10		82		209		273		278
2004	M	10		86		213		266		286
2004	Q	13	82	84	205	207	263	263	284	286
2004	M	13		86		209		263		282
2004	M	13		84		209		263		288
2004	M	13		92		209		266		288
2004	M	13		84		207		263		288
2004	M	13		86		209		274		-1
2004	Q	15	86	88	205	209	273	278	274	276
2004	M	15		86		207		266		294
2004	M	15		86		207		278		298
2004	M	15		78		207		266		298
2004	M	15		86		205		278		286
2004	M	15		78		205		266		285
2004	Q	16	84	88	207	209	263	266	285	285
2004	M	16		78		205		263		290
2004	M	16		86		205		263		274
2004	M	16		86		209		269		282
2004	M	16		84		205		266		288
2004	M	16		88		205		273		272
2004	M	16		78		207		273		286
2004	M	16		88		207		266		290
2004	M	16		82		205		266		284
2004	Q	18	82	84	205	207	269	276	276	296
2004	M	18		84		209		273		-1
2004	M	18		86		209		276		290
2004	M	18		86		211		263		308
2004	M	18		84		207		273		296
2004	M	18		86		209		269		290
2004	Q	19	82	88	207	209	262	269		-1
2004	M	19		82		207		-1		-1
2004	M	19		86		205		278		-1
2004	M	19		88		205		266		-1
2004	M	19		88		209		269		-1
2004	M	19		86		211		-1		-1
2004	M	19		86		211		273		-1
2004	M	19		86		207		273		-1

Table A-2 continued

Year	Caste	Colony	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2004	Q	23	82	82	209	213	263	273	286	292
2004	M	23		88		205		-1		-1
2004	M	23		88		215		266		298
2004	M	23		82		215		273		286
2004	M	23		84		215		266		285
2004	M	23		82		211		266		298
2004	M	23		88		211		266		286
2004	Q	24	82	82	205	209	269	273	272	284
2004	M	24		86		207		262		285
2004	M	24		84		215		263		290
2004	M	24		84		207		263		-1
2004	M	24		90		215		263		-1
2004	M	24		84		213		263		298
2004	M	24		86		205		269		296
2004	M	24		90		215		278		298
2004	Q	28	78	82	205	205	276	284	298	298
2004	M	28		84		211		273		290
2004	M	28		84		211		-1		290
2004	M	28		82		211		273		288
2004	M	28		86		209		262		290
2004	M	28		86		211		273		302
2004	Q	29	82	86	209	209	263	266	284	294
2004	M	29		86		207		266		298
2004	M	29		82		205		278		290
2004	M	29		86		205		278		290
2004	Q	43	78	86	207	211	260	266	290	290
2004	M	43		86		207		266		294
2004	M	43		88		207		269		-1
2004	M	43		86		207		263		290
2004	M	43		84		205		266		-1
2004	M	43		86		209		286		270
2004	Q	45	82	86	209	213	266	273	282	290
2004	M	45		82		207		266		279
2004	M	45		82		207		269		282
2004	M	45		88		205		266		282
2004	M	45		84		209		266		280
2004	M	45		82		207		269		312
2006	Q	63	82	88	207	207	271	276	290	290
2006	M	63		82		207		263		284
2006	M	63		82		209		273		290
2006	M	63		82		207		269		280
2006	M	63		82		209		276		278
2006	M	63		88		207		278		294
2006	M	63		86		205		263		272
2006	M	63		86		205		263		272
2006	Q	65	78	82	207	209	252	269	286	290
2006	M	65		86		205		278		280
2006	M	65		84		209		276		286
2006	M	65		84		205		278		298

Table A-2 continued

Year	Caste	Colony	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2006	M	65		84		207		262		296
2006	Q	66	86	88	207	213	271	278	286	292
2006	M	66		82		205		274		292
2006	M	66		82		213		278		288
2006	M	66		84		211		271		292
2006	M	66		86		207		263		286
2006	Q	67	82	84	207	211	260	266	286	298
2006	M	67		78		215		274		274
2006	M	67		84		215		269		280
2006	M	67		-1		207		274		274
2006	M	67		82		211		263		290
2006	M	67		86		209		260		282
2006	M	67		88		205		273		286
2006	M	67		88		215		271		280
2006	Q	68	82	84	209	211	273	273	270	285
2006	M	68		84		211		279		276
2006	M	68		82		207		271		282
2006	M	68		84		207		271		285
2006	M	68		-1		211		271		285
2006	M	68		-1		211		273		-1
2006	M	68		84		-1		269		282
2006	Q	69	82	88	205	215	269	273	288	294
2006	M	69		78		207		269		312
2006	M	69		82		211		269		312
2006	M	69		82		211		269		312
2006	M	69		82		211		273		312
2006	M	69		82		211		269		312
2006	Q	70	82	88	207	211	269	278	272	292
2006	M	70		88		211		269		288
2006	M	70		82		211		263		294
2006	M	70		82		213		269		288
2006	M	70		88		207		263		272
2006	M	70		82		207		269		280
2006	M	70		88		-1		278		-1
2006	M	70		-1		211		278		288
2006	Q	72	82	88	207	209	263	271	281	312
2006	M	72		84		207		279		282
2006	M	72		82		207		262		272
2006	M	72		82		205		269		293
2006	M	72		82		211		263		290
2006	Q	76	82	88	207	211	263	279	282	284
2006	M	76		88		211		278		288
2006	M	76		88		207		263		279
2006	M	76		84		215		269		288
2006	M	76		82		215		269		288
2006	M	76		82		-1		271		-1
2006	M	76		82		209		284		280
2006	M	76		82		209		266		280
2006	M	76		82		215		273		302

Table A-2 continued

Year	Caste	Colony	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2006	M	76		86		211		269		308
2006	M	76		82		207		269		292
2006	Q	77	82	84	207	209	279	279	294	296
2006	M	77		82		215		263		272
2006	M	77		84		209		279		294
2006	M	77		78		215		263		285
2006	M	77		84		209		279		282
2006	M	77		82		209		279		285
2006	M	77		84		205		279		272
2006	M	77		82		209		279		294
2006	M	77		104		207		260		285
2006	M	77		-1		211		269		272
2006	M	77		-1		205		-1		-1
2006	Q	79	84	86	205	213	278	279	290	294
2006	M	79		88		213		271		290
2006	M	79		86		205		278		278
2006	M	79		82		205		271		270
2006	M	79		84		207		279		278
2006	M	79		84		209		278		312
2006	M	79		86		213		278		292
2006	M	79		86		205		279		292
2006	M	79		84		211		263		292
2017	Q	195	82	86	205	207	269	278	284	284
2017	M	195		82		209		269		288
2017	M	195		86		207		269		274
2017	M	195		82		207		273		288
2017	M	195		88		211		263		274
2017	M	195		82		205		278		284
2017	M	195		82		209		263		312
2017	Q	196	82	82	213	215	266	274	272	294
2017	M	196		82		205		269		274
2017	M	196		82		207		266		285
2017	M	196		82		205		269		288
2017	M	196		82		205		273		294
2017	M	196		86		211		274		285
2017	M	196		82		207		279		274
2017	Q	201	78	88	205	207	273	273	285	293
2017	M	201		84		211		-1		288
2017	M	201		82		213		-1		288
2017	M	201		82		213		263		282
2017	M	201		88		211		278		288
2017	M	201		82		211		263		272
2017	M	201		88		211		273		-1
2017	M	201		88		211		278		288
2017	M	201		82		213		263		288
2017	Q	204	-1	-1	-1	-1	269	274	284	286
2017	M	204		-1		-1		269		284
2017	M	204		-1		-1		278		288
2017	M	204		-1		-1		269		-1

Table A-2 continued

Year	Caste	Colony	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2017	M	204		-1		-1		269		270
2017	Q	205	82	88	205	215	266	274	276	278
2017	M	205		82		207		271		274
2017	M	205		84		207		269		274
2017	M	205		84		207		276		288
2017	M	205		82		207		271		-1
2017	M	205		82		207		271		274
2017	Q	226	82	84	205	205	263	263	268	296
2017	M	226		82		211		269		282
2017	M	226		88		207		269		285
2017	M	226		84		207		276		294
2017	M	226		86		213		273		274
2017	M	226		88		205		271		279
2017	Q	228	82	88	209	211	263	276	274	278
2017	M	228		84		209		273		296
2017	M	228		82		209		273		282
2017	M	228		86		205		262		274
2017	Q	229	78	82	199	207	263	278	282	296
2017	M	229		82		207		263		288
2017	M	229		88		207		266		279
2017	M	229		84		207		284		282
2017	M	229		-1		-1		-1		-1
2017	M	229		82		-1		263		-1
2017	M	229		82		207		266		294
2017	M	229		88		207		266		279
2017	Q	230	82	84	205	209	269	279	288	312
2017	M	230		84		209		263		278
2017	M	230		86		211		-1		286
2017	M	230		82		205		278		298
2017	M	230		82		205		271		294
2017	M	230		84		-1		269		-1
2017	M	230		82		215		278		285
2017	Q	193	86	88	207	209	263	266	270	288
2017	M	193		82		207		273		288
2017	M	193		82		211		263		270
2017	M	193		82		205		273		268
2017	M	193		82		205		269		270
2017	Q	198	82	84	207	209	266	273	286	308
2017	M	198		82		207		269		294
2017	M	198		82		207		276		274
2017	M	198		88		199		269		296
2017	M	198		78		207		274		274
2017	Q	199	82	84	209	213	271	278	270	298
2017	M	199		82		209		269		-1
2017	M	199		82		209		278		268
2017	M	199		82		209		278		274
2017	M	199		82		209		278		268
2017	M	199		88		207		260		270
2017	M	199		88		211		278		268

Table A-2 continued

Year	Caste	Colony	Rufa12	Rufa12	Rufa19	Rufa19	VMA3	VMA3	VMA6	VMA6
2017	M	199		82		209		278		302
2017	Q	200	84	84	207	215	266	273	294	302
2017	M	200		78		-1		278		282
2017	M	200		86		209		266		288
2017	M	200		82		207		276		274
2017	M	200		86		209		274		285
2017	M	200		84		215		260		288
2017	M	200		84		211		279		286
2017	M	200		82		209		269		288
2017	M	200		82		209		269		282
2017	M	200		84		213		-1		294
2017	Q	203	78	82	199	205	269	278	274	282
2017	M	203		82		213		263		274
2017	M	203		82		207		263		274
2017	M	203		82		207		279		294
2017	M	203		-1		215		-1		278
2017	Q	209	82	86	205	207	271	279	278	298
2017	M	209		82		-1		-1		-1
2017	M	209		86		207		266		288
2017	M	209		82		209		271		288
2017	M	209		84		207		271		282
2017	M	209		82		207		266		272
2017	M	209		82		-1		266		278
2017	M	209		84		205		266		288
2017	M	209		84		207		271		284
2017	M	209		84		205		278		284
2017	Q	210	84	86	205	207	262	271	285	294
2017	M	210		82		211		266		288
2017	M	210		86		209		278		288
2017	M	210		82		211		276		288
2017	Q	215	84	86	209	215	266	276	288	294
2017	M	215		82		207		276		282
2017	M	215		86		205		269		288
2017	M	215		82		205		273		288
2017	M	215		84		207		269		282
2017	M	215		82		209		269		294
2017	M	215		86		205		262		285
2017	M	215		84		207		260		270
2017	M	215		84		207		266		285
2017	Q	217	82	84	199	213	274	276	294	294
2017	M	217		82		205		278		288
2017	M	217		82		205		269		284
2017	M	217		82		205		274		298
2017	M	217		82		205		269		284
2017	M	217		104		205		278		288
2017	Q	221	82	84	205	207	260	279	274	288
2017	M	221		82		207		266		288
2017	M	221		82		211		262		288
2017	M	221		82		205		262		288
2017	M	221		88		205		263		284
2017	M	221		82		207		269		274
2017	M	221		86		207		273		288
2017	Q	227	82	84	207	215	263	263	285	296
2017	M	227		84		209		278		296
2017	M	227		82		215		266		294
2017	M	227		86		207		-1		294
2017	M	227		82		211		266		285

Table A-2 continued

Year	Caste	Colony	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2004	Q	8	242	250	178	181	197	220	153	174
2004	M	8		-1		175		208		168
2004	M	8		-1		181		208		168
2004	M	8		262		178		-1		156
2004	M	8		262		184		197		153
2004	M	8		258		178		-1		165
2004	Q	10	242	250	172	184	200	204	146	159
2004	M	10		256		184		197		150
2004	M	10		260		172		197		149
2004	M	10		256		-1		197		153
2004	M	10		262		184		197		168
2004	M	10		246		175		197		174
2004	Q	13	250	266	166	175	200	208	153	174
2004	M	13		250		184		200		146
2004	M	13		258		178		-1		156
2004	M	13		262		178		192		156
2004	M	13		250		-1		197		153
2004	M	13		-1		-1		-1		156
2004	Q	15	242	258	163	190	204	210		-1
2004	M	15		-1		-1		204		-1
2004	M	15		-1		190		204		-1
2004	M	15		250		172		-1		-1
2004	M	15		250		169		210		-1
2004	M	15		250		169		210		-1
2004	Q	16	250	256	172	181	192	200	156	156
2004	M	16		-1		175		192		156
2004	M	16		242		187		208		-1
2004	M	16		258		178		200		146
2004	M	16		242		181		208		162
2004	M	16		250		181		200		159
2004	M	16		258		-1		200		159
2004	M	16		242		181		192		153
2004	M	16		256		166		200		159
2004	Q	18	250	260	169	193	200	204	153	153
2004	M	18		250		-1		-1		159
2004	M	18		264		-1		204		162
2004	M	18		242		172		192		156
2004	M	18		250		193		208		165
2004	M	18		-1		-1		204		162
2004	Q	19		-1	181	184	192	204	156	159
2004	M	19		-1		-1		-1		156
2004	M	19		-1		166		204		156
2004	M	19		-1		172		200		162
2004	M	19		-1		169		204		149
2004	M	19		-1		175		192		146
2004	M	19		-1		172		200		165
2004	M	19		-1		187		200		165

Table A-2 continued

Year	Caste	Colony	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2004	Q	23	250	266	169	178	192	208	153	159
2004	M	23		256		169		210		159
2004	M	23		-1		172		210		165
2004	M	23		274		163		208		159
2004	M	23		250		163		204		178
2004	M	23		250		-1		200		165
2004	M	23		236		172		208		146
2004	Q	24	236	250	178	178	197	210	153	159
2004	M	24		242		193		216		162
2004	M	24		256		178		208		171
2004	M	24		262		175		200		159
2004	M	24		242		178		197		153
2004	M	24		262		172		208		153
2004	M	24		258		178		192		153
2004	M	24		258		178		184		153
2004	Q	28	238	250	169	178	204	208		-1
2004	M	28		252		184		200		-1
2004	M	28		252		184		197		-1
2004	M	28		252		178		192		-1
2004	M	28		258		184		204		-1
2004	M	28		252		175		192		-1
2004	Q	29	248	250	178	184	204	210	146	156
2004	M	29		242		184		216		-1
2004	M	29		258		175		-1		-1
2004	M	29		-1		175		204		159
2004	Q	43	242	260	178	184	197	200	153	159
2004	M	43		242		181		200		149
2004	M	43		238		190		210		171
2004	M	43		260		184		208		156
2004	M	43		236		181		-1		159
2004	M	43		252		184		200		153
2004	Q	45	240	250	172	178	200	204	153	156
2004	M	45		252		187		200		153
2004	M	45		258		184		210		165
2004	M	45		-1		169		208		147
2004	M	45		240		184		200		153
2004	M	45		258		169		210		165
2006	Q	63	242	250	269	187	192	208	156	159
2006	M	63		266		-1		197		149
2006	M	63		266		190		204		153
2006	M	63		266		175		197		156
2006	M	63		250		184		208		147
2006	M	63		270		178		210		147
2006	M	63		236		196		208		153
2006	M	63		236		196		-1		153
2006	Q	65	242	268	278	284	204	208	153	153
2006	M	65		250		181		192		156
2006	M	65		238		187		204		149
2006	M	65		250		187		-1		159

Table A-2 continued

Year	Caste	Colony	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2006	M	65		250		181		204		159
2006	Q	66	250	270	184	199	180	208	153	156
2006	M	66		-1		199		204		159
2006	M	66		250		199		204		150
2006	M	66		254		199		210		168
2006	M	66		270		175		208		162
2006	Q	67	232	268	172	184	192	208	149	156
2006	M	67		254		172		200		162
2006	M	67		240		190		208		156
2006	M	67		-1		172		210		159
2006	M	67		248		178		204		159
2006	M	67		232		175		208		-1
2006	M	67		256		184		210		146
2006	M	67		248		175		-1		159
2006	Q	68	264	268	178	178	204	216	153	156
2006	M	68		254		181		-1		162
2006	M	68		264		178		204		156
2006	M	68		268		190		-1		-1
2006	M	68		268		178		-1		-1
2006	M	68		278		-1		-1		153
2006	M	68		254		184		200		159
2006	Q	69	236	252	172	181	204	220	153	168
2006	M	69		250		178		200		149
2006	M	69		236		178		200		149
2006	M	69		252		-1		200		149
2006	M	69		-1		190		200		149
2006	M	69		252		190		200		-1
2006	Q	70	250	256	172	184	204	210	146	162
2006	M	70		256		199		204		153
2006	M	70		250		184		204		156
2006	M	70		256		172		210		178
2006	M	70		-1		-1		204		178
2006	M	70		256		184		204		-1
2006	M	70		-1		-1		-1		146
2006	M	70		-1		-1		216		-1
2006	Q	72	250	274	178	181	204	208		-1
2006	M	72		250		181		-1		-1
2006	M	72		250		-1		-1		-1
2006	M	72		250		178		204		-1
2006	M	72		-1		181		-1		-1
2006	Q	76	250	256	187	193	192	200	153	153
2006	M	76		250		187		192		156
2006	M	76		250		187		200		159
2006	M	76		-1		187		204		171
2006	M	76		-1		178		216		159
2006	M	76		-1		-1		216		153
2006	M	76		278		184		204		159
2006	M	76		270		178		210		159
2006	M	76		250		175		200		-1

Table A-2 continued

Year	Caste	Colony	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2006	M	76		276		-1		208		-1
2006	M	76		256		178		216		-1
2006	Q	77	250	274	178	184	192	200	156	159
2006	M	77		-1		199		210		156
2006	M	77		250		178		200		153
2006	M	77		274		178		184		156
2006	M	77		274		199		200		156
2006	M	77		274		178		200		159
2006	M	77		274		190		192		156
2006	M	77		-1		178		192		159
2006	M	77		-1		-1		204		-1
2006	M	77		-1		175		200		-1
2006	M	77		-1		-1		208		-1
2006	Q	79	250	260	178	178	204	210	149	174
2006	M	79		260		181		-1		153
2006	M	79		-1		178		204		153
2006	M	79		232		175		188		153
2006	M	79		250		178		-1		153
2006	M	79		-1		184		192		153
2006	M	79		-1		178		204		165
2006	M	79		-1		178		-1		156
2006	M	79		256		178		204		153
2017	Q	195	250	256	178	178	197	197	153	156
2017	M	195		252		178		204		171
2017	M	195		250		-1		197		156
2017	M	195		254		178		210		153
2017	M	195		-1		190		204		147
2017	M	195		250		178		197		153
2017	M	195		252		178		197		159
2017	Q	196	238	258		-1	197	210	153	156
2017	M	196		236		-1		204		159
2017	M	196		232		-1		200		156
2017	M	196		236		-1		204		178
2017	M	196		-1		-1		204		178
2017	M	196		236		-1		204		159
2017	M	196		-1		-1		210		165
2017	Q	201	246	250	175	178	200	204	146	174
2017	M	201		272		178		197		174
2017	M	201		242		184		210		-1
2017	M	201		242		178		197		153
2017	M	201		-1		178		-1		156
2017	M	201		272		175		204		153
2017	M	201		256		178		200		156
2017	M	201		256		175		204		156
2017	M	201		242		178		197		153
2017	Q	204	246	272	172	178	180	197	146	146
2017	M	204		248		169		192		159
2017	M	204		-1		178		180		146
2017	M	204		250		-1		180		-1

Table A-2 continued

Year	Caste	Colony	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2017	M	204		250		-1		188		-1
2017	Q	205	250	256	169	184	192	204	159	165
2017	M	205		270		169		204		159
2017	M	205		-1		184		216		168
2017	M	205		246		169		192		159
2017	M	205		270		169		204		-1
2017	M	205		270		172		192		159
2017	Q	226	238	258	172	184	197	200	153	159
2017	M	226		258		169		200		162
2017	M	226		278		175		204		159
2017	M	226		242		172		197		-1
2017	M	226		242		190		197		159
2017	M	226		258		190		200		150
2017	Q	228	242	248	178	178	192	197	153	174
2017	M	228		242		178		210		162
2017	M	228		248		178		210		162
2017	M	228		250		178		210		162
2017	Q	229	250	252	178	181	192	210	159	159
2017	M	229		250		184		204		162
2017	M	229		250		190		204		149
2017	M	229		250		178		204		156
2017	M	229		252		178		192		147
2017	M	229		258		184		-1		178
2017	M	229		258		178		-1		178
2017	M	229		258		178		192		159
2017	Q	230	250	270	178	184	200	208	156	159
2017	M	230		250		184		216		159
2017	M	230		250		178		-1		159
2017	M	230		250		172		216		159
2017	M	230		256		187		204		156
2017	M	230		-1		184		204		159
2017	M	230		236		184		208		168
2017	Q	193	252	252	187	190	192	210	147	147
2017	M	193		258		169		192		168
2017	M	193		250		187		204		162
2017	M	193		252		178		200		156
2017	M	193		242		178		197		153
2017	Q	198	250	250	169	190	200	204	159	159
2017	M	198		242		169		208		149
2017	M	198		232		-1		204		153
2017	M	198		258		178		204		153
2017	M	198		250		169		197		156
2017	Q	199	250	262	178	178	208	210		-1
2017	M	199		250		181		216		-1
2017	M	199		254		199		197		-1
2017	M	199		254		-1		192		-1
2017	M	199		250		181		210		-1
2017	M	199		-1		190		210		-1
2017	M	199		262		178		192		-1

Table A-2 continued

Year	Caste	Colony	VMA8	VMA8	LIST2002	LIST2002	LIST2003	LIST2003	LIST2004	LIST2004
2017	M	199		244		-1		204		-1
2017	Q	200	250	264	178	181	197	200	159	159
2017	M	200		244		199		-1		153
2017	M	200		264		190		204		153
2017	M	200		250		178		204		156
2017	M	200		244		190		204		153
2017	M	200		250		190		192		156
2017	M	200		250		190		210		156
2017	M	200		250		181		210		-1
2017	M	200		244		190		204		149
2017	M	200		244		-1		210		147
2017	Q	203	250	252	169	169	192	210	149	153
2017	M	203		240		178		192		162
2017	M	203		256		190		192		149
2017	M	203		252		181		200		156
2017	M	203		250		178		204		159
2017	Q	209	250	262	175	190	200	216	147	178
2017	M	209		262		184		-1		156
2017	M	209		250		-1		208		147
2017	M	209		250		-1		208		171
2017	M	209		258		178		197		159
2017	M	209		246		184		200		162
2017	M	209		274		-1		200		174
2017	M	209		-1		-1		200		-1
2017	M	209		274		178		197		162
2017	M	209		258		178		197		147
2017	Q	210	256	276	178	184	210	216	156	174
2017	M	210		268		178		220		159
2017	M	210		250		181		197		156
2017	M	210		258		178		204		147
2017	Q	215	236	236	172	181	204	210	168	174
2017	M	215		256		172		200		153
2017	M	215		278		172		200		147
2017	M	215		236		169		204		150
2017	M	215		252		172		204		147
2017	M	215		242		169		204		153
2017	M	215		242		172		-1		159
2017	M	215		252		172		208		150
2017	M	215		278		172		204		159
2017	Q	217	238	238	169	178	204	208	156	159
2017	M	217		-1		172		208		153
2017	M	217		252		190		200		156
2017	M	217		262		172		200		153
2017	M	217		262		190		200		156
2017	M	217		-1		172		204		156
2017	Q	221	242	250	178	190	192	200	153	156
2017	M	221		258		172		210		150
2017	M	221		258		172		204		174
2017	M	221		258		181		204		156
2017	M	221		242		181		192		162
2017	M	221		258		178		192		159
2017	M	221		250		172		192		-1
2017	Q	227	252	254	172	178	197	204	156	156
2017	M	227		252		187		208		168
2017	M	227		-1		172		208		162
2017	M	227		252		178		204		178
2017	M	227		250		172		204		159

Table A-2 continued

Year	Caste	Colony	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2004	Q	8	152	152	147	151	183	191	201	207
2004	M	8		154		151		183		207
2004	M	8		156		141		183		203
2004	M	8		154		145		189		201
2004	M	8		148		157		195		201
2004	M	8		156		147		193		185
2004	Q	10	150	156	147	149	191	191	203	206
2004	M	10		150		147		173		185
2004	M	10		158		145		173		203
2004	M	10		158		147		187		206
2004	M	10		152		151		181		206
2004	M	10		158		153		193		207
2004	Q	13	154	156	147	151	179	193	201	203
2004	M	13		150		153		185		201
2004	M	13		152		141		193		203
2004	M	13		156		151		195		201
2004	M	13		-1		149		195		-1
2004	M	13		150		141		193		207
2004	Q	15	158	158	151	153	173	193	207	213
2004	M	15		148		155		195		201
2004	M	15		160		147		173		207
2004	M	15		-1		157		-1		207
2004	M	15		154		149		-1		207
2004	M	15		154		149		193		206
2004	Q	16	152	154	141	145	191	193	203	207
2004	M	16		154		147		187		206
2004	M	16		156		155		189		206
2004	M	16		156		155		195		207
2004	M	16		150		147		187		203
2004	M	16		146		141		199		203
2004	M	16		150		147		-1		203
2004	M	16		154		141		187		207
2004	M	16		158		149		191		201
2004	Q	18	148	154	147	149	185	193	203	208
2004	M	18		158		141		189		207
2004	M	18		156		153		-1		202
2004	M	18		158		153		185		206
2004	M	18		154		153		185		202
2004	M	18		156		147		185		207
2004	Q	19	148	150	149	153	191	193	203	207
2004	M	19		156		147		185		203
2004	M	19		154		145		197		201
2004	M	19		156		155		197		201
2004	M	19		154		149		195		193
2004	M	19		154		-1		181		203
2004	M	19		-1		149		187		203
2004	M	19		148		155		187		203

Table A-2 continued

Year	Caste	Colony	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2004	Q	23	154	156	147	147	191	193	203	207
2004	M	23		154		147		-1		-1
2004	M	23		150		145		197		193
2004	M	23		154		151		183		203
2004	M	23		150		149		181		193
2004	M	23		150		151		183		193
2004	M	23		150		141		185		201
2004	Q	24	150	152	147	149	179	193	203	206
2004	M	24		154		141		191		201
2004	M	24		150		141		189		204
2004	M	24		156		149		173		203
2004	M	24		150		149		195		207
2004	M	24		154		149		191		207
2004	M	24		156		149		193		203
2004	M	24		152		149		195		207
2004	Q	28	162	162	149	149	181	191	203	207
2004	M	28		160		-1		195		189
2004	M	28		160		-1		181		189
2004	M	28		158		149		195		203
2004	M	28		158		145		187		201
2004	M	28		158		147		187		189
2004	Q	29	156	158	141	147	185	191	203	207
2004	M	29		148		149		187		203
2004	M	29		146		149		179		206
2004	M	29		152		149		191		207
2004	Q	43	148	156	149	149	191	195	207	207
2004	M	43		148		153		195		203
2004	M	43		156		-1		195		207
2004	M	43		156		155		183		203
2004	M	43		154		149		191		206
2004	M	43		156		141		195		203
2004	Q	45	150	158	141	147	195	195	203	207
2004	M	45		154		147		191		203
2004	M	45		158		145		-1		207
2004	M	45		158		149		191		203
2004	M	45		156		159		187		206
2004	M	45		150		147		195		203
2006	Q	63	154	156	147	159	191	193	203	205
2006	M	63		154		-1		199		203
2006	M	63		152		147		179		205
2006	M	63		158		147		185		201
2006	M	63		150		147		187		205
2006	M	63		162		155		191		211
2006	M	63		158		141		189		203
2006	M	63		158		141		189		203
2006	Q	65	148	150	147	149	193	193	205	207
2006	M	65		154		147		189		207
2006	M	65		146		149		193		189
2006	M	65		154		147		193		207

Table A-2 continued

Year	Caste	Colony	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2006	M	65		156		149		193		201
2006	Q	66	152	156	141	149	193	193	202	205
2006	M	66		152		153		193		207
2006	M	66		158		147		187		205
2006	M	66		148		153		193		207
2006	M	66		150		153		173		205
2006	Q	67	152	154	149	153	179	187	205	207
2006	M	67		148		147		193		205
2006	M	67		152		149		173		201
2006	M	67		154		141		193		201
2006	M	67		158		149		179		205
2006	M	67		152		149		179		-1
2006	M	67		154		149		193		207
2006	M	67		158		149		185		201
2006	Q	68	152	156	147	149	179	191	189	205
2006	M	68		154		147		193		207
2006	M	68		154		147		185		207
2006	M	68		154		151		189		202
2006	M	68		152		147		-1		207
2006	M	68		-1		149		193		202
2006	M	68		-1		141		193		189
2006	Q	69	150	156	141	149	189	193	201	205
2006	M	69		152		151		193		203
2006	M	69		152		145		193		203
2006	M	69		158		147		193		203
2006	M	69		158		141		193		203
2006	M	69		152		147		193		203
2006	Q	70	152	152	141	147	185	193	206	213
2006	M	70		152		147		173		213
2006	M	70		152		147		185		206
2006	M	70		152		147		193		203
2006	M	70		152		157		193		206
2006	M	70		152		147		185		189
2006	M	70		152		141		-1		-1
2006	M	70		152		147		193		-1
2006	Q	72	154	156	147	149	187	193	206	209
2006	M	72		150		149		173		193
2006	M	72		154		147		193		203
2006	M	72		152		147		179		206
2006	M	72		150		147		187		185
2006	Q	76	148	152	149	157	191	193	201	206
2006	M	76		152		151		193		206
2006	M	76		148		151		193		203
2006	M	76		156		149		191		203
2006	M	76		154		149		191		208
2006	M	76		152		147		193		208
2006	M	76		148		149		193		206
2006	M	76		156		151		191		-1
2006	M	76		152		145		185		201

Table A-2 continued

Year	Caste	Colony	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2006	M	76		152		149		191		203
2006	M	76		154		141		191		-1
2006	Q	77	154	156	145	147	185	193	203	207
2006	M	77		154		149		193		207
2006	M	77		154		147		185		207
2006	M	77		152		153		185		201
2006	M	77		156		147		193		203
2006	M	77		156		147		185		203
2006	M	77		154		149		187		207
2006	M	77		154		145		193		203
2006	M	77		152		141		191		209
2006	M	77		154		147		191		205
2006	M	77		154		-1		189		201
2006	Q	79	152	154	147	147	181	189	201	203
2006	M	79		148		147		181		203
2006	M	79		154		147		189		203
2006	M	79		148		149		191		206
2006	M	79		152		147		189		204
2006	M	79		156		147		191		203
2006	M	79		154		147		181		203
2006	M	79		152		147		181		206
2006	M	79		156		149		193		185
2017	Q	195	150	154	147	149	191	193	207	209
2017	M	195		152		149		173		207
2017	M	195		148		149		185		209
2017	M	195		154		149		189		211
2017	M	195		158		147		193		209
2017	M	195		154		147		191		209
2017	M	195		154		141		191		205
2017	Q	196	156	156	151	159	191	193	201	205
2017	M	196		156		147		179		203
2017	M	196		146		149		191		209
2017	M	196		148		147		189		206
2017	M	196		156		145		191		205
2017	M	196		148		147		199		211
2017	M	196		150		147		179		206
2017	Q	201	154	154	147	151	173	193	206	211
2017	M	201		154		-1		193		211
2017	M	201		152		-1		191		211
2017	M	201		156		153		181		201
2017	M	201		156		147		181		209
2017	M	201		150		153		179		201
2017	M	201		156		153		181		209
2017	M	201		152		153		181		209
2017	M	201		156		153		181		201
2017	Q	204	178	178	141	155	187	193		-1
2017	M	204		162		145		187		-1
2017	M	204		-1		147		193		-1
2017	M	204		-1		149		193		-1

Table A-2 continued

Year	Caste	Colony	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2017	M	204		-1		149		191		-1
2017	Q	205	150	152	147	157	173	191	205	205
2017	M	205		152		157		185		208
2017	M	205		162		147		191		203
2017	M	205		152		149		191		203
2017	M	205		152		149		185		208
2017	M	205		152		149		185		208
2017	Q	226	152	154	147	151	179	193	205	207
2017	M	226		158		147		191		203
2017	M	226		150		147		185		209
2017	M	226		154		151		187		207
2017	M	226		154		149		191		207
2017	M	226		154		147		185		193
2017	Q	228	152	154	147	149	191	193	206	211
2017	M	228		158		151		193		205
2017	M	228		158		157		185		205
2017	M	228		158		141		191		209
2017	Q	229	152	154	147	153	173	193	203	209
2017	M	229		148		147		185		209
2017	M	229		154		149		193		205
2017	M	229		152		141		181		203
2017	M	229		152		-1		191		209
2017	M	229		148		147		-1		-1
2017	M	229		154		147		189		213
2017	M	229		154		149		193		209
2017	Q	230	152	154	147	149	179	193	213	213
2017	M	230		154		159		181		205
2017	M	230		154		147		179		205
2017	M	230		158		149		193		203
2017	M	230		154		155		193		206
2017	M	230		158		151		189		185
2017	M	230		158		151		179		185
2017	Q	193	152	156	147	153	173	191	207	209
2017	M	193		154		153		185		207
2017	M	193		152		147		193		207
2017	M	193		148		147		187		209
2017	M	193		154		149		191		209
2017	Q	198	154	156	147	151	189	193	205	207
2017	M	198		154		147		185		207
2017	M	198		-1		141		173		207
2017	M	198		156		153		191		207
2017	M	198		158		149		191		207
2017	Q	199	154	156	151	151	185	191	207	209
2017	M	199		154		147		179		193
2017	M	199		152		151		193		209
2017	M	199		154		-1		189		205
2017	M	199		150		151		193		209
2017	M	199		150		149		189		205
2017	M	199		154		151		193		205

Table A-2 continued

Year	Caste	Colony	LIST2007	LIST2007	LIST2008	LIST2008	LIST2010	LIST2010	LIST2013	LIST2013
2017	M	199		154		151		189		207
2017	Q	200	152	154	141	151	179	189	203	211
2017	M	200		154		153		193		205
2017	M	200		154		147		187		208
2017	M	200		150		141		189		208
2017	M	200		150		147		193		211
2017	M	200		146		149		179		208
2017	M	200		154		147		173		211
2017	M	200		154		147		193		208
2017	M	200		154		151		187		211
2017	M	200		154		147		189		205
2017	Q	203	152	156		-1	193	193	209	209
2017	M	203		156		-1		191		211
2017	M	203		156		-1		181		202
2017	M	203		156		-1		193		211
2017	M	203		152		-1		193		205
2017	Q	209	154	158	149	149	181	191	202	211
2017	M	209		152		-1		185		206
2017	M	209		152		159		193		206
2017	M	209		156		151		173		206
2017	M	209		150		147		189		206
2017	M	209		154		141		181		209
2017	M	209		154		159		179		209
2017	M	209		156		149		193		189
2017	M	209		150		159		189		209
2017	M	209		150		149		189		189
2017	Q	210	156	158	147	153	179	193	209	211
2017	M	210		162		149		179		209
2017	M	210		156		151		189		205
2017	M	210		156		149		191		189
2017	Q	215	152	158	147	149	191	191	189	204
2017	M	215		152		147		191		211
2017	M	215		148		147		173		209
2017	M	215		152		153		185		203
2017	M	215		152		153		191		204
2017	M	215		150		153		187		207
2017	M	215		152		149		191		209
2017	M	215		150		149		185		211
2017	M	215		156		147		193		193
2017	Q	217	152	158	147	153	189	191	203	203
2017	M	217		148		149		191		211
2017	M	217		154		147		193		211
2017	M	217		158		149		191		189
2017	M	217		154		153		193		189
2017	M	217		148		149		-1		-1
2017	Q	221	154	158	141	149	191	191	205	209
2017	M	221		148		149		193		213
2017	M	221		148		147		193		205
2017	M	221		154		151		179		207
2017	M	221		148		147		199		213
2017	M	221		148		147		199		213
2017	M	221		148		149		193		207
2017	Q	227	148	148	141	149	173	193	203	209
2017	M	227		152		149		193		205
2017	M	227		156		149		181		209
2017	M	227		152		159		193		209
2017	M	227		156		159		191		209

Table A-2 continued

Year	Caste	Colony	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2004	Q	8	160	168	160	164	139	148	238	252
2004	M	8		164		164		148		234
2004	M	8		166		154		148		232
2004	M	8		166		154		148		-1
2004	M	8		166		154		152		242
2004	M	8		170		158		148		234
2004	Q	10	168	170	154	158	139	152	238	252
2004	M	10		170		166		148		236
2004	M	10		168		158		148		236
2004	M	10		168		158		148		236
2004	M	10		164		158		148		236
2004	M	10		164		172		148		234
2004	Q	13	166	168	154	154	139	139	236	252
2004	M	13		164		176		148		246
2004	M	13		166		160		148		244
2004	M	13		166		166		148		246
2004	M	13		166		154		148		-1
2004	M	13		164		158		148		252
2004	Q	15	166	168	154	158	139	148	238	238
2004	M	15		160		154		139		238
2004	M	15		164		166		148		236
2004	M	15		168		166		144		234
2004	M	15		164		154		137		234
2004	M	15		160		166		137		234
2004	Q	16	168	168	154	158	139	152	234	236
2004	M	16		166		158		148		238
2004	M	16		168		166		135		246
2004	M	16		166		166		148		238
2004	M	16		166		166		148		248
2004	M	16		168		164		148		238
2004	M	16		166		166		148		234
2004	M	16		168		166		148		238
2004	M	16		166		158		148		250
2004	Q	18	166	170	158	158	139	139	236	238
2004	M	18		166		160		148		234
2004	M	18		170		154		148		246
2004	M	18		166		154		152		226
2004	M	18		168		166		148		244
2004	M	18		166		160		148		236
2004	Q	19	168	168	154	158	148	152	236	246
2004	M	19		168		158		148		232
2004	M	19		164		166		135		236
2004	M	19		164		158		144		232
2004	M	19		168		158		148		232
2004	M	19		164		158		148		232
2004	M	19		168		154		148		246
2004	M	19		168		166		148		234

Table A-2 continued

Year	Caste	Colony	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2004	Q	23	166	168	154	158	139	148	236	244
2004	M	23		168		158		148		236
2004	M	23		170		160		139		244
2004	M	23		170		154		152		236
2004	M	23		170		160		139		246
2004	M	23		170		160		152		236
2004	M	23		170		158		148		232
2004	Q	24	166	168	154	158	148	148	234	246
2004	M	24		166		154		139		232
2004	M	24		164		154		148		240
2004	M	24		164		160		148		242
2004	M	24		174		154		139		246
2004	M	24		168		154		148		236
2004	M	24		164		154		148		246
2004	M	24		170		154		139		244
2004	Q	28	166	168	154	154	148	152	234	248
2004	M	28		160		168		139		248
2004	M	28		160		168		139		238
2004	M	28		160		154		152		238
2004	M	28		160		158		139		248
2004	M	28		162		158		139		248
2004	Q	29	166	166	154	156	139	139	236	246
2004	M	29		166		164		148		246
2004	M	29		168		154		139		236
2004	M	29		166		154		139		238
2004	Q	43	166	168	154	154	148	152	238	246
2004	M	43		166		156		148		236
2004	M	43		168		162		148		234
2004	M	43		166		154		139		226
2004	M	43		168		164		148		234
2004	M	43		168		154		148		-1
2004	Q	45	166	168	154	158	139	139	232	234
2004	M	45		170		176		148		244
2004	M	45		166		158		139		252
2004	M	45		168		154		139		240
2004	M	45		166		154		148		244
2004	M	45		166		154		139		234
2006	Q	63	164	166	154	158	139	139	232	238
2006	M	63		170		158		139		236
2006	M	63		168		158		135		234
2006	M	63		166		164		152		238
2006	M	63		168		158		148		-1
2006	M	63		168		158		139		238
2006	M	63		168		154		139		238
2006	M	63		168		154		139		232
2006	Q	65	168	168	158	158	139	148	246	246
2006	M	65		166		164		139		240
2006	M	65		168		162		148		232
2006	M	65		164		160		148		236

Table A-2 continued

Year	Caste	Colony	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2006	M	65		168		158		148		238
2006	Q	66	164	166	154	158	139	148	236	244
2006	M	66		168		164		139		246
2006	M	66		168		176		148		252
2006	M	66		166		166		139		236
2006	M	66		166		154		139		238
2006	Q	67	160	166	154	168	139	139	232	252
2006	M	67		160		154		139		-1
2006	M	67		166		158		152		238
2006	M	67		168		158		139		234
2006	M	67		164		160		139		238
2006	M	67		166		164		144		234
2006	M	67		168		166		139		234
2006	M	67		164		154		152		234
2006	Q	68	166	168	154	162	139	148	240	244
2006	M	68		166		162		144		238
2006	M	68		166		158		139		246
2006	M	68		-1		154		152		-1
2006	M	68		166		158		144		246
2006	M	68		166		158		135		236
2006	M	68		166		154		139		248
2006	Q	69	166	168	154	158	148	148	246	248
2006	M	69		164		164		152		226
2006	M	69		162		164		148		252
2006	M	69		162		154		148		252
2006	M	69		162		154		155		252
2006	M	69		162		158		152		252
2006	Q	70	166	168	154	164	139	139	226	236
2006	M	70		166		154		152		246
2006	M	70		166		164		139		234
2006	M	70		166		154		148		236
2006	M	70		168		154		139		-1
2006	M	70		166		164		148		234
2006	M	70		164		166		-1		-1
2006	M	70		164		168		-1		236
2006	Q	72	164	168	154	158	139	152	226	238
2006	M	72		166		164		139		248
2006	M	72		170		156		148		248
2006	M	72		166		158		144		234
2006	M	72		168		156		139		252
2006	Q	76	164	166	154	158	139	148	232	240
2006	M	76		166		158		152		238
2006	M	76		164		154		148		-1
2006	M	76		166		154		148		236
2006	M	76		168		154		152		236
2006	M	76		166		160		-1		-1
2006	M	76		168		164		148		242
2006	M	76		170		154		148		236
2006	M	76		168		154		139		232

Table A-2 continued

Year	Caste	Colony	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2006	M	76		-1		158		152		232
2006	M	76		168		154		152		236
2006	Q	77	166	168	154	160	137	148	236	244
2006	M	77		164		154		148		232
2006	M	77		168		154		148		246
2006	M	77		166		158		139		238
2006	M	77		168		154		139		232
2006	M	77		168		160		148		236
2006	M	77		168		158		139		236
2006	M	77		168		160		148		244
2006	M	77		168		158		152		236
2006	M	77		168		154		152		238
2006	M	77		164		168		-1		232
2006	Q	79	166	168	158	172	135	139	232	234
2006	M	79		164		158		153		-1
2006	M	79		166		158		139		236
2006	M	79		166		154		139		236
2006	M	79		166		172		139		236
2006	M	79		166		172		152		246
2006	M	79		168		172		152		236
2006	M	79		166		172		139		234
2006	M	79		166		158		144		252
2017	Q	195	164	166	158	160	139	144	234	246
2017	M	195		164		166		148		242
2017	M	195		166		162		139		236
2017	M	195		164		158		139		246
2017	M	195		166		154		148		244
2017	M	195		166		158		144		234
2017	M	195		166		164		152		238
2017	Q	196	160	168	154	164	144	148	234	234
2017	M	196		160		156		139		244
2017	M	196		166		172		139		234
2017	M	196		166		154		139		244
2017	M	196		166		162		139		244
2017	M	196		168		156		144		236
2017	M	196		168		158		152		238
2017	Q	201	160	160	156	160	139	148	238	252
2017	M	201		164		154		144		234
2017	M	201		166		158		148		248
2017	M	201		166		154		148		236
2017	M	201		168		160		139		236
2017	M	201		168		158		135		244
2017	M	201		168		156		139		236
2017	M	201		168		166		153		236
2017	M	201		166		154		139		236
2017	Q	204	164	166	158	162		-1	238	240
2017	M	204		166		154		-1		240
2017	M	204		166		154		-1		-1
2017	M	204		164		154		-1		238

Table A-2 continued

Year	Caste	Colony	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2017	M	204		164		162		-1		-1
2017	Q	205	166	168	154	158	139	152	234	236
2017	M	205		166		154		139		234
2017	M	205		168		162		148		234
2017	M	205		166		158		152		244
2017	M	205		168		154		139		236
2017	M	205		168		154		139		234
2017	Q	226	164	166	154	154	152	152	234	236
2017	M	226		162		160		152		244
2017	M	226		168		162		152		234
2017	M	226		166		154		148		248
2017	M	226		168		154		139		236
2017	M	226		164		166		-1		232
2017	Q	228	166	168	154	160		-1	226	266
2017	M	228		166		158		-1		238
2017	M	228		166		162		-1		244
2017	M	228		164		164		-1		238
2017	Q	229	162	168	154	158	139	148	232	238
2017	M	229		166		158		139		238
2017	M	229		166		154		139		238
2017	M	229		166		164		139		236
2017	M	229		166		-1		-1		238
2017	M	229		-1		-1		-1		-1
2017	M	229		166		164		139		232
2017	M	229		166		158		139		238
2017	Q	230	164	168	160	160	139	139	248	248
2017	M	230		168		158		152		236
2017	M	230		-1		158		148		236
2017	M	230		164		154		152		234
2017	M	230		164		158		155		244
2017	M	230		-1		158		148		234
2017	M	230		-1		160		139		234
2017	Q	193	166	168	154	164	139	148	232	234
2017	M	193		164		154		139		238
2017	M	193		166		154		148		226
2017	M	193		164		160		148		238
2017	M	193		168		160		148		236
2017	Q	198	166	168	164	166	135	139	234	238
2017	M	198		166		158		139		246
2017	M	198		166		164		152		236
2017	M	198		166		164		139		234
2017	M	198		166		160		139		236
2017	Q	199	164	166	154	158	139	148	238	246
2017	M	199		164		162		139		234
2017	M	199		166		-1		139		238
2017	M	199		166		166		148		232
2017	M	199		166		166		148		238
2017	M	199		166		154		139		246
2017	M	199		166		162		139		238

Table A-2 continued

Year	Caste	Colony	LIST2015	LIST2015	LIST2017	LIST2017	LIST2019	LIST2019	LIST2020	LIST2020
2017	M	199		168		158		148		246
2017	Q	200	160	166	154	158	139	151	232	246
2017	M	200		168		162		148		234
2017	M	200		166		158		148		240
2017	M	200		168		164		148		234
2017	M	200		164		158		148		252
2017	M	200		170		154		139		236
2017	M	200		160		158		148		232
2017	M	200		168		158		148		232
2017	M	200		168		158		139		234
2017	M	200		168		156		148		238
2017	Q	203	166	168	154	158	135	137	240	240
2017	M	203		160		158		139		244
2017	M	203		164		154		148		246
2017	M	203		168		158		139		236
2017	M	203		160		158		139		234
2017	Q	209	164	166	158	158	139	148	236	238
2017	M	209		-1		-1		-1		232
2017	M	209		166		166		144		234
2017	M	209		166		166		-1		234
2017	M	209		166		168		152		248
2017	M	209		166		162		144		236
2017	M	209		168		-1		152		248
2017	M	209		166		160		139		-1
2017	M	209		166		160		139		-1
2017	M	209		166		162		139		-1
2017	Q	210	166	168	154	164	135	139	246	248
2017	M	210		162		158		148		236
2017	M	210		160		168		148		236
2017	M	210		166		158		148		246
2017	Q	215	166	168	154	158	139	152	232	246
2017	M	215		168		168		148		234
2017	M	215		160		158		152		246
2017	M	215		160		168		148		250
2017	M	215		164		158		152		242
2017	M	215		166		158		152		236
2017	M	215		160		162		148		246
2017	M	215		166		164		148		238
2017	M	215		170		160		148		244
2017	Q	217	164	168	154	160	148	148	244	246
2017	M	217		166		164		148		236
2017	M	217		166		168		148		236
2017	M	217		-1		158		148		236
2017	M	217		166		168		148		236
2017	M	217		166		164		148		236
2017	Q	221	166	170	158	166	139	148	234	246
2017	M	221		166		164		152		236
2017	M	221		166		154		152		236
2017	M	221		168		158		148		244
2017	M	221		166		160		148		232
2017	M	221		164		166		139		246
2017	M	221		166		158		139		250
2017	Q	227	166	168	158	160	144	144	234	236
2017	M	227		166		158		152		252
2017	M	227		168		158		-1		248
2017	M	227		170		154		-1		236
2017	M	227		166		164		-1		252

Table A-3. Number of *V. maculifrons* workers per colony genotyped (N), observed number of patrilines (P_o), effective number of patrilines (P_e), male reproductive skew (B), relatedness between nestmate workers (r_{ww}), and relatedness between males (r_{mm})

Year	Colony ID	Latitude	Longitude	Collection Date	N	P_o	P_e	B	r_{ww}	r_{mm}
2004	8	33.8315	-84.7018	9-Sep-04	16	5	3.914	0.008	0.376	0.000
	10	34.5877	-84.0029	15-Sep-04	16	5	4.453	-0.023	0.379	0.116
	13	34.5877	-84.0029	15-Sep-04	16	5	3.075	0.078	0.442	0.058
	15	34.5877	-84.0029	15-Sep-04	16	5	4.036	0.000	0.358	0.106
	16	33.7850	-84.3176	24-Sep-04	16	8	6.797	-0.031	0.357	0.026
	18	33.8314	-84.2572	28-Sep-04	16	5	4.036	0.000	0.290	0.064
	19	33.8918	-84.4850	1-Oct-04	16	7	4.453	0.030	0.309	0.018
	23	33.7330	-84.3737	1-Oct-04	16	6	4.967	-0.016	0.314	0.152
	24	33.7497	-84.2922	1-Oct-04	16	7	6.150	-0.032	0.337	0.035
	28	34.1717	-84.4212	11-Oct-04	16	5	3.914	0.008	0.442	0.275
	29	33.7740	-84.3956	12-Oct-04	16	3	2.636	0.008	0.426	0.019
	43	34.0027	-84.3816	27-Oct-04	16	5	4.612	-0.031	0.378	0.000
	45	33.8312	-84.3934	28-Oct-04	16	5	3.491	0.039	0.339	0.028
Mean					**16**	**5.46**	**4.350**	**0.003**	**0.365**	**0.069**
2006	63	33.7900	-84.3727	7-Sep-06	15	7	5.544	-0.018	0.299	0.023
	65	33.7900	-84.3727	7-Sep-06	13	4	3.986	-0.053	0.395	0.079
	66	33.7900	-84.3727	7-Sep-06	13	4	3.986	-0.053	0.313	0.052
	67	33.8455	-84.3517	7-Sep-06	14	7	6.612	-0.051	0.298	0.022
	68	33.7900	-84.3727	25-Sep-06	14	6	4.959	-0.022	0.354	0.064
	69	33.7900	-84.3727	25-Sep-06	14	5	3.099	0.069	0.566	0.327
	70	34.0692	-84.6584	4-Oct-06	16	7	5.615	-0.017	0.477	0.060
	72	34.5877	-84.0029	18-Oct-06	14	4	3.420	-0.008	0.349	0.069
	76	33.8709	-84.4187	19-Oct-06	25	10	6.602	0.016	0.277	0.028
	77	33.8709	-84.4187	19-Oct-06	28	10	7.862	-0.005	0.326	0.045
	79	33.8709	-84.4187	19-Oct-06	16	8	7.597	-0.047	0.379	0.063
Mean					**16.55**	**6.55**	**5.390**	**-0.017**	**0.367**	**0.076**
2017	193	33.8060	-84.4215	27-Jun-2017	16	4	3.491	-0.008	0.406	0.011
	195	33.7764	-84.3172	13-Jul-2017	16	6	4.166	0.023	0.354	0.000
	196	33.9682	-84.4753	19-Jul-2017	16	6	4.036	0.031	0.395	0.047
	198	34.1365	-84.2680	19-Jul-2017	16	4	3.150	0.023	0.433	0.040
	199	34.2476	-84.0319	19-Jul-2017	16	7	6.150	-0.032	0.397	0.095
	200	34.2476	-84.0319	19-Jul-2017	16	9	7.597	-0.034	0.288	0.049
	201	34.2476	-84.0319	19-Jul-2017	16	8	6.457	-0.023	0.418	0.150
	203	34.0017	-84.0214	19-Jul-2017	16	4	2.870	0.055	0.496	0.040
	204	33.8431	-84.5558	26-Jul-2017	16	4	3.491	-0.008	0.447	0.059
	205	33.8388	-84.2736	26-Jul-2017	16	5	4.967	-0.047	0.452	0.199
	209	33.9565	-84.4612	11-Aug-2017	15	9	7.839	-0.042	0.271	0.073
	210	33.9523	-84.3322	11-Aug-2017	16	3	1.928	0.148	0.407	0.002
	215	34.0160	-84.4303	6-Sep-2017	16	8	6.797	-0.031	0.316	0.039
	217	33.9561	-84.4262	6-Sep-2017	16	5	4.036	0.000	0.488	0.175
	221	33.8673	-84.5365	27-Sep-2017	16	6	5.166	-0.023	0.317	0.079
	226	33.8240	-84.3091	16-Nov-2017	14	5	3.673	0.018	0.389	0.000
	227	33.8240	-84.3091	16-Nov-2017	15	4	3.988	-0.047	0.414	0.052
	228	33.8124	-84.3309	16-Nov-2017	16	3	2.870	-0.023	0.457	0.188
	229	33.8727	-84.5134	16-Nov-2017	16	7	4.612	0.022	0.375	0.078
	230	33.8725	-84.5147	16-Nov-2017	15	6	4.289	0.013	0.401	0.057
Mean					**15.75**	**5.65**	**4.580**	**0.001**	**0.396**	**0.072**
Grand mean					**16.02**	**5.818**	**4.713**	**-0.003**	**0.379**	**0.072**

Table A-4. P-values for tests of linkage disequilibrium for all loci at each of three timepoints. Values represent mean p-values for pairwise comparisons between the

Locus	Timepoint		
	2004	**2006**	**2017**
Rufa12	0.7053	0.7823	0.6946
Rufa19	0.6573	0.6977	0.7975
VMA3	0.9245	x	0.8466
VMA6	x	x	x
VMA8	0.8275	0.8177	0.7789
LIST2002	0.9146	0.8414	0.6691
LIST2003	0.8480	0.8390	0.6774
LIST2004	0.8308	0.8540	0.6106
LIST2007	0.8828	0.7540	0.7058
LIST2008	0.6949	0.8270	0.7662
LIST2010	0.7518	0.8178	0.7569
LIST2013	0.5839	1	0.7858
LIST2015	0.5571	0.7803	0.7231
LIST2017	0.6415	0.7827	0.7062
LIST2019	0.7714	0.6945	0.6865
LIST2020	0.8877	1	0.7529
Mean	**0.7653**	**0.8206**	**0.7305**

APPENDIX B. SUPPLEMENTAL MATERIALS FOR CHAPTER 4

Supplemental Materials and Methods 1

Gene family and DNA methylation datasets

Gene family information from OrthoDB v9.1 (Zdobnov, et al. 2017) was used to identify genes that were found in single- and multiple-copy in *A. mellifera* based off orthologous genes in related taxa (Chau and Goodisman 2017). The set of duplicated genes obtained consisted of all multiple-copy, functional genes in *A. mellifera,* including genes that underwent a duplication event following divergence from other insect species within Apoidea. Following identification of gene families, genes with the lowest pairwise dS value in the family were identified as duplicate pairs within those larger family contexts.

Whole-genome bisulfite sequencing data from three studies of DNA methylation in the honeybee were downloaded from the European Nucleotide Archive (Leinonen, et al. 2010). The three studies focused on (1) DNA methylation differences between queen and worker brains (Lyko F. 2010), (2) the effects of RNA-i knockdown of DNMT3 in workers (Li-Byarlay, et al. 2013), and (3) epigenetic state reversal between worker subclasses (Herb, et al. 2012). In total, 16 datasets from these studies were used for further analyses.

All bisulfite samples were analyzed with FastQC (Andrews 2010) to determine the need for custom trimming parameters. Sequences were trimmed using Trim Galore! (Krueger 2015) and primers and extraneous sequence repeats were removed using Cutadapt (Martin 2011). Trimming was completed with parameters -q 20 -e 0.1 -l 20

(representing a Phred quality score cutoff of 20, a maximum error rate of 0.1, and a minimum post-trim read length of 20bp). The reference genome used for read alignment was *A. mellifera* genome assembly Amel_4.5, downloaded from the Beebase Hymenoptera Genome Database (Munoz-Torres, et al. 2010) and converted to an indexed reference using Bismark Genome Preparation (Krueger and Andrews 2011) with default parameters.

Trimmed FastQ files were aligned to the indexed reference using Bismark and Bowtie2 (Langmead and Salzberg 2012). The alignment was performed using the most stringent parameters for allowed mismatches and seed length. In addition, the --ambiguous tag was applied in order to discard any reads with more than one unique alignment. This alignment setting was included to eliminate multimapping of reads which mapped to multiple areas of the reference genome with the same quality of alignment, which may be an issue when mapping duplicated genes with high sequence similarity. Due to the stringency of alignment parameters, this led to less than 5% of reads needing removal due to non-unique alignments. The analyses were also tested with these reads included, as a control against the data being skewed from this step. CpG methylation calls from the alignment were then extracted using Bismark Methylation Extractor (see supplemental table S6 for data file and alignment information).

Methylation calls from Bismark were imported into SeqMonk (Andrews) for visualization and quantification. Reading frames (probes) were created at the level of individual genes, as defined by the *A. mellifera* Official Gene Set Version 3.2 (Elsik, et al. 2014). Read coverage outliers were removed using SeqMonk following developer recommendations to avoid skewing of the results by data of questionable quality. The

Seqmonk Bisulphite Methylation Pipeline was used to calculate the mean percentage of bisulfite-converted CpG sites out of all CpG dinucleotides for each gene.

The correlations between DNA methylation calls between each of the 16 WGBS datasets were calculated using JMP in order to measure the consistency of the data across the different studies, tissues, and individuals (supplemental table S7). A new dataset was created by finding the mean CpG methylation level for each *A. mellifera* gene across the 16 datasets. This new mean set was used as representative data for all other analyses.

Analyses of DNA methylation in duplicated genes and singletons

ANOVA was used to determine if the mean methylation level of genes belonging to families of different sizes differed significantly. CpG methylation for this analysis was defined as the percentage of CpG dinucleotides that were methylated within each gene. Methylation status of genes ("high" vs "low") was determined by estimating the midpoint of the bimodally distributed level of gene methylation in the honeybee. This midpoint was identified as 5% CpG methylation across all datasets, and this value was thus used as the threshold for determining if a gene showed high or low levels of methylation. χ^2 tests of independence were used to test the association between "duplication status" (singletons vs. duplicates) and "methylation status" (high vs. low) of duplicated genes, and to determine whether either of these variables could be predictive of the other. χ^2 analysis was completed using the "Chi-Square Test Calculator" (Social Science Statistics, 2018).

We next investigated a relative measure of divergence between paralogs. Relative divergence (D_r) was calculated as (V1 – V2)/(V1 + V2) where V1 and V2 were the

values for the focal gene copies (Keller and Yi 2014). We tested if the relative methylation divergence between duplicate gene pairs differed from null expectations using a randomization test. Specifically, all duplicated genes were pooled together into a single gene set and two of these genes were then randomly selected to produce new pairs of "pseudoparalogs". This procedure was repeated until all genes in the set had been randomly paired. The divergence in methylation level between the pseudoparalogs was calculated, and a mean was found for all the pseudoparalog pairs in the set. This entire trial was repeated 10,000 times to produce a null distribution of mean methylation divergence for pairs of randomly selected duplicate genes. The actual observed mean methylation D_r between paralogs was then compared to the null distribution of mean D_r of randomly paired genes. The observed value of mean methylation D_r was deemed significant if it fell outside of 95% of the randomly generated mean methylation D_r values. This randomization trial was then replicated using singleton genes randomly paired into pseudoparalogs, with a normal distribution again being created from 10,000 trials.

We next examined the relationship between relative methylation divergence of paralogs and their sequence divergence since duplication. We used custom Bioperl scripts to extract nucleotide sequence information from genome sequencing files. Multiple protein alignments between duplicate genes were generated using MUSCLE aligner (Edgar 2004) for each gene. Codon alignments were then created from aligned protein files and multifasta nucleotide sequences using PAL2NAL (Suyama, et al. 2006). Codon alignments were used to calculate synonymous substitution rate ratios between paralogs using the PAML yn00 package (Yang 2007). Duplicate pairs with a dS value greater than

3 were discarded from analyses to avoid genes saturated with substitutions. Genes were placed into one of three bins dependent on dS value from 0-1, 1-2, or 2-3. ANOVA was used to test the difference in the means of each bin and determine whether there was an association between relative methylation divergence and synonymous substitution rate ratio (dS).

Analyses of DNA methylation and gene expression

Sequenced RNA data was obtained from previous studies that examined gene expression differences in *A. mellifera* between drone, worker, and queen larvae (Ashby, et al. 2016) and drone, worker, and queen pupae (Vleurinck, et al. 2016). The reads from these datasets were quantified in SeqMonk (Andrews) using the RNA-seq pipeline to generate a read count per gene value. Genes were identified as differentially expressed genes (DEGs) using a statistical cutoff of FDR >= 0.05 between their differential raw expression. These genes were further subdivided into singletons and duplicates, as well as "high methylation" and "low methylation" as described previously. χ^2 tests of independence were used to determine whether there were associations between gene methylation status and gene expression bias between castes for singletons and for duplicates. We also conducted sample-size corrected analyses of these statistical tests to control for differences in sample sizes for singleton and duplicate genes. These re-analyses showed the same general associations as those in the full datasets.

Divergence of paralogs from outgroup orthologs

OrthoDB v9.1 (Zdobnov, et al. 2017) was used to identify *A. mellifera* duplicate genes that had single copy orthologs in the bee *C. calcarata*. *C. calcarata* was chosen for this analysis due to its relative close relation to *A. mellifera* and the availability of

sequences for its DNA methylation and gene expression. Genes lacking methylation or expression data were excluded from the dataset. *C. calcarata de novo* genome, methylome, and transcriptome files were obtained from previous studies on the carpenter bee species (Rehan, et al. 2014; Rehan, et al. 2016).

C. calcarata BS-seq reads were trimmed for quality and adapters with default parameters using cutadapt (Martin 2011), and aligned to the *C. calcarata* reference genome using Bismark and Bowtie2 (Langmead and Salzberg 2012) as previously described. SeqMonk (Andrews) BS-seq quantification pipeline was used to quantify the percentage of methylated CpG dinucleotides per gene location, as annotated in Rehan et al. (2016). Values were parsed to find methylation data of gene locations in *C. calcarata* that corresponded to *A. mellifera* genes, creating a subset of 92 genes. Spearman's rank correlations were used to determine whether relationships existed between the methylation levels of the three orthologous genes. Nonparametric tests were used to mitigate the effects of the differences in the overall levels of genome methylation that are found across insects.

C. calcarata transcriptome reads were trimmed for quality and adapters with default parameters using cutadapt (Martin 2011) and aligned to the *C. calcarata* reference genome using Bismark and Bowtie2 (Langmead and Salzberg 2012). SeqMonk (Andrews) RNA-seq quantification pipeline was used to count the raw reads per gene location, as annotated in Rehan, et al. 2016. These values were parsed to find expression raw counts for *C. calcarata* singleton gene locations that are orthologous to *A. mellifera* duplicates from the gene subset previously created using methylation data. This resulted in a subset of 90 genes that had expression data available. Spearman's rank correlations

were used to determine whether relationships existed between the expression levels of the orthologous and paralogous genes.

Spearman's rank correlations were used to find correlations between relative divergence of percent genic methylation and levels of expression. The distributions in the relative divergence of methylation and expression between *C. calcarata* and each *A. mellifera* paralog was tested against a normal distribution using a Shapiro – Wilcoxon Goodness of Fit test to determine whether divergence values significantly differed from a normal distribution centered around a mean of 0.

Analyses of DNA methylation and location of paralogs

Metadata regarding chromosomal location and gene start and end sites was extracted from OrthoDB gene annotations for downstream analysis using custom perl scripts. ANOVA was used to determine whether the 16 *A. mellifera* chromosomes differed in their mean cytosine methylation percentage. Duplicated genes were assigned as being syntenic (located on the same chromosome) or non-syntenic (on different chromosomes) depending on whether the paralogs were located within the same linkage group. The chromosomal location of each gene was taken from OrthoDB v9.1 orthologous groups and combined into seventeen bins: sixteen *A. mellifera* linkage groups (chromosomes) and one unassociated group for genes that were not annotated with a chromosome location (unassociated genes were not used in further analyses).

Mean methylation divergence levels were calculated for syntenic and non-syntenic duplicate pairs. We used ANOVA to determine if the divergence of syntenic duplicates differed significantly from the divergence of non-syntenic duplicates. To

establish a control group as a reference for the duplicate gene analyses, 734 singletons were paired at random to mirror the sample size of duplicates. These singletons were then assigned as being on the syntenic or non-syntenic based on their linkage group designation from OrthoDB v9.1 orthology. Mean methylation divergence was calculated for these pseudoparalogs. ANOVA was then used to determine whether methylation divergence differed significantly between pseudoparalogs on the same or different chromosomes. This randomization trial was repeated ten times and the mean of the ANOVA results was obtained to produce a control value for comparison with the ANOVA results of the true duplicate pairs.

Analyses of DNA methylation and gene length

Gene length was defined as the nucleotide sequence difference between the start and end sites of a gene, with start and end sites of genes extracted from orthoDB metadata. CpG methylation percentage for each gene was defined as the percentage of CpG dinucleotides within each gene that were methylated. Genes were placed into one of eight bins based on length, with a width of 40kb per bin. ANOVA was used to test the difference in the mean gene body methylation level of each length bin. This test was performed separately using duplicate and singleton genes. Additionally, ANOVA was used to determine if singleton genes and duplicated genes differed in length overall.

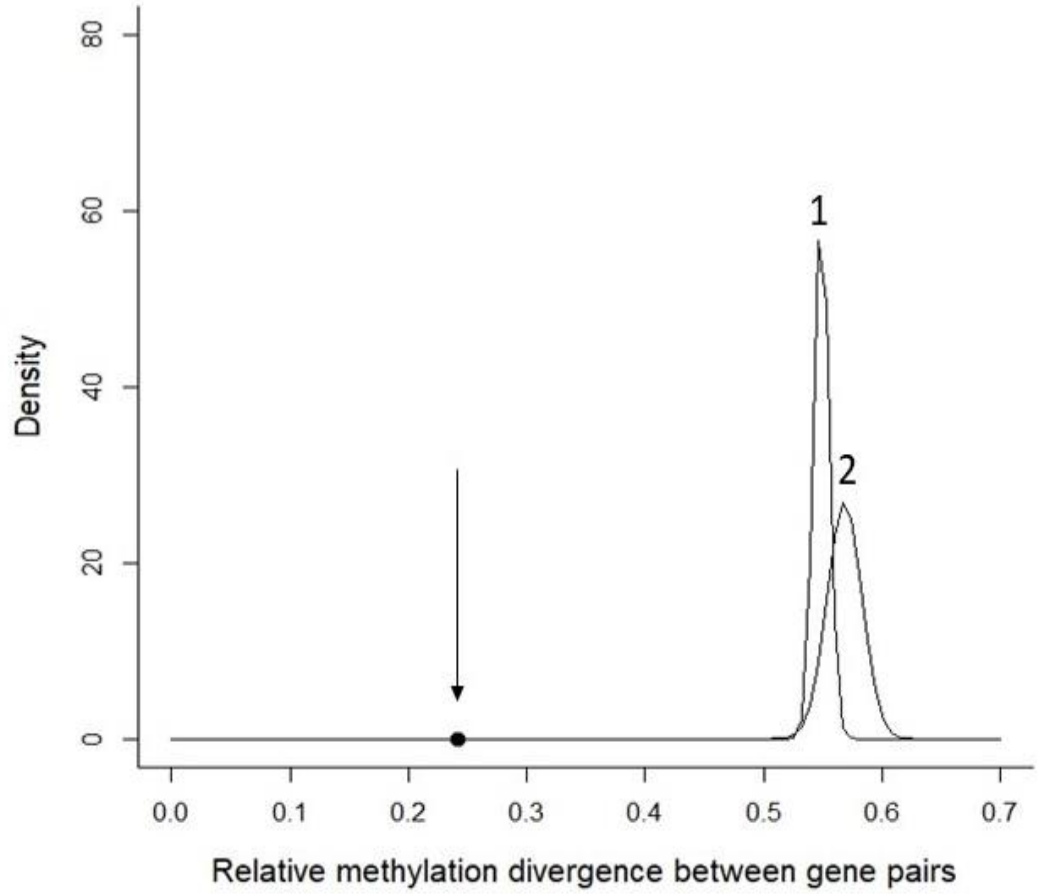

Figure B-1. Null distributions of relative divergence in methylation for 10,000 randomly paired (1) singletons and (2) duplicates. Arrow indicates actual value of relative divergence (D_r) in methylation between duplicate genes in *Apis mellifera*. The true mean value differed significantly from the distributions of randomly generated mean values ($p < 0.0001$).

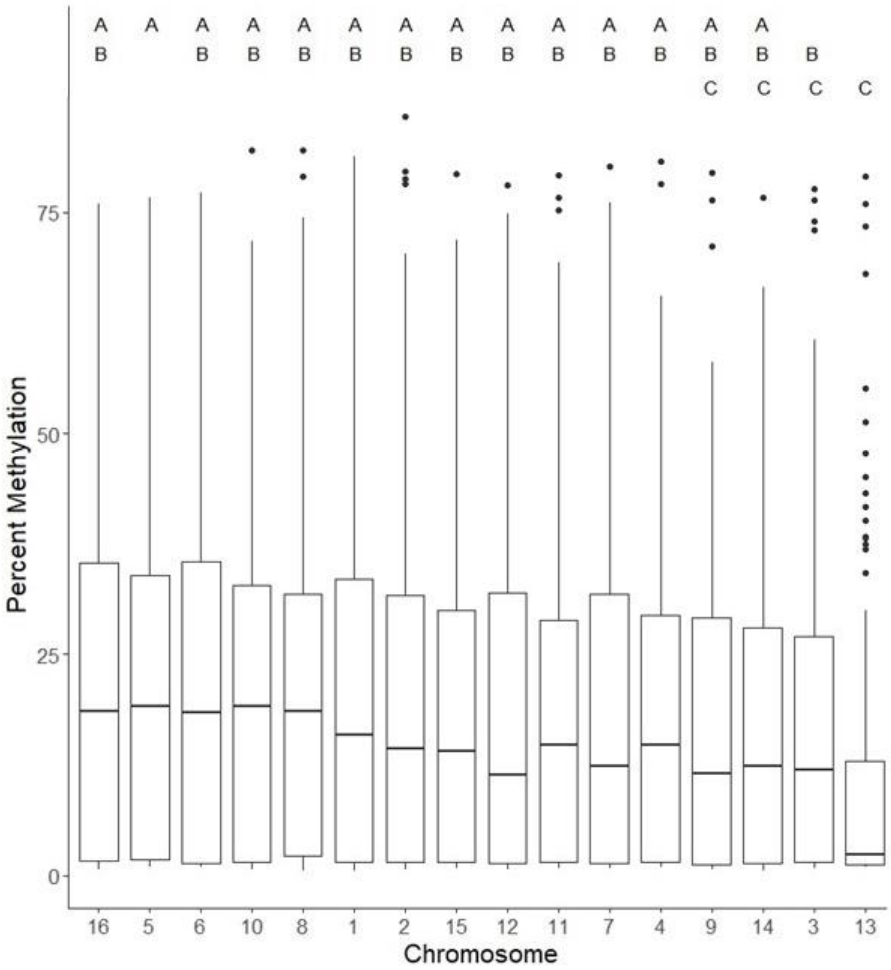

Figure B-2. Percentage of CpG methylation for genes on the 16 chromosomes, ordered from highest to lowest mean percent methylation. The genes on chromosomes denoted with different letters differed significantly in mean percent methylation. Box represents first quartile, median, and third quartile values, while whiskers represent values within 1.5 x the interquartile range, and points represent outliers.

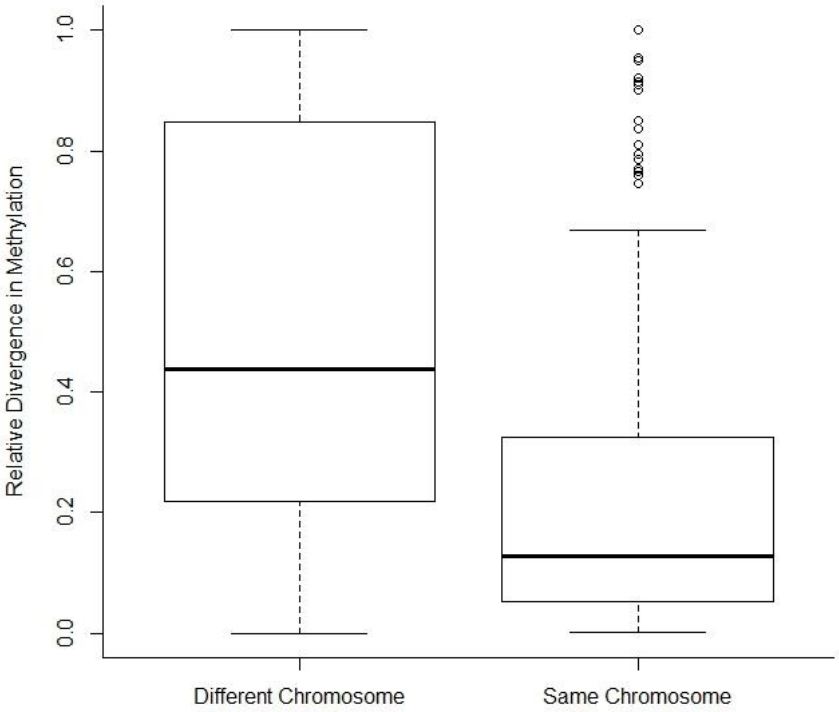

Figure B-3. Duplicated genes on the same chromosome show significantly lower levels of methylation divergence than those on different chromosomes ($p < 0.0001$). Boxes provide first quartile, median, and third quartile values, while whiskers represent values within 1.5 x the interquartile range, and points represent outliers.

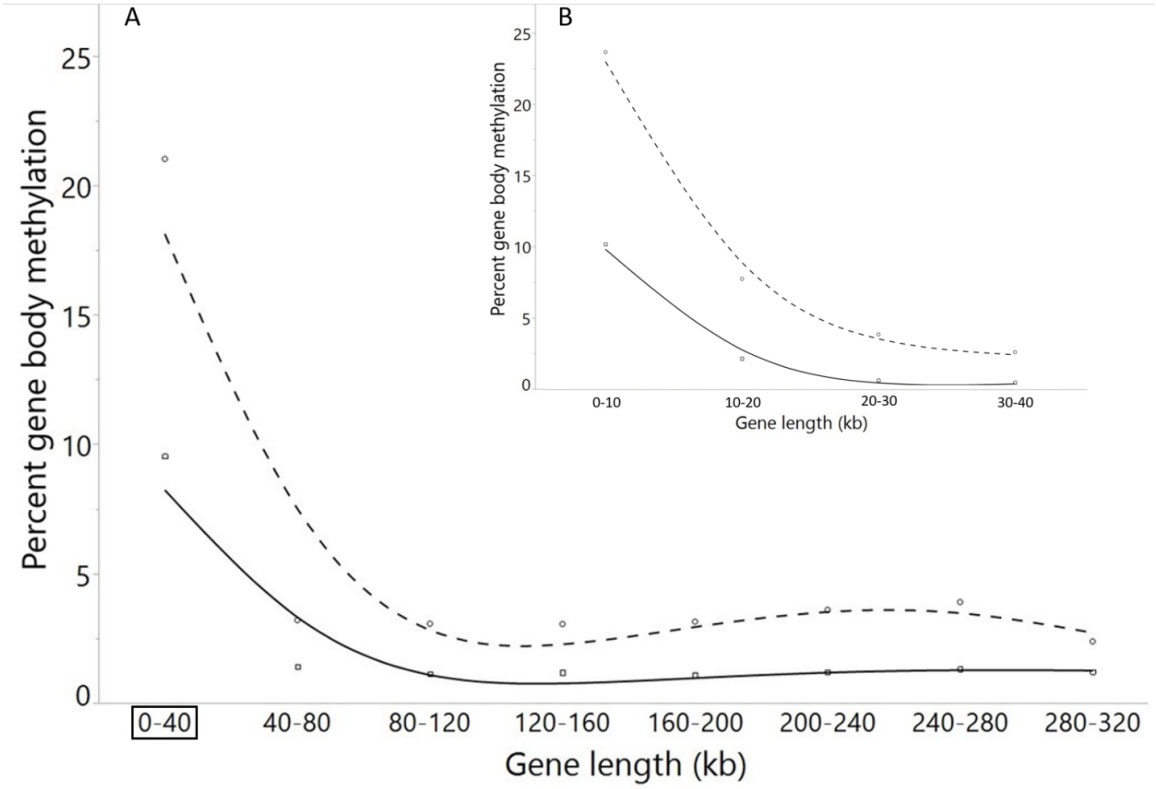

Figure B-4. (A) Relationship between gene body methylation and gene body length in singleton (○, dashed line) and duplicate (□, solid line) genes. Longer singletons and duplicate genes tended to have significantly lower levels of DNA methylation. Points represent mean gene body methylation level for genes in each bin. Lines represent cubic spline fit with lambda (smoothing parameter) of 0.05. ANOVA of means shows that singletons vary significantly in mean methylation across bins ($p < 0.0001$), while duplicates do not ($p = 0.3173$). (B) Relationship between gene length and percent genic methylation of shorter genes (0-40kb) shows a significant negative association in both singletons ($p < 0.0001$) and duplicates ($p = 0.0008$).

Table B-1. Mean ± SEM[a] methylation statistics across all datasets for genes of different family sizes in the honeybee *Apis mellifera*.

Family Size	Number of Genes	CpG Methylation Level (%) ± SEM
Singletons	5235	19.46 ± 0.2619
Duplicates	734	8.603 ± 0.6633
Larger Families (3+)	271	3.364 ± 0.5765

[a]Standard Error of the Mean

Table B-2. Observed (and expected) counts of high- and low-methylation singleton and duplicate genes illustrating the significant ($p < 0.001$) nonrandom associations between methylation level and gene duplication status.

Methylation Level	Singleton	Duplicate	Total
Low	1919 (2162.9)	534 (290.1)	2453
High	3315 (3071.1)	168 (411.9)	3483
Total	5234	702	5936

Table B-3. χ2 tests of independence between actual and (expected) counts for DNA methylation level and duplication status in 16 whole genome bisulfite

	Methylation Level	Singletons	Duplicates	Total	Significance
Queen[a]	Low	1600 (1827.57)	464 (236.43)	2064	χ^2 = 380.1; df = 1; p < 0.001
	High	3633 (3405.43)	213 (440.57)	3846	
	Total	5233	677	5910	
Worker[a]	Low	1875 (2109.88)	509 (274.12)	2384	χ^2 = 381.0; df = 1; p < 0.001
	High	3359 (3124.12)	171 (405.88)	3530	
	Total	5234	680	5914	
F1[b]	Low	1985 (2213.8)	505 (276.2)	2490	χ^2 = 369.5; df = 1; p < 0.001
	High	3249 (3020.2)	148 (376.8)	3397	
	Total	5234	653	5887	
F2[b]	Low	1971 (2194.63)	500 (276.37)	2471	χ^2 = 350.9; df = 1; p < 0.001
	High	3262 (3038.37)	159 (382.63)	3421	
	Total	5233	659	5892	
F3[b]	Low	1975 (2202.34)	503 (275.66)	2478	χ^2 = 364.3; df = 1; p < 0.001
	High	3258 (3030.66)	152 (379.34)	3410	
	Total	5233	655	5888	
F4[b]	Low	1958 (2186.49)	503 (274.51)	2461	χ^2 = 367.7; df = 1; p < 0.001
	High	3275 (3046.51)	154 (382.49)	3429	
	Total	5233	657	5890	
F5[b]	Low	1960 (2187.81)	505 (277.19)	2465	χ^2 = 362.5; df = 1; p < 0.001
	High	3273 (3045.19)	158 (385.81)	3431	
	Total	5233	663	5896	
F6[b]	Low	1963 (2190.1)	505 (277.9)	2468	χ^2 = 359.7; df = 1; p < 0.001
	High	3270 (3042.9)	159 (386.1)	3429	
	Total	5233	664	5897	
N1[b]	Low	1970 (2203.51)	511 (277.49)	2481	χ^2 = 382.2; df = 1; p < 0.001
	High	3263 (3029.49)	148 (381.51)	3411	
	Total	5233	659	5892	
N2[b]	Low	1944 (2171.99)	504 (276.01)	2448	χ^2 = 362.9; df = 1; p < 0.001
	High	3289 (3061.01)	161 (388.99)	3450	
	Total	5233	665	5898	
N3[b]	Low	1965 (2195.29)	508 (277.71)	2473	χ^2 = 370.6; df = 1; p < 0.001
	High	3268 (3037.71)	154 (384.29)	3422	
	Total	5233	662	5895	
N4[b]	Low	1956 (2184.63)	505 (276.37)	2461	χ^2 = 365.8; df = 1; p < 0.001
	High	3277 (3048.37)	157 (385.63)	3434	
	Total	5233	662	5895	
N5[b]	Low	1969 (2197.75)	503 (274.25)	2472	χ^2 = 370.0; df = 1; p < 0.001
	High	3264 (3035.25)	150 (378.75)	3414	
	Total	5233	653	5886	
N6[b]	Low	1976 (2201.92)	499 (273.08)	2475	χ^2 = 362.7; df = 1; p < 0.001
	High	3257 (3031.08)	150 (375.92)	3407	
	Total	5233	649	5882	
Control[c]	Low	596 (788.19)	291 (98.81)	887	χ^2 = 495.3; df = 1; p < 0.001
	High	4637 (4444.81)	365 (557.19)	5002	
	Total	5233	656	5889	
Knockdown[c]	Low	1503 (1724.47)	436 (214.53)	1939	χ^2 = 383.4; df = 1; p < 0.001
	High	3730 (3508.53)	215 (436.47)	3945	
	Total	5233	651	5884	

[a]Lyko, et al. 2010
[b]Herb, et al. 2012
[c]Li-Byarlay, et al. 2013

Table B-4. χ2 tests of independence between actual and (expected) counts for DNA methylation level and gene expression bias for singletons and duplicates in six gene expression datasets from the

		Singletons				Duplicates			
	Expression Bias	Low Methylation	High Methylation	Total	Significance	Low Methylation	High Methylation	Total	Significance
Drone/Queen[a]	Unbiased	916 (1049.61)	1659 (1525.39)	2575	χ^2 = 309.65; df = 1; p < 0.001	49 (52.48)	33 (29.52)	82	χ^2 = 2.611; df = 1; p = 0.1061
	Biased	240 (106.39)	21 (154.61)	261		15 (11.52)	3 (6.48)	18	
	Total	1156	1680	2836		64	36	100	
Drone/Worker[a]	Unbiased	1073 (1255.31)	1989 (1806.69)	3062	χ^2 = 413.97; df = 1; p < 0.001	51 (60.75)	45 (35.25)	96	χ^2 = 15.34; df = 1; p < 0.001
	Biased	334 (151.69)	36 (218.31)	370		30 (20.25)	2 (11.75)	32	
	Total	1407	2025	3432		81	47	128	
Queen/Worker[a]	Unbiased	1005 (1168.89)	1987 (1823.11)	2992	χ^2 = 399.25; df = 1; p < 0.001	54 (55.94)	37 (35.06)	91	χ^2 = 0.5792; df = 1; p = 0.4466
	Biased	285 (121.11)	25 (188.89)	310		13 (11.06)	5 (6.94)	18	
	Total	1290	2012	3302		67	42	109	
Drone/Queen[b]	Unbiased	1102 (1229.71)	2028 (1900.29)	3130	χ^2 = 304.35; df = 1; p < 0.001	64 (64.3)	41 (40.7)	105	χ^2 = 0.0084; df = 1; p = 0.9269
	Biased	222 (94.29)	18 (145.71)	240		15 (14.7)	9 (9.3)	24	
	Total	1324	2046	3370		79	50	129	
Drone/Worker[b]	Unbiased	636 (656.61)	411 (390.39)	1047	χ^2 = 27.85; df = 1; p < 0.001	30 (32.2)	20 (17.8)	50	χ^2 = 1.660; df = 1; p = 0.1977
	Biased	62 (41.39)	4 (24.61)	66		8 (5.8)	1 (3.2)	9	
	Total	698	415	1113		38	21	59	
Queen/Worker[b]	Unbiased	1075 (1222)	1818 (1671)	2893	χ^2 = 347.96; df = 1; p < 0.001	66 (67.1)	45 (43.9)	111	χ^2 = 0.078; df = 1; p = 0.7797
	Biased	264 (117)	13 (160)	277		15 (13.9)	8 (9.1)	23	
	Total	1339	1831	3170		81	53	134	

[a]Ashby, et al. 2016
[b]Vleurinck, et al. 2016

Table B-5. χ2 tests of independence between actual and (expected) counts for gene copy number and gene expression bias for genes showing high or low levels of gene body methylation in six gene expression datasets from the honeybee *Apis mellifera*.

		Low Methylation				High Methylation			
	Expression Bias	Singleton	Duplicate	Total	Significance	Singleton	Duplicate	Total	Significance
Drone/Queen[a]	Unbiased	916 (914.38)	49 (50.62)	965	$\chi^2 = 0.2627$; df = 1; p = 0.6083	1659 (1656.5)	33 (35.50)	1692	$\chi^2 = 12.8231$; df = 1; p = 0.0003
	Biased	240 (241.62)	15 (13.38)	255		21 (23.50)	3 (0.50)	24	
	Total	1156	64	1220		1680	36	1716	
Drone/Worker[a]	Unbiased	1073 (1062.81)	51 (61.19)	1124	$\chi^2 = 7.334$; df = 1; p = 0.0068	1989 (1987.86)	45 (46.14)	2034	$\chi^2 = 1.5661$; df = 1; p = 0.2108
	Biased	334 (344.19)	30 (19.81)	364		36 (37.14)	2 (0.86)	38	
	Total	1407	81	1488		2025	47	2072	
Queen/Worker[a]	Unbiased	1005 (1006.71)	54 (52.29)	1059	$\chi^2 = 0.2689$; df = 1; p = 0.6040	1987 (1982.61)	37 (41.39)	2024	$\chi^2 = 32.4968$; df = 1; p = < 0.00001
	Biased	285 (283.29)	13 (14.71)	298		25 (29.39)	5 (0.61)	30	
	Total	1290	67	1357		2012	42	2054	
Drone/Queen[b]	Unbiased	1102 (1100.34)	64 (65.66)	1166	$\chi^2 = 0.2617$; df = 1; p = 0.6089	2028 (2019.64)	41 (49.36)	2069	$\chi^2 = 112.5025$; df = 1; p < 0.00001
	Biased	222 (223.66)	15 (13.34)	237		18 (26.36)	9 (0.64)	27	
	Total	1324	79	1403		2046	50	2096	
Drone/Worker[b]	Unbiased	636 (631.61)	30 (34.39)	666	$\chi^2 = 6.202$; df = 1; p = 0.0128	411 (410.24)	20 (20.76)	431	$\chi^2 = 2.5435$; df = 1; p = 0.11075
	Biased	62 (66.39)	8 (3.61)	70		4 (4.76)	1 (0.24)	5	
	Total	698	38	736		415	21	436	
Queen/Worker[b]	Unbiased	1075 (1075.91)	66 (65.09)	1141	$\chi^2 = 0.0694$; df = 1; p = 0.7922	1818 (1810.59)	45 (52.41)	1863	$\chi^2 = 96.6926$; df = 1; p < 0.00001
	Biased	264 (263.09)	15 (15.91)	279		13 (20.41)	8 (0.59)	21	
	Total	1339	81	1420		1831	53	1884	

[a]Ashby, et al. 2016
[b]Vleurinck, et al. 2016

Table B-6. Alignment statistics for whole genome bisulfite sequence datasets used in methylation analyses.

Dataset Name	Sequence Run File	Coverage Depth	Publication Source	Mapping efficiency	Total read pairs	Unique best hits	No alignments	Non-unique alignments	% Multimapping	CpG%	CHG%	CHH%
Queen	SRR039815	> 20x	Lyko, et al. (2010)	60.8	32148761	19534890	10727000	1886871	5.87	0.5	0.1	0.1
	SRR353494	> 20x	Lyko, et al. (2010)	7.2	3581330	256120	3038567	286643	8.00	4.3	13.6	40.4
	SRR353496	> 20x	Lyko, et al. (2010)	15.7	139766555	21993032	113887798	3885725	2.78	3.2	9.6	19.9
Worker	SRR039813	> 20x	Lyko, et al. (2010)	74.3	10152748	7542798	1194373	1415577	13.94	0.8	0.4	1.1
	SRR039814	> 20x	Lyko, et al. (2010)	55.3	17723734	9798175	6895574	1029985	5.81	0.4	0.1	0.1
	SRR353497	> 20x	Lyko, et al. (2010)	7.1	3325536	235942	2863212	226382	6.81	3.3	13	36.3
	SRR353498	> 20x	Lyko, et al. (2010)	14.2	115636657	16390850	96739708	2506099	2.17	2.6	8.2	13.7
Forager(F)1	SRR445767	> 50x	Herb, et al. (2012)	54.8	14522329	7957660	5986568	578101	3.98	1.2	0.1	0.1
F2	SRR445768	> 50x	Herb, et al. (2012)	51.1	55796003	28495456	25369544	1931003	3.46	1.4	0.1	0.2
F3	SRR445769	> 50x	Herb, et al. (2012)	54.1	34222119	18511610	14409838	1300671	3.80	1.2	0.1	0.1
F4	SRR445770	> 50x	Herb, et al. (2012)	50.8	67796519	34417838	30890255	2488426	3.67	1.3	0.1	0.1
F5	SRR445771	> 50x	Herb, et al. (2012)	54.5	80485242	43836816	33641985	3006441	3.74	1.3	0.1	0.1
F6	SRR445773	> 50x	Herb, et al. (2012)	57.6	72464793	41720786	27589284	3154723	4.35	1.1	0.1	0.1
Nurse(N)1	SRR445774	> 50x	Herb, et al. (2012)	52.9	27966415	14788994	12049179	1128242	4.03	1	0.1	0.1
N2	SRR445775	> 50x	Herb, et al. (2012)	54.0	122466855	66161167	51524521	4781167	3.90	1.2	0.1	0.2
N3	SRR445776	> 50x	Herb, et al. (2012)	49.7	107420965	53408471	50212734	3799760	3.54	1.3	0.1	0.1
N4	SRR445777	> 50x	Herb, et al. (2012)	49.8	66284166	33037966	30717736	2528464	3.81	1.3	0.1	0.1
N5	SRR445778	> 50x	Herb, et al. (2012)	49.3	62047761	30574926	29305661	2167174	3.49	1.3	0.1	0.1
N6	SRR445799	> 50x	Herb, et al. (2012)	23.9	61176519	14623152	45521263	1032104	1.69	1.4	0.1	0.1
Control	SRR1270128	> 5x	Li-Byarlay, et al. (2013)	69.5	225134678	156521111	49170178	19443389	8.64	1	0.6	0.8
Knockdown	SRR1270129	> 5x	Li-Byarlay, et al. (2013)	73.3	205289099	150552909	38318736	16417454	8.00	0.8	0.5	0.7

Table B-7. Correlations of genic methylation levels between bisulfite sequence datasets (above diagonal) in the honeybee *Apis mellifera* show high consistency in CpG methylation calling[a]. The correlation was highly significant in every pairwise comparison (below diagonal)

	Queen	Worker	F1	F2	F3	F4	F5	F6	N1	N2	N3	N4	N5	N6	Control	KD
Queen[b]		0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.95	0.95
Worker[b]	***[e]		0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.96	0.97	0.95	0.95
F1[c]	***	***		0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.97	0.98	0.97	0.98	0.95	0.96
F2[c]	***	***	***		0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.95	0.95
F3[c]	***	***	***	***		0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.95	0.95
F4[c]	***	***	***	***	***		0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.95	0.95
F5[c]	***	***	***	***	***	***		0.98	0.98	0.98	0.98	0.98	0.98	0.98	0.95	0.95
F6[c]	***	***	***	***	***	***	***		0.98	0.98	0.98	0.98	0.98	0.98	0.96	0.95
N1[c]	***	***	***	***	***	***	***	***		0.98	0.98	0.98	0.98	0.98	0.95	0.95
N2[c]	***	***	***	***	***	***	***	***	***		0.98	0.98	0.97	0.98	0.95	0.95
N3[c]	***	***	***	***	***	***	***	***	***	***		0.98	0.97	0.98	0.95	0.95
N4[c]	***	***	***	***	***	***	***	***	***	***	***		0.98	0.98	0.95	0.95
N5[c]	***	***	***	***	***	***	***	***	***	***	***	***		0.98	0.95	0.95
N6[c]	***	***	***	***	***	***	***	***	***	***	***	***	***		0.95	0.95
Control[d]	***	***	***	***	***	***	***	***	***	***	***	***	***	***		0.96
KD[d]	***	***	***	***	***	***	***	***	***	***	***	***	***	***	***	

[a]Correlations between CpG methylation calls for singleton, duplicate, and larger families genes of *Apis mellifera*

[b]Lyko, et al. (2010)

[c]Herb, et al. (2012)

[d]Knockdown, Li-Byarlay, et al. (2013)

[e] $p < 0.001$

APPENDIX C. SUPPLEMENTAL MATERIALS FOR CHAPTER 5

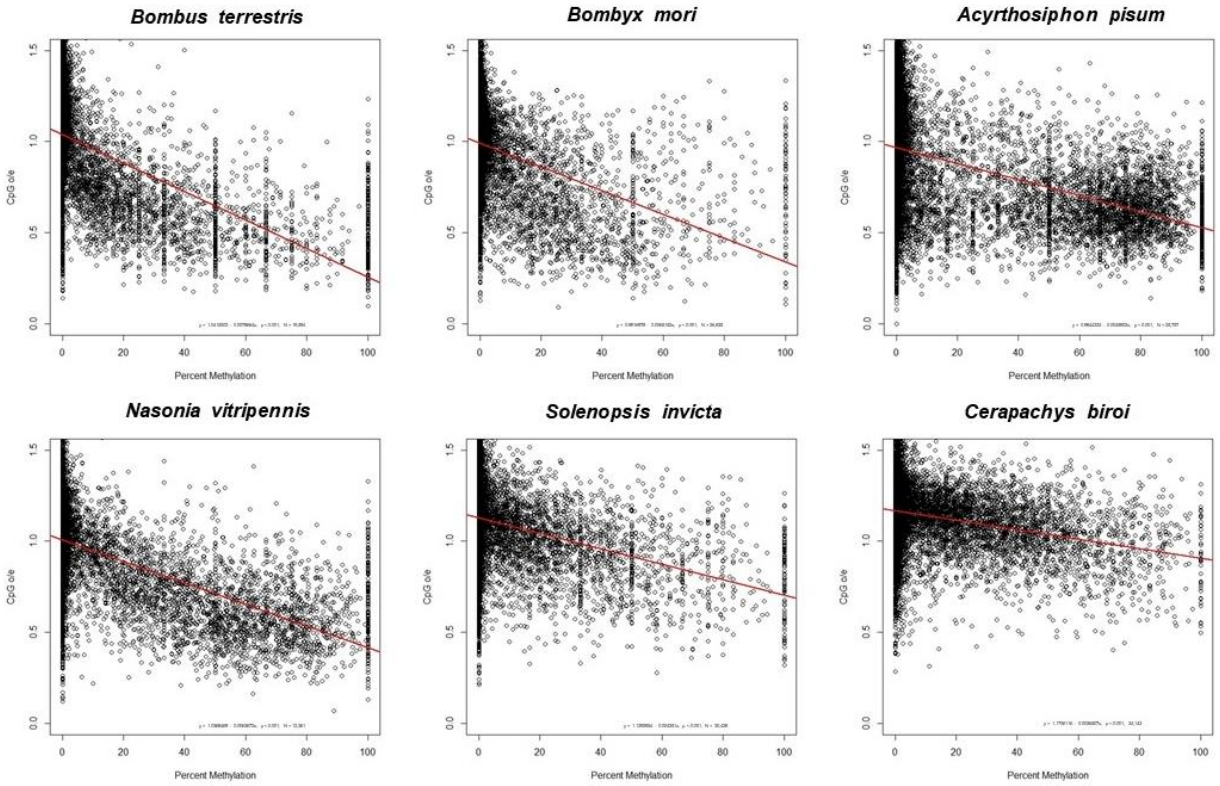

Figure C-1. Correlations between CpGo/e and DNA methylation of genes in 6 insect species used in analyses.

Table C-1. CpGo/e analysis of chromosomal methylation distribution in 25 insect species

Species	Order	Chrom count	Gene count	Welch's F	Welch's P	Levene's F	Levene's P	CV
Acyrthosiphon pisum	Hemiptera	4	25757	2.34	0.072	1.85	0.136	35.60
Apolygus lucorum	Hemiptera	17	20113	32.19	***	9.36	***	23.11
Nilaparvata lugens	Hemiptera	16	30521	59.50	***	19.30	***	27.03
Rhopalosiphum maidis	Hemiptera	4	19481	10.95	***	4.36	0.004	38.25
Aphidius gifuensis	Hymenoptera	6	11405	2.95	0.012	1.35	0.242	40.21
Apis mellifera	Hymenoptera	16	23361	33.74	***	24.17	***	38.39
Bombus terrestris	Hymenoptera	18	19594	19.08	***	14.50	***	33.79
Solenopsis invicta	Hymenoptera	16	30428	24.71	***	15.69	***	20.16
Cerapachys biroi	Hymenoptera	14	24143	16.99	***	4.03	***	15.24
Nasonia vitripennis	Hymenoptera	5	12361	12.66	***	18.04	***	25.59
Belonocnema treatae	Hymenoptera	10	25172	16.61	***	16.12	***	20.07
Monomorium pharaonis	Hymenoptera	11	27800	20.94	***	12.22	***	20.32
Vespula germanica	Hymenoptera	25	17128	3.96	***	3.60	***	36.31
Vespula pensylvanica	Hymenoptera	25	18441	4.44	***	3.19	***	35.25
Vespula vulgaris	Hymenoptera	25	15484	4.67	***	4.48	***	35.25
Zerene cesonia	Lepidoptera	29	15776	17.30	***	18.07	***	26.96
Danaus plexippus plexippus	Lepidoptera	30	19655	13.33	***	12.37	***	21.19
Manduca sexta	Lepidoptera	28	22115	6.00	***	17.92	***	23.18
Aricia agestis	Lepidoptera	23	21274	6.67	***	7.99	***	24.28
Pararge aegeria	Lepidoptera	30	20763	11.97	***	14.76	***	23.86
Bombyx mori	Lepidoptera	28	26835	16.08	***	24.94	***	22.64
Spodoptera frugiperda	Lepidoptera	32	29941	24.19	***	19.38	***	23.58
Spodoptera litura	Lepidoptera	26	250	3.02	0.005	1.60	0.05	19.79
Trichoplusia ni	Lepidoptera	28	21668	5.79	***	8.36	***	22.79
Drosophila melanogaster	Diptera	3	25262	11.36	***	0.4826	0.617	14.43

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