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Temporal Analysis of Effective Population Size and Mating System in a Social Wasp

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Abstract

Highly social species are successful because they cooperate in obligately integrated societies. We examined temporal genetic variation in the eusocial wasp *Vespula maculifrons* 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 3 separate timepoints 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 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.

Subject area: Molecular Adaptation and Selection
Keywords: DNA microsatellite, Eusocial, Hymenoptera, polyandry, social behavior, yellowjacket wasp

Introduction

The evolution of advanced societies represented an important and successful major transition in biological history (Wilson 1971; Maynard Smith and Szathmary 1998). 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; Luikart et al. 2010; Ellegren and Galtier 2016).

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 (Wilson 1971; Crozier 1979; Crozier and Pamilo 1996).

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 forthcoming). 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 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) (Strassmann 2001; Kraus and Moritz 2010). 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; Strassmann 2001; Ratnieks et al. 2006).

However, multiple mating by queens may also have advantages (Boomsma and Ratnieks 1996; Crozier and Fjerdingstad 2001; Baer 2016; 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 (Crozier and Fjerdingstad 2001; Tarpy and Seeley 2006; Baer 2016; Dobelmann et al. 2017; 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 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 (MacDonald and Matthews 1981; Greene 1991). 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 (Ross 1986; Ross and Carpenter 1991; Kovacs and Goodisman 2007).

V. maculifrons queens mate with multiple males (MacDonald and Matthews 1981; Greene 1991). Within-colony genetic diversity is, therefore, a function of queen mate number (Ross 1986; Goodisman et al. 2007; Goodisman et al. 2007; Kovacs et al. 2008; Johnson et al. 2009; Kovacs and Goodisman 2012). 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). *Vespula* queens are estimated to travel 1000–5000 m 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 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 2 aims are of importance to further understanding the evolution of eusocial species in light of environmental change and associated susceptibility to selective pressures. 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.

Materials and Methods

Sample Collection

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

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 (Foster et al. 2001; Daly et al. 2002; 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 1).

Microsatellite Genotyping

PCRs were used to amplify DNA at each of the 16 microsatellite loci. PCRs were conducted using a 15 µL reaction composed of: 6.4 µL deionized water, 2.4 µL 25 mM $MgCl_2$, 1.5 µL 10× PCR buffer, 1.2 µL 2.5 mM 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 s at 94 °C, 30 s at T_a, 30 s at 72 °C, and a final extension for 5.5 min at 72 °C. Annealing temperatures (T_a) are provided in Table 1.

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 Supplementary Table S1.

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 F_1 generation genotypes. Reconstructed queen and male genotypes are found in Supplementary Table S2.

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

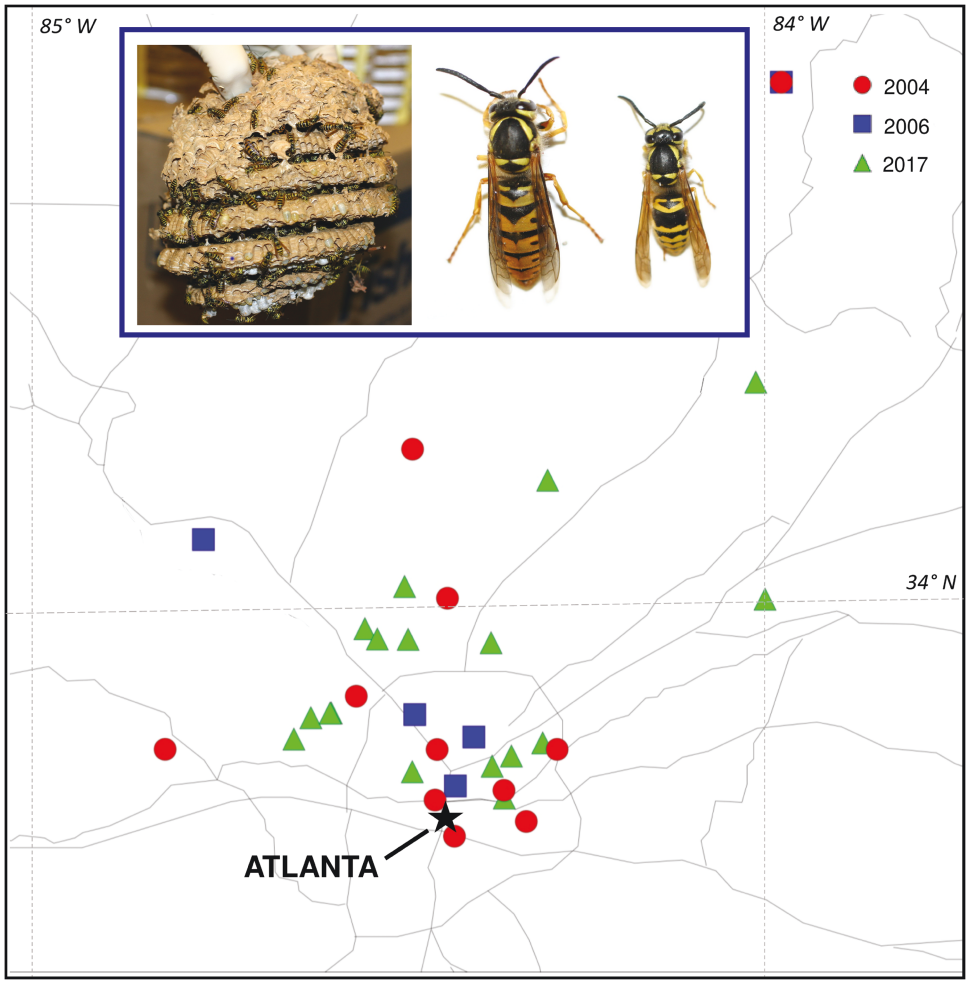

Figure 1. Locations of *V. maculifrons* nests collected around Atlanta, GA, USA at each of three timepoints. Inset: *Vespula maculifrons* small, mature nest, and *V. maculifrons* queen and worker. See online version for full color.

Table 1. Allelic variability metrics at 16 microsatellite loci in *Vespula maculifrons*

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

Values represent means across 3 timepoints.

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.

male genotypes violated Hardy-Weinberg equilibrium (HWE) 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.

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 (Krimbas and Tsakas 1971; Jorde and Ryman 2007; 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 timepoints. These measures of genetic differentiation were then used to estimate genetic drift and produce a pairwise harmonic mean estimate of $N_e = t/\{2[F_s - 1/(2n_x) - 1/(2n_y)]\}$ (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 n_x and n_y 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 (CIs) were produced by jackknifing across the 16 loci 10 000 times. Jacknifing 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 N_e 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.

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 analysis of variance (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 timepoint was calculated using the equation from Nielsen et al. (2003): $k_{e3} = (n-1)^2/[\Sigma p_i^2(n+1)(n-2)+3-n]$, where p is the proportion of offspring in a colony sired by male "i". Effective paternity across timepoints 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% CIs 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 multilocus estimator of 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).

Results

Population Genetic Analysis

We tested if the genotypes of individuals deviated from expectations under HWE. 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 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, Supplementary Table S4).

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 3 timepoints (p_{2004} = 0.3620; p_{2006} = 0.8460; p_{2017} = 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 (Goodisman et al. 2001; Chau et al. 2015). Thus, our results in this study are consistent with the idea that *Vespula* wasps are able to move relatively freely throughout their geographic range.

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 3 comparisons using the genetically independent queen/male dataset (04-06, 04-17, 06-17, respectively; Table 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 2). The queen-only dataset also displayed the largest CIs.

Table 2. Estimates of effective population size (N_e) in *Vespula maculifrons* with 95% confidence intervals in brackets

Comparison	Sample	F_s	F'	N_e [CI]
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]

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. F_s, *F*-statistic measuring allele frequency differentiation between years; F', unbiased estimate of F_s accounting for sampling scheme; INF, infinity.

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 2, Supplementary Table S3). 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).

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 3 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 timepoint. We found that there was no significant difference in mean effective paternity across timepoints (F(2,41) = 1.5632, P = 0.2217, Figure 2, Supplementary Table S3). In addition, there was no significant paternity skew found at any of the three timepoints, and no difference in skew across years (F(2,41) = 0.9211, P = 0.4062, Figure 3, Supplementary Table S3).

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, Supplementary Table S3). 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, Supplementary Table S3). 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.

Discussion

This study investigated genetic and life history changes in a eusocial insect population. We estimated the effective population size (N_e)

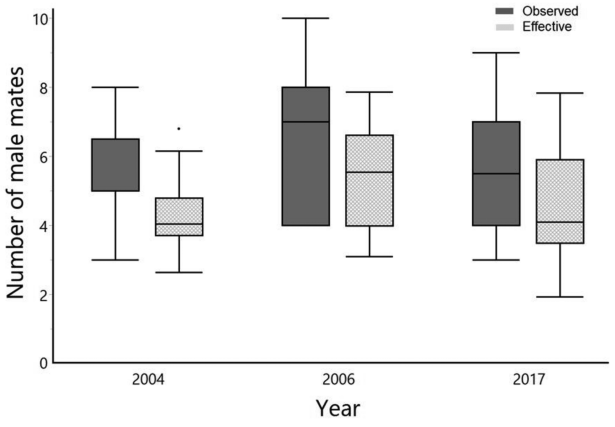

Figure 2. Number of male mates (dark gray) and effective number of male mates (light gray) per queen at three timepoints (N = 13, 11, and 20 colonies, respectively). Boxes display first quartile, median, and third quartile values, whereas whiskers represent values within 1.5X the interquartile range.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

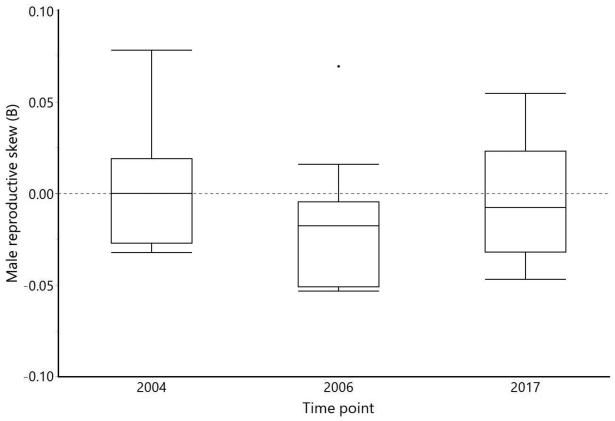

Figure 3. Reproductive skew (*B*) of males mated to single queens in *Vespula maculifrons*. Boxes display first quartile, median, and third quartile values, whereas whiskers represent values within 1.5X the interquartile range. Mating skew did not vary significantly from 0 (dashed line) in any of the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

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

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

of a eusocial taxon 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.

Effective Population Size in Eusocial Species

We found that the effective population size for *V. maculifrons* ranged in the 100s of individuals (Table 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 (Nei and Tajima 1981; Ellegren and Galtier 2016; Galtier and Rousselle 2020). 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; Echodu et al. 2011; Rasic et al. 2015; Saarman et al. 2017; Bergamo et al. 2020), 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.

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 2). The skew among *V. maculifrons* male mates (Figure 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 (Ross 1986; Hoffman et al. 2008).

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; Strassmann 2001; Goodisman et al. 2002; Wenseleers et al. 2005; Hoffman et al. 2008), 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 (Goodisman et al. 2007; Loope et al. 2014; Dobelmann et al. 2017) 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 (Jaffe et al. 2012; Jaffe 2014; Baer 2016; Jacobs and Schrempf 2017). Our results indicate that skew is generally nonsignificant in *V. maculifrons* (Figure 3, Supplementary Table S3). 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. 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 (Field et al. 2010; El-Niweiri and Moritz 2011; Purcell 2011; Schurch et al. 2016; Shen et al. 2017; Aviles and Guevara 2017; Dew et al. 2018; Groom and Rehan 2018). In addition, geographic differences in queen mate number in eusocial bees may arise from environmental differences affecting mating behaviors (DeFelice et al. 2015; Crowther et al. 2019). This may be particularly relevant in cases where invasive and native populations are compared (Inoue et al. 2012; Delaplane et al. 2015; Tsuchida et al. 2019). Notably, however, other studies have failed to find evidence for variation in queen mate number in different geographic regions (Tarpy et al. 2010; Rattanawannee et al. 2012; Tarpy et al. 2015; Ding et al. 2017).

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.

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.

Supplementary Material

Supplementary material can be found at *Journal of Heredity* online.

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Authors' Contributions

C.J.D., O.L.P., R.M.D., V.J.T., C.H.J., and M.A.D.G. performed sample collections and laboratory analyses. C.J.D. performed data analyses, and C.J.D. and M.A.D.G. wrote the manuscript.

Data Availability

Genetic data are provided in Supplementary Tables S1 and S2.

References

Arnqvist G, Nilsson T. 2000. The evolution of polyandry: multiple mating and female fitness in insects. *Anim Behav*. 60:145–164.

Aviles L, Guevara J. 2017. Sociality in spiders. In: *Comparative social evolution*. Cambridge (UK): Cambridge University Press. p. 188–223.

Baer B. 2016. Proximate and ultimate consequences of polyandry in ants (Hymenoptera: Formicidae). *Myrmecol News*. 22:1–9.

Barker JS. 2011. Effective population size of natural populations of *Drosophila buzzatii*, with a comparative evaluation of nine methods of estimation. *Mol Ecol*. 20:4452–4471.

Bergamo LW, Silva-Brandao KL, Vicentini R, Fresia P, Azeredo-Espin AML. 2020. Genetic differentiation of a new world screwworm fly population from Uruguay detected by SNPs, mitochondrial DNA and microsatellites in two consecutive years. *Insects*. 11:ARTN 539.

Boomsma JJ, Ratnieks FLW. 1996. Paternity in eusocial Hymenoptera. *Philos Trans R Soc Lond*. 351:947–975.

Bromham L, Leys R. 2005. Sociality and the rate of molecular evolution. *Mol Biol Evol*. 22:1393–1402.

Calderón I, Palacín C, Turon X. 2009. Microsatellite markers reveal shallow genetic differentiation between cohorts of the common sea urchin *Paracentrotus lividus* (Lamarck) in northwest Mediterranean. *Mol Ecol*. 18:3036–3049.

Chak STC, Harris SE, Hultgren KM, Duffy J, Rubenstein DR. 2020. Demographic inference provides insights into the extirpation and ecological dominance of eusocial snapping shrimps. *bioRxiv*

Chapman NC, Dos Santos Cocenza R, Blanchard B, Nguyen LM, Lim J, Buchmann G, Oldroyd BP. 2019. Genetic diversity in the progeny of commercial Australian queen honey bees (Hymenoptera: Apidae) produced in autumn and early spring. *J Econ Entomol*. 112:33–39.

Charlesworth B. 2009. Effective population size and patterns of molecular evolution and variation. *Nat Rev Genet*. 10:195–205.

Chau LM, Hanna C, Jenkins LT, Kutner RE, Burns EA, Kremen C, Goodisman MA. 2015. Population genetic structure of the predatory, social wasp *Vespula pensylvanica* in its native and invasive range. *Ecol Evol*. 5:5573–5587.

Crowther LP, Wright DJ, Richardson DS, Carvell C, Bourke AFG. 2019. Spatial ecology of a range-expanding bumble bee pollinator. *Ecol Evol*. 9:986–997.

Crozier RH. 1979. Genetics of sociality. In: Hermann HR, editor. *Social insects*. Vol. 1. New York: Academic Press. p. 223–286

Crozier RH, Fjerdingstad EJ. 2001. Polyandry in social Hymenoptera-disunity in diversity? *Ann Zool Fenn*. 38:267–285.

Crozier RH, Pamilo P. 1996. *Evolution of social insect colonies: sex allocation and kin selection*. Oxford: Oxford University Press.

Daly D, Archer ME, Watts PC, Speed MP, Hughes MR, Barker FS, Jones J, Odgaard K, Kemp SJ. 2002. Polymorphic microsatellite loci for eusocial wasps (Hymenoptera: Vespidae). *Mol Ecol Notes*. 2:273–275.

DeFelice DS, Ross C, Simone-Finstrom M, Warrit N, Smith DR, Burgett M, Sukumalanand P, Rueppell O. 2015. Geographic variation in polyandry of the Eastern Honey Bee, *Apis cerana*, in Thailand. *Insectes Soc*. 62:37–42.

Delaplane KS, Pietravalle S, Brown MA, Budge GE. 2015. Honey bee colonies headed by Hyperpolyandrous queens have improved brood rearing efficiency and lower infestation rates of parasitic *Varroa* mites. *PLoS One*. 10:e0142985.

Dew RM, Shell WA, Rehan SM. 2018. Changes in maternal investment with climate moderate social behaviour in a facultatively social bee. *Behav Ecol Sociobiol*. 72.

Ding G, Xu H, Oldroyd BP, Gloag RS. 2017. Extreme polyandry aids the establishment of invasive populations of a social insect. *Heredity (Edinb)*. 119:381–387.

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. *Mol Ecol Resour*. 14:209–214.

Dobelmann J, Loope KJ, Wilson-Rankin E, Quinn O, Baty JW, Gruber MAM, Lester PJ. 2017. Fitness in invasive social wasps: the role of variation in viral load, immune response and paternity in predicting nest size and reproductive output. *Oikos*. 126:1208–1218.

Echodu R, Beadell JS, Okedi LM, Hyseni C, Aksoy S, Caccone A. 2011. Temporal stability of *Glossina fuscipes fuscipes* populations in Uganda. *Parasit Vectors*. 4:19.

Ellegren H, Galtier N. 2016. Determinants of genetic diversity. *Nat Rev Genet*. 17:422–433.

El-Niweiri MAA, Moritz RFA. 2011. Mating in the rain? Climatic variance for polyandry in the honeybee (Apis mellifera jemenitica). *Popul Ecol*. 53:421–427.

Field J, Paxton RJ, Soro A, Bridge C. 2010. Cryptic plasticity underlies a major evolutionary transition. *Curr Biol*. 20:2028–2031.

Foster KR, Ratnieks FL, Gyllenstrand N, Thorén PA. 2001. Colony kin structure and male production in Dolichovespula wasps. *Mol Ecol*. 10:1003–1010.

Foster KR, Ratnieks FLW. 2001. Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions. *Behav Ecol Sociobiol*. 50:1–8.

Francisco SM, Robalo JI. 2020. Time matters: genetic composition and evaluation of effective population size in temperate coastal fish species. *PeerJ*. 8:e9098.

Galtier N, Rousselle M. 2020. How much does N_e vary among species? *Genetics*. 216:559–572.

Goodisman MA, Kovacs JL, Hoffman EA. 2007. Lack of conflict during queen production in the social wasp *Vespula maculifrons*. *Mol Ecol*. 16:2589–2595.

Goodisman MA, Kovacs JL, Hoffman EA. 2007. The significance of multiple mating in the social wasp *Vespula maculifrons*. *Evolution*. 61:2260–2267.

Goodisman MA, Matthews RW, Crozier RH. 2001. Hierarchical genetic structure of the introduced wasp *Vespula germanica* in Australia. *Mol Ecol*. 10:1423–1432.

Goodisman MAD, Matthews RW, Crozier RH. 2002. Mating and reproduction in the wasp *Vespula germanica*. *Behav Ecol Sociobiol*. 51:497–502.

Greene A. 1991. Dolichovespula and Vespula. In: Ross KG, Matthews RW, editors. *The social biology of wasps*. Ithaca (NY): Comstock Publishing Associates. p. 263–305.

Groom SVC, Rehan SM. 2018. Climate-mediated behavioural variability in facultatively social bees. *Biol J Linn Soc*. 125:165–170.

Habel JC, Husemann M, Finger A, Danley PD, Zachos FE. 2014. The relevance of time series in molecular ecology and conservation biology. *Biol Rev Camb Philos Soc*. 89:484–492.

Hardy OJ, Vekemans X. 2002. SPAGEDi: a versatile computer program to analyse spatial genetic structure at the individual or population levels. *Mol Ecol Notes*. 2:618–620.

Hasegawa E, Takahashi J-I. 2002. Microsatellite loci for genetic research in the hornet *Vespa mandarinia* and related species. *Mol Ecol Notes*. 2:306–308.

Hedgecock D, Pan FTC. 2021. Genetic divergence of selected and wild populations of Pacific oysters (Crassostrea gigas) on the West Coast of North America. *Aquaculture*. 530. doi:10.1016/j.aquaculture.2020.735737

Hoffman EA, Kovacs JL, Goodisman MA. 2008. Genetic structure and breeding system in a social wasp and its social parasite. *BMC Evol Biol*. 8:239.

Hölldobler B, Wilson EO. 1990. *The Ants*. Cambridge (MA): The Belknap Press of Harvard University Press.

Imrit MA, Dogantzis KA, Harpur BA, Zayed A. 2020. Eusociality influences the strength of negative selection on insect genomes. *Proc Biol Sci*. 287:20201512.

Inoue MN, Saito F, Tsuchida K, Goka K. 2012. Potential increase in mating frequency of queens in feral colonies of *Bombus terrestris* introduced into Japan. *Naturwissenschaften*. 99:853–861.

Jacobs S, Schrempf A. 2017. Male-male competition in ants (Hymenoptera: Formicidae). *Myrmecol News*. 24:31–42.

Jaffe R. 2014. An updated guide to the study of polyandry in social insects. *Sociobiology*. 61:1–8.

Jaffé R, Garcia-Gonzalez F, den Boer SP, Simmons LW, Baer B. 2012. Patterns of paternity skew among polyandrous social insects: what can they tell us about the potential for sexual selection? *Evolution*. 66:3778–3788.

Jansson E, Ruokonen M, Kojola I, Aspi J. 2012. Rise and fall of a wolf population: genetic diversity and structure during recovery, rapid expansion and drastic decline. *Mol Ecol*. 21:5178–5193.

Jara L, Muñoz I, Cepero A, Martín-Hernández R, Serrano J, Higes M, De la Rúa P. 2015. Stable genetic diversity despite parasite and pathogen spread in honey bee colonies. *Naturwissenschaften*. 102:53.

Jennions MD, Petrie M. 2000. Why do females mate multiply? A review of the genetic benefits. *Biol Rev Camb Philos Soc*. 75:21–64.

Johnson EL, Cunningham TW, Marriner SM, Kovacs JL, Hunt BG, Bhakta DB, Goodisman MA. 2009. Resource allocation in a social wasp: effects of breeding system and life cycle on reproductive decisions. *Mol Ecol*. 18:2908–2920.

Jorde PE, Ryman N. 1995. Temporal allele frequency change and estimation of effective size in populations with overlapping generations. *Genetics*. 139:1077–1090.

Jorde PE, Ryman N. 2007. Unbiased estimator for genetic drift and effective population size. *Genetics*. 177:927–935.

Kanuch P, Berggren A, Cassel-Lundhagen A. 2020. A clue to invasion success: genetic diversity quickly rebounds after introduction bottlenecks. *Biol Invasions*. 23:1141–1156

Kovacs JL, Goodisman MA. 2007. Irregular brood patterns and worker reproduction in social wasps. *Naturwissenschaften*. 94:1011–1014.

Kovacs JL, Goodisman MA. 2012. Effects of size, shape, genotype, and mating status on queen overwintering survival in the social wasp *Vespula maculifrons*. *Environ Entomol*. 41:1612–1620.

Kovacs JL, Hoffman EA, Goodisman MAD. 2008. Mating success in the polyandrous social wasp *Vespula maculifrons*. *Ethology*. 114:340–350.

Kraus FB, Moritz RFA. 2010. Extreme polyandry in social Hymenoptera: evolutionary causes and consequences for colony organisation. In: *Animal behaviour: evolution and mechanisms*. Berlin, Heidelberg: Springer.

Kraus FB, Neumann P, Moritz RFA. 2004. Genetic variance of mating frequency in the honeybee (Apis mellifera L.). *Insectes Soc*. 51:1–5.

Krimbas CB, Tsakas S. 1971. The genetics of *Dacus oleae*. v. changes of esterase polymorphism in a natural population following insecticide control-selection or drift? *Evolution*. 25:454–460.

Kvarnemo C, Simmons LW. 2013. Polyandry as a mediator of sexual selection before and after mating. *Philos Trans Roy Soc B*. 368. doi:10.1098/rstb.2012.0042

Ledoux JB, Frias-Vidal S, Montero-Serra I, Antunes A, Bueno CC, Civit S, Lopez-Sendino P, Linares C, Garrabou J. 2020. Assessing the impact of population decline on mating system in the overexploited Mediterranean red coral. *Aquat Conserv Mar Freshw Ecosyst*. 30:1149–1159.

Loope KJ, Chien C, Juhl M. 2014. Colony size is linked to paternity frequency and paternity skew in yellowjacket wasps and hornets. *BMC Evol Biol*. 14:277.

Loope KJ, Lopez-Osorio F, Dvořák L. 2017. Convergent reversion to single mating in a wasp social parasite. *Am Nat*. 189:E138–E151.

Lozier JD. 2014. Revisiting comparisons of genetic diversity in stable and declining species: assessing genome-wide polymorphism in North American bumble bees using RAD sequencing. *Mol Ecol*. 23:788–801.

Luikart G, Ryman N, Tallmon DA, Schwartz MK, Allendorf FW. 2010. Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conserv Genet*. 11:355–373.

MacDonald JF, Matthews RW. 1981. Nesting biology of the Eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae). *J Kans Entomol Soc*. 54:433–457.

Maebe K, Haramboure M, Lucia M, Alvarez LJ, Smagghe G. 2019. Temporal drop of genetic diversity in *Bombus pauloensis*. *Apidologie*. 50:526–537.

Masciocchi M, Martinez AS, Pereira AJ, Villacide JM, Corley JC. 2018. Dispersal behavior of yellowjacket (*Vespula germanica*) queens. *Insect Sci*. 25:109–116.

Maynard Smith J, Szathmary E. 1998. *The major transitions in evolution*. Oxford (UK): Oxford University Press.

Molnár T, Benedek I, Kovács B, Zsolnai A, Lehoczky I. 2020. Genetic consequences of pond production of a pikeperch (*Sander lucioperca* L.) stock with natural origin: the effects of changed selection pressure and reduced population size. *PeerJ*. 8:e8745.

Nei M, Tajima F. 1981. Genetic drift and estimation of effective population size. *Genetics*. 98:625–640.

Nielsen R, Tarpy DR, Reeve HK. 2003. Estimating effective paternity number in social insects and the effective number of alleles in a population. *Mol Ecol*. 12:3157–3164.

Nonacs P. 2003. Measuring the reliability of skew indices: is there one best index? *Anim Behav*. 65:615–627.

Olah G, Stojanovic D, Webb MH, Waples RS, Heinsohn R. 2020. Comparison of three techniques for genetic estimation of effective population size in a critically endangered parrot. *Anim Conserv*. 24:491–498

Palinkas-Bodzsar N, Sztan N, Molnar T, Hidas A. 2020. Gene conservation of six Hungarian local chicken breeds maintained in small populations over time. *PLoS One*. 15:e0238849.

Paxton RJ, Thorén PA, Estoup A, Tengö J. 2001. Queen-worker conflict over male production and the sex ratio in a facultatively polyandrous bumblebee, *Bombus hypnorum*: the consequences of nest usurpation. *Mol Ecol*. 10:2489–2498.

Peakall R, Smouse PE. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. *Bioinformatics*. 28:2537–2539.

Pizzari T, Wedell N. 2013. The polyandry revolution. *Philos Trans R Soc Lond B Biol Sci*. 368:20120041.

Purcell J. 2011. Geographic patterns in the distribution of social systems in terrestrial arthropods. *Biol Rev*. 86:475–491.

Queller DC, Goodnight KF. 1989. Estimating relatedness using genetic markers. *Evolution*. 43:258–275.

Rašić G, Endersby-Harshman N, Tantowijoyo W, Goundar A, White V, Yang Q, Filipović I, Johnson P, Hoffmann AA, Arguni E. 2015. Aedes aegypti has spatially structured and seasonally stable populations in Yogyakarta, Indonesia. *Parasit Vectors*. 8:610.

Ratnieks FL, Foster KR, Wenseleers T. 2006. Conflict resolution in insect societies. *Annu Rev Entomol*. 51:581–608.

Rattanawannee A, Chanchao C, Wongsiri S, Oldroyd BP. 2012. No evidence that habitat disturbance affects mating frequency in the giant honey bee *Apis dorsata*. *Apidologie*. 43:761–770.

Romiguier J, Lourenco J, Gayral P, Faivre N, Weinert LA, Ravel S, Ballenghien M, Cahais V, Bernard A, Loire E, *et al.* 2014. Population genomics of eusocial insects: the costs of a vertebrate-like effective population size. *J Evol Biol*. 27:593–603.

Romiguier J, Weyna A. Forthcoming. Relaxation of purifying selection suggests low effective population size in eusocial Hymenoptera and pollinating bees. *bioRxiv*. doi:10.1101/2020.04.14.038893

Ross KG. 1983. Laboratory studies of the mating biology of the Eastern yellowjacket, *Vespula maculifrons* (Hymenoptera: Vespidae). *J Kans Entomol Soc*. 56:523–537.

Ross KG. 1986. Kin selection and the problem of sperm utilization in social insects. *Nature*. 323:798–800.

Ross KG, Carpenter JM. 1991. Population genetic structure, relatedness, and breeding systems. In: Ross KG, Matthews RW, editors. *The social biology of wasps*. Ithaca (NY): Comstock Publishing Associates. p. 451–479

Ross KG, Matthews RW. 1991 *The social biology of wasps*. Ithaca (NY): Comstock Publishing Associates.

Rousset F. 2008. genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol Ecol Resour*. 8:103–106.

Saarinen EV, Austin JD, Daniels JC. 2010. Genetic estimates of contemporary effective population size in an endangered butterfly indicate a possible role for genetic compensation. *Evol Appl*. 3:28–39.

Saarman NP, Gloria-Soria A, Anderson EC, Evans BR, Pless E, Cosme LV, Gonzalez-Acosta C, Kamgang B, Wesson DM, Powell JR. 2017. Effective population sizes of a major vector of human diseases, *Aedes aegypti*. *Evol Appl*. 10:1031–1039.

Saga T, Okuno M, Loope KJ, Tsuchida K, Ohbayashi K, Shimada M, Okada Y. 2020. Polyandry and paternity affect disease resistance in eusocial wasps. *Behav Ecol*. 31:1172–1179.

SAS Inc. 2019. *JMP. Version 15.0.0*. Cary (NC): SAS Inc.

Schürch R, Accleton C, Field J. 2016. Consequences of a warming climate for social organisation in sweat bees. *Behav Ecol Sociobiol*. 70:1131–1139.

Settepani V, Bechsgaard J, Bilde T. 2016. Phylogenetic analysis suggests that sociality is associated with reduced effectiveness of selection. *Ecol Evol*. 6:469–477.

Shen SF, Emlen ST, Koenig WD, Rubenstein DR. 2017. The ecology of cooperative breeding behaviour. *Ecol Lett*. 20:708–720.

Sih A, Ferrari MC, Harris DJ. 2011. Evolution and behavioural responses to human-induced rapid environmental change. *Evol Appl*. 4:367–387.

Strassmann J. 2001. The rarity of multiple mating by females in the social Hymenoptera. *Insectes Soc*. 48:1–13.

Sutter A, Travers LM, Weedon M, Oku K, Price TAR, Wedell N. 2019. No selection for change in polyandry under experimental evolution. *J Evol Biol*. 32:717–730.

Tarpy DR, Caren JR, Delaney DA, Sammataro D, Finley J, Loper GM, DeGrandi-Hoffman G. 2010. Mating frequencies of Africanized honey bees in the south western USA. *J Apic Res*. 49:302–310.

Tarpy DR, Delaney DA, Seeley TD. 2015. Mating frequencies of honey bee queens (*Apis mellifera* L.) in a population of feral colonies in the Northeastern United States. *PLoS One*. 10:e0118734.

Tarpy DR, Seeley TD. 2006. Lower disease infections in honeybee (*Apis mellifera*) colonies headed by polyandrous vs monandrous queens. *Naturwissenschaften*. 93:195–199.

Tong C, Najm GM, Pinter-Wollman N, Pruitt JN, Linksvayer TA. 2020. Comparative genomics identifies putative signatures of sociality in spiders. *Genome Biol Evol*. 12:122–133.

Tsuchida K, Yamaguchi A, Kanbe Y, Goka K. 2019. Reproductive interference in an introduced bumblebee: polyandry may mitigate negative reproductive impact. *Insects*. 10:59.

Vollet-Neto A, Imperatriz-Fonseca VL, Ratnieks FLW. 2019. Queen execution, diploid males, and selection for and against polyandry in the Brazilian stingless bee *Scaptotrigona depilis*. *Am Nat*. 194:725–735.

Wenseleers T, Badcock NS, Erven K, Tofilski A, Nascimento FS, Hart AG, Burke TA, Archer ME, Ratnieks FL. 2005. A test of worker policing theory in an advanced eusocial wasp, *Vespula rufa*. *Evolution*. 59:1306–1314.

Wilson EO. 1971. *The insect societies*. Cambridge (MA): Harvard University Press.

Wilson EO. 1990. *Success and dominance in ecosystems: The case of social insects*. Oldendorf/Luhe (Germany): Ecology Institute.

Wong BBM, Candolin U. 2015. Behavioral responses to changing environments. *Behav Ecol*. 26:665–673.

Wood DA, Rose JP, Halstead BJ, Stoelting RE, Swaim KE, Vandergast AG. 2020. Combining genetic and demographic monitoring better informs conservation of an endangered urban snake. *PLoS One*. 15:e0231744.