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Resource allocation in a social wasp: effects of breeding system and life cycle on reproductive decisions

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Abstract

Organisms must make important decisions on how to allocate resources to reproduction. We investigated allocation decisions in the social wasp *Vespula maculifrons* to understand how social insects make reproductive choices. We first determined how annual colonies apportioned resources to growth and reproduction by analysing developing brood. In contrast to expectations, colonies invested in both growth (workers) and reproduction (males) simultaneously. In addition, colonies showed evidence of producing males in pulses and reversing their reproductive choices by decreasing investment in males late in the season. This reversal is consistent with theory suggesting that colonies decrease production in males if fitness of late emerging males is low. To further investigate reproductive decisions within colonies, we determined if the male mates of multiply-mated queens varied in their reproductive success over time. Sperm use by queens did vary over time suggesting that male success may depend on sperm clumping within the female reproductive tract. Finally, we tested if colony sex ratio conformed to expectations under kin selection theory that nestmate relatedness would positively correlate with investment in new queens if workers controlled sex allocation. Surprisingly, the proportion of queens produced by colonies was negatively correlated with nestmate relatedness, suggesting that allocation may be shaped by advantages arising from increased genetic diversity resulting from multiple mating by queens. Overall, our study suggests that the reproductive decisions of colonies are flexible and may depend both on environmental cues arising from energetic needs of the colony and genetic cues arising from mating behaviours of queens.

Keywords: kin selection, life-history strategy, optimal resource allocation, polyandry, reproductive competition, sex ratio, social insect

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Introduction

Organisms face a fundamental ecological problem of optimally allocating resources to reproduction (Roff 1992; Stearns 1992; Perrin & Sibly 1993). Social insects present particularly interesting systems in which to investigate the problem of resource allocation because insect societies can be viewed both as 'superorganisms' and as groups of distinct individuals. Thus, on the one hand, insect societies sometimes appear to function as single organisms attempting to balance investment in growth, by producing sterile workers and soldiers, and investment in reproduction, by producing reproductive queens and males (Oster & Wilson 1978). On the other hand, social insect colonies are actually collections of genetically distinct individuals that may have contrasting interests. Such divergent interests may lead to reproductive conflict, which can affect how colonies ultimately allocate their resources towards growth and reproduction (Bourke & Franks 1995; Crozier & Pamilo 1996; Ratnieks *et al.* 2006).

Questions concerning allocation of resources in social insects are further complicated when multiple reproductives contribute to offspring production within societies. For example, in hymenopteran social insects (ants, social bees, and social wasps), reproductive competition may occur among queens if multiple queens reside within colonies, among male mates of queens if queens mate multiply (polyandry), or between queens and their worker offspring

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if workers are reproductively capable. In fact, conflict over reproduction likely represents an important feature of colonial life that can lead to selfish behaviours, which can potentially degrade the cooperative actions that typically characterize highly social animals (Beekman & Ratnieks 2003; Boomsma & Franks 2006; Ratnieks *et al*. 2006).

Allocation strategies in the social Hymenoptera contain a final layer of complexity because of the unusual relationships among colonymates that arise as a consequence of their haplodiploid genetic system. Haplodiploid species are characterized as having haploid males produced parthenogenetically by females and diploid females that are sexually produced. Consequently, females are related to their full sisters by 3/4 but only related to their brothers by 1/4. In contrast, a queen mother is related to both her female and male offspring by 1/2. These asymmetries in relatedness can lead to conflict over sex ratio because workers are generally expected to invest more in the production of new queens than males, whereas the mother queen should prefer a more equal investment in the sexes (Trivers & Hare 1976; Crozier & Pamilo 1996; Mehdiabadi *et al*. 2003; Bourke 2005).

Overall, social insects thus provide a rich framework for studying how organisms allocate resources to growth and reproduction. The purpose of this study was to understand how these decisions are made in a social wasp. Wasps hold a place of central importance to the study of social behaviour because of the diversity of social systems that they display (Ross & Matthews 1991; Turillazzi & West-Eberhard 1996). Social wasps in the genus *Vespula* are particularly useful for study because of their known natural history, social biology, and behaviour (reviewed by Spradbery 1973; Edwards 1980; Greene 1991; Foster & Ratnieks 2001a).

We took advantage of the life-history characteristics of *Vespula* to test three hypotheses concerning how allocation to reproduction occurs in social animals. First, our main aim was to understand how colonies transitioned from allocation to growth (i.e. producing workers) to allocation to reproduction (producing new queens and males). Simple theoretical predictions suggest that annual colonies, such as those displayed by *Vespula*, should display a 'bang-bang' mode of reproduction (Macevicz & Oster 1976; Oster & Wilson 1978; Mitesser *et al*. 2007b). That is, colonies should put all resources into worker production until one generation before the colony is expected to fail. Then, the colony should invest all resources into the production of reproductives.

However, colonies may also display graded control of reproduction, whereby allocation to both growth and reproduction occurs simultaneously (King & Roughgarden 1982a). Graded control may be adaptive if the growing season is of uncertain length or if robust reproduction requires some concurrent investment in growth (King & Roughgarden 1982a; Kozlowski 1992; Mitesser *et al*. 2007a). Additionally, switching between growth and reproduction may occur under certain distributions of growing seasons or if predictable loss of growth components (i.e. workers) occurs during the season (King & Roughgarden 1982b; Mitesser *et al*. 2007a). Finally, colonies may invest differentially in new queen and male production over time if mating success or survival of males and new queens depends on when they emerge (Bulmer 1983; Duchateau & Velthuis 1988; Bourke 1997). Consequently, multiple processes may affect investment in growth and reproduction in social insects. Our goal was thus to distinguish among these multiple hypotheses by testing if investment in reproduction showed graded control and if switching between growth and reproduction occurred.

The second aim of this study was to investigate if competition over allocation occurred among reproductives within colonies. *Vespula* is a particularly interesting taxon in which to investigate reproductive competition, because colonies are headed by a single, multiply mated queen. The single queen is responsible for virtually all reproduction within the colony as long as she is alive (Wenseleers & Ratnieks 2006). Thus there is no obvious, direct competition over reproduction between the queen and her workers as sometimes occurs in other social taxa (Beekman & Ratnieks 2003; Ratnieks *et al*. 2006).

However, reproductive competition may occur among the deceased male mates of the queen (Boomsma *et al*. 2005). That is, males may vary in reproductive success due to sperm competition or female choice even though the male reproductives have long since perished. Such variation in sperm use could affect male fitness and within-cohort relatedness, thereby influencing colony social structure and, ultimately, individual behaviours and colony-level reproductive allocation strategies (Boomsma & Ratnieks 1996; Boomsma & Sundström 1998). Alternatively, individual selection operating on male reproductive success may be overridden by selection at the level of the colony leading to relatively unbiased use of sperm by queens. Indeed, there should be little selection for male sperm to compete for success in worker production, because males gain little direct fitness return by producing workers, which generally do not reproduce. Moreover, strongly biased sperm use would potentially diminish advantages arising from having a genetically diverse worker force (Crozier & Fjerdingstad 2001; Oldroyd & Fewell 2007). The available theory thus suggests that males should not necessarily show variation in sperm use during the reproductive season. In this study, we tested this prediction by determining if *Vespula* males mated to specific queens contributed differentially to worker production over time.

Finally, our third aim was to investigate predictions from kin selection theory regarding how colonies invest in producing new queens and males. As discussed above, the haplodiploid genetic system displayed by hymenopteran social insects such as *Vespula* may lead to conflict over sex investment ratio between queens and workers (Boomsma & Ratnieks 1996; Crozier & Pamilo 1996; Boomsma & Sundström 1998; Sundström & Boomsma 2001; Beekman & Ratnieks 2003). However, multiple mating by queens depresses this level of conflict. When queens mate with multiple males, the queen sex investment ratio optimum remains at 1:1 (queens:males) but the worker optimum falls below 3:1 and ultimately converges on 1:1 if the queen mates many times. Indeed, it has been suggested that a possible advantage to polyandry is that it reduces queen–worker conflict over sex ratio within colonies (Boomsma & Grafen 1991; Ratnieks & Boomsma 1995; Sundström & Ratnieks 1998). Consequently, in the absence of other factors that may affect sex ratio, colonies should produce equal investment in the sexes if the queen controls reproductive output. However, most colonies should bias reproductive output towards females if workers are in charge of reproductive decisions, although the worker optimum is expected to be close to the queen optimum with high queen mate number.

Vespula workers may have a substantial impact on sex ratio decisions within colonies because they build the cells in which larvae are reared and care for the developing offspring. Thus, sex investment ratio may be at least partially worker controlled. Consequently, colonies are expected to display female-biased sex ratios (in the absence of split sex ratio effects; Boomsma & Grafen 1991), and queen mate number should be negatively correlated with investment in new queens. Surprisingly, although factors affecting sex ratio in hymenopteran social insects have been intensely studied (Chapuisat & Keller 1999; Meunier *et al*. 2008), the specific prediction that sex investment ratio should be affected by queen mate number has rarely been investigated in highly polyandrous social insects (but see Dijkstra & Boomsma 2008). In this study, we determined if patterns of sex investment ratio in *Vespula* colonies conformed to predictions and were consistent with queen or worker control.

We studied resource allocation in the eastern yellowjacket, *Vespula maculifrons* (reproductive biology reviewed by MacDonald & Matthews 1981; Goodisman *et al*. 2007a, b; Hoffman *et al*. 2008; Kovacs *et al*. 2008). In the location where this study was conducted (Atlanta, Georgia, USA), a typical new *V. maculifrons* colony is initiated by a single, mated queen that emerges from hibernation in April. The queen constructs a new nest made of worker cells and lays diploid eggs in these cells that ultimately develop into workers. The developmental time for a *Vespula* larva in a worker cell ranges from 24 to 39 days depending on cell size, food availability, and time of year. The colony grows through the spring and summer and eventually contains a few thousand workers. Then, in late August, the colony switches from producing exclusively workers to producing gynes (prereproductive queens) and males. Males are typically reared in already-constructed worker cells and thus emerge before gynes. In contrast, gynes are reared in specially constructed queen cells that are built when males are first produced. Workers tend developing larvae and thus may have some control over rearing decisions of the sexuals. New gynes and males leave the nest to mate after they emerge. Gynes mate with multiple males and then hibernate to pass the winter. The males, workers, and old queens within the population die. Consequently, *V. maculifrons* colonies behave as semelparous organisms and reproduce only once. Thus, overall, *V. maculifrons* displays many characteristics that are useful for studying allocation to reproduction in social insects.

Methods

Sampling

We collected *Vespula maculifrons* colonies between the months of September and November of 2006. The reproductive queen heading the colony was successfully identified in some nests collected early in the season. We sampled individuals of different developmental stages from worker cells of all colonies in order to understand how the reproductive decisions of colonies varied over time. Specifically, we collected eggs, first to third instar 'young' larvae, fourth to fifth instar 'old' larvae, and pupae from randomly selected worker cells in each nest. Eggs were frozen at –20 °C, whereas young larvae, old larvae, and pupae were stored in 95% ethanol for subsequent genetic analysis.

We collected and dried the adult workers, gynes, and males from each colony at 60 °C for 24–48 h to obtain estimates of the total number and mass of adults of each caste. All combs from each nest were photographed and the total areas of the queen and worker comb were estimated using tools within the Canvas 9.0.4 computer package. These data were then used to estimate the number of queen and worker cells that made up each nest.

Genetic analyses

Chelex extractions were used to obtain DNA from eggs, as described by Kovacs & Goodisman (2007), and from pupae using the methods of Goodisman *et al*. (2001). Chelex extractions were not effective in obtaining high quality DNA from larvae. Consequently, whole young larvae or heads of old larvae were ground in 300 μL of lysis solution (0.1 M NaCl, 0.2 M Sucrose, 0.1 M Tris-Cl pH = 9.0, 0.05 M EDTA, 0.5% SDS) and then heated to 70 °C for 30 m. A total of 150 μL of NaOAc pH = 5.0 was added to this homogenate and the sample was placed on ice for 20 m. The cellular material was removed from the homogenate by centrifugation. A total of 900 μL of 95% ethanol was then mixed with the supernatant. The resulting solution was centrifuged at room temperature to pellet the genomic DNA. The DNA pellet was washed once with 70% ethanol, dried, and then resuspended in 200 μL of TE.

The genotypes of sampled individuals were then obtained at the five microsatellite loci LIST2004, LIST2013, LIST2019, LIST2020, and VMA-6 (Daly *et al*. 2002; Hasegawa & Takahashi 2002; Goodisman *et al*. 2007a, b). The genetic data were used for three related purposes. First, we used the genetic markers to distinguish developing males from workers. Haploid males display only a single allele at any given locus, whereas females usually display two alleles at highly polymorphic loci. Second, the markers were used to discriminate between workers sired by different males of multiply mated queens. Paternity analyses in hymenopteran taxa are particularly straightforward because males are haploid and full siblings always display the same multilocus haplotype derived from their father. Third, we used the genetic data to distinguish queen-produced males from worker-produced males. Queen-produced males can be identified because they possess alleles of the queen only. In contrast, worker-produced males almost always display alleles derived from the queen's male mates when sufficient numbers of loci are analysed. We used the methods of Foster *et al*. (2001b) to determine the probability of detecting a worker-produced male in each colony. Colonies were considered queenright if the queen heading the colony was located or if the colony contained almost exclusively queen-produced males. In contrast, colonies were considered queenless if they contained an abundance of worker-produced males (Wenseleers & Ratnieks 2006).

Statistical analyses

We first investigated how allocation to reproduction changed over time within queenright colonies. We considered colonies that reared workers in worker cells to be allocating resources towards growth. In contrast, colonies that reared males in worker cells were deemed to be allocating resources towards reproduction. We used *G*-tests of heterogeneity to determine if the ratio of males to workers differed among life stages. Statistical calculations were conducted using the software program JMP.

We next used the molecular genetic data derived from the worker genotypes to investigate if the contributions of the different male mates of queens differed among life stages within colonies. We used *G*-tests of heterogeneity as implemented by the program JMP to determine if the distribution of patrilines differed over time. The information from all colonies was then combined using the unweighted *Z*-transform approach (Whitlock 2005) to obtain an overall *P* value across all colonies.

We next investigated if the sperm from certain males was more persistent than that of others. Variation in persistence could occur if sperm from some males competed or survived better than sperm from other males, if the amount of sperm transmitted by males varied, or if females varied in the sperm they used over time through cryptic female choice. To test for variation in sperm persistence, we determined if the number of males contributing to worker production decreased over time. We first calculated the effective paternity (k_{e3}) for workers in each developmental stage using the method of Nielsen *et al*. (2003). This information was then used to determine Spearman's correlation between k_{e3} and the relative age of different life stages (Eggs = 1, Young larvae = 2, Old larvae = 3, Pupae = 4) within each colony. These individual colony correlations were subsequently used in a one-tailed sign test to determine if they were positive more often than expected by chance, as might occur if certain males ultimately outcompeted other males for reproductive success over time.

Finally, we investigated the factors associated with variation in colony sex ratio in *V. maculifrons*. We first used Spearman's correlation coefficient to assess the relationship between number of queen or worker cells within a nest and k_{e3} (Goodisman *et al*. 2007b). We next calculated the relatedness of workers in queenright colonies (r_{WW}) using the program Relatedness 4.2 (Queller & Goodnight 1989). Statistical analyses were then conducted on numerical sex ratio (i.e. the number of gynes divided by the total number of gynes plus males) within the seven queenright colonies that produced adults of both sexes using logistic regression as implemented by the program SAS (Boomsma & Nachman 2002). The following variables were considered as possibly affecting sex ratio within colonies: 'relatedness of workers (r_{WW})', 'number of male mates (k)', 'effective number of male mates (k_{e3})', 'number of worker cells', 'number of queen cells', 'number of workers', 'number of gynes', 'number of males', and 'number of gynes plus males (i.e. colony productivity)'. Backwards elimination was used to identify variables that significantly explained numerical sex ratio (Boomsma & Nachman 2002).

We then investigated if the population-level sex investment ratio (as opposed to the colony-level sex ratio discussed above) differed from that expected under either queen or worker control. To estimate the investment in the sexes, we first calculated the relative cost of producing gynes and males (c) as the ratio of the mean dry mass of gynes to the mean dry mass of males. The cost ratio, c, was not corrected (cf. Boomsma 1989) because it likely varies among species, corrections are not known for this taxon, and sexual dimorphism in this species is modest (Helms 1994; Boomsma *et al*. 1995). We then determined the optimal proportion of energetic allocation that should go to females (o) under queen or worker control, which is expected to vary as a function of the relatedness of workers to the developing brood within colonies if workers control allocation, but to remain invariant if queens control sex investment ratio. We

Table 1 Characteristics of 15 *Vespula maculifrons* colonies sampled in this study

		Total mass of adults (g)			Total number of adults			Queen mate number	
Colony ID	Date collected	Workers	Gynes	Males	Workers	Gynes	Males	Observed (k)	Effective (k_{e3})
63	7-Sep-06	16.07	0	0	908	0	0	7	5.44
65	7-Sep-06	26.35	0	0	1578	0	0	4	3.84
66	7-Sep-06	6.14	0	0	405	0	0	4	3.73
67	7-Sep-06	0.62	0	0	52	0	0	7	6.93
68*	25-Sep-06	6.51	0	N/D†	423	0	9	5	4.65
69	25-Sep-06	17.31	0	0	861	0	0	2	1.55
70*	4-Oct-06	4.58	0	0	265	0	0	7	6.68
72	18-Oct-06	4.93	14.16	9.74	277	169	274	4	3.66
73	18-Oct-06	1.53	10.12	11.99	82	113	336	7	6.38
74	18-Oct-06	0.20	4.67	3.54	11	51	118	7	6.24
76	19-Oct-06	7.14	6.01	10.60	359	74	284	9	3.06
77	19-Oct-06	6.82	10.20	20.80	311	133	506	8	5.53
78	19-Oct-06	0.50	2.97	11.29	29	33	326	7	4.83
79	19-Oct-06	3.60	5.85	5.91	163	78	172	7	5.35
81*	3-Nov-06	1.03	7.88	3.59	56	77	82	5	3.95
Mean		6.88	4.12	5.53	385.33	48.53	140.46	6.00	4.79
Sum		103.32	61.86	77.46	5780	728	2107		

*Colony judged to be queenless. See text for details. †No data. Nine males were present but their total mass was not obtained.

obtained estimates of the proportion of females (f) expected under queen or worker control of sex investment, given c and the mean value of o for all colonies, using the formula $f = o/(o + c - oc)$ (modified from Ichinose *et al.* 2007).

The resulting output from the logistic regression analyses was used to test if the population sex ratio differed from that expected under either queen or worker control. The point estimate of population sex ratio under the model was estimated by taking the mean proportion of gynes from each colony, weighted by the total number of progeny in each colony. The standard error for this mean point estimate was obtained using the method of Hosmer & Lemeshow (2000). This estimate was then compared to that expected under queen or worker control via a t-test.

Results

Samples

We sampled individuals from 15 *Vespula maculifrons* colonies. As expected, colonies collected early in the season tended to have few adult gynes or males in residence (Table 1). In contrast, colonies collected later in the season contained many adult sexuals. We obtained the genotypes of a mean (± SD) of 30.73 ± 7.86 eggs, 31.13 ± 9.22 young larvae, 33.40 ± 6.92 old larvae, and 32.67 ± 8.41 pupae per colony. Thus, we genotyped a total of 461 eggs, 467 young larvae, 501 old larvae, and 490 pupae for a grand total of 1919 individuals.

Our genetic markers were highly polymorphic. The expected heterozygosities for loci LIST2004, LIST2013, LIST2019, LIST2020, and VMA-6 were 0.885, 0.862, 0.744, 0.864, and 0.944 in this population, respectively (Hoffman *et al.* 2008), and thus provided substantial power to achieve our objectives. For instance, the probability of a particular female being homozygous at all five loci was < 0.0001. Consequently, haploid males could easily be distinguished from diploid females genetically. Also, the probability of two distinct males having the same genotype at all five loci was < 0.0001. Thus, workers produced by distinct male mates of queens could readily be identified. Finally, our genetic markers were sufficiently variable to provide reasonable power to distinguish queen-produced from worker-produced males within colonies (Table 2; Foster *et al.* 2001b).

Individuals showing unusual or rare genotypes were rerun in order to confirm the existence of infrequent patrilines. Moreover, no patriline was identified based on data from only a single locus. For example, two individual workers (one in each of two colonies) that displayed putative patrilines that differed from a more common patriline at only a single locus were considered to have arisen via mutation and lumped into the more common patriline. In addition, genetic data from one colony, which could have arisen from two different males sharing alleles with their putative queen mate, were judged to be more parsimoniously explained by the presence of a null allele in a single male mate.

Table 2 Number of male brood that could have been produced by the queen (Q) or were produced by workers (W) in 15 *Vespula maculifrons* colonies. P is the probability of distinguishing a worker-produced male from a queen-produced male

		Egg		Young larva		Old larva		Pupa		Total	
Colony ID	P	Q	W	Q	W	Q	W	Q	W	Q	W
63	0.832	12	7	1	0	0	0	0	0	13	7
65	0.899	7	0	7	0	2	0	3	0	19	0
66	0.839	1	0	1	0	0	0	0	0	2	0
67	0.923	0	0	0	0	0	0	0	0	0	0
68*	0.910	9	25	4	32	3	37	0	0	16	94
69	0.938	17	0	12	0	9	2	2	0	40	2
70*	0.895	4	29	3	36	4	29	3	9	14	103
72	0.947	15	0	21	0	12	0	31	0	79	0
73	0.845	17	0	24	0	10	0	33	0	84	0
74	0.884	5	0	11	0	6	0	33	0	55	0
76	0.921	15	0	24	0	3	0	14	0	56	0
77	0.777	17	0	33	0	14	0	29	0	93	0
78	0.868	22	0	20	0	9	0	34	0	85	0
79	0.939	17	0	5	0	4	0	19	0	45	0
81*	0.875†	6	5	1	15	4	11	15	21	26	52

*Colony judged to be queenless. See text for details. †Estimate attained using genetic information from adult workers.

We successfully discovered the queen heading colonies #63, #66, and #67. In addition, we differentiated queenright from queenless colonies based on patterns of worker reproduction. In particular, we judged colonies #68, #70, and #81 to be queenless because they contained an abundance of worker-produced males (Table 2). In contrast, the presumably queenright colonies contained almost no worker-produced males.

Examination of the time course of worker reproduction in colonies #63, #68, and #69, which showed transitions from queen to worker production of males, indicated that queen production of males always preceded worker production of males. We also note that the appearance of worker-produced males started relatively abruptly in colony #68 but more gradually in colony #81 (i.e. a relatively high proportion of males in pupae could still be queen produced) indicating that the transition to worker reproduction may not always occur in the same way in all colonies.

Allocation to growth and reproduction

We analysed the genotypes of individuals sampled from worker cells to determine how colonies allocated resources towards growth (workers) and reproduction (males). Not surprisingly, most queenright colonies showed significant differences in the production of males in different life stages (Fig. 1; $G3 > 12.50$; $P < 0.01$ for all colonies tested except for colonies #65 and #66 where $G3 < 7.20$, $P > 0.05$). However, the patterns of allocation to male production differed considerably depending on when colonies were collected. In particular, the proportion of males reared in worker cells in colonies collected early in the reproductive season (early colonies: #63, #65, #66, and #69) increased more or less monotonically over time, with more males being reared in younger life stages (Fig. 1). However, colonies collected later in the season (late colonies: #72–#74 and #76–#79) showed more complex patterns of allocation. Male brood was actually less common in the younger life stages of egg and young larva than in the pupal stages in many late colonies.

We tested if the variation in proportion of males produced among life stages was associated with overall colony productivity in late colonies, as may be expected if colony size affected allocation patterns. However, we found no significant correlation between standard deviation in proportion of males among life stages and total number of sexuals produced ($r_S = -0.286$, $P = 0.534$). In addition, males made up a strikingly small proportion of old larvae in late colonies relative to the proportions in the other life stages (Fig. 1).

Allocation by male mates of the queen

Queens mated with a mean of 6.00 ± 2.03 males with a range of two to nine male mates. The mean effective mate number of queens, k_{e3}, was 4.79 ± 1.50 with a range of 1.54–6.93 (Table 1). We determined if the contributions of males to workers varied over time in the 12 colonies that produced at least some workers in all life stages. Within single colonies, we uncovered little evidence of such variation. Indeed, only colony #73 showed strongly

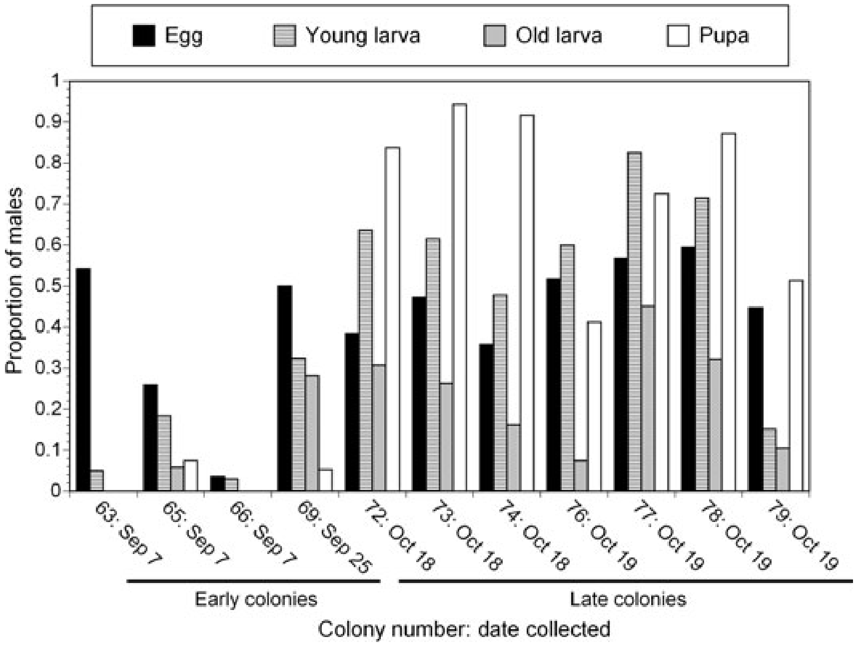

Fig. 1 Proportion of males in four developmental stages sampled from worker cells of queenright *Vespula maculifrons* colonies. Colony number and collection date are provided. Colonies showed nonmonotonic increases in male production in late colonies.

significant variation in male reproductive success over time (Fig. 2). However, when data from all colonies were considered, we found that males did, in fact, show significant variation in reproductive success over time ($Z = -2.409, P = 0.008$). This significant combined result was generated by the individual colony-level *P* values, which were frequently less than 0.5 (Fig. 2). In contrast, *P* values are expected to fall above or below 0.5 with equal frequency if the null hypothesis is correct (Whitlock 2005).

We next investigated if the sperm from particular males showed evidence of being more persistent than that of others by determining if colonies showed decreasing effective paternity (k_{e3}) over time. However, correlations between k_{e3} and relative age of different life stages were positive in only five colonies and negative in seven colonies. Thus, even though there was significant variation in sperm use by queens over time, there was no significant evidence of trends in effective paternity in different life stages (binomial test, $P > 0.05$).

Allocation to gynes and males

We used data collected in this study to determine if queen effective mate number (k_{e3}) was predictive of queen cell number in queenright, mature colonies (those that contained adult sexuals; Goodisman *et al.* 2007b). Consistent with prior results, we found no correlation between k_{e3} and number of worker cells ($r_S = 0.250, P = 0.589$). However, in contrast to previous results, we also failed to uncover a significant correlation between k_{e3} and number of queen cells in these colonies ($r_S = -0.071, P = 0.879$).

We then turned toward investigating factors that affected investment in adult sexuals. In general, queenright colonies produced more males than gynes (Table 1). However, there was considerable range in the sex ratios produced, with the proportion of gynes (= total number of gynes/total number of sexuals) ranging from 0.092 to 0.381 in queenright colonies that produced both sexes. This numerical investment in males is typical for this group of wasps (MacDonald & Matthews 1981; Greene 1991). Regardless, gynes were more massive than males (ratio of gyne to male mass varied between 1.87 and 3.05 with a mean of 2.39). Thus, the mass ratios (total mass of gynes/ total mass of all sexuals) produced by queenright colonies varied from a low of 0.208 to a high of 0.593.

We used logistic regression to determine which colony variables aided in predicting colony numerical sex ratio (Boomsma & Nachman 2002). We found that a subset of the potential parameters were able to significantly predict colony sex ratio (Table 3, $P < 0.0001$). In particular, elimination of nonsignificant parameters ultimately revealed that worker relatedness (r_{WW}) and number of male mates of queens (k) were both significantly associated with sex ratio (logistic model parameters: intercept $= 1.467$, $P < 0.0001$; $r_{WW} = -5.668, P < 0.0001$; $k = -0.110, P = 0.0003$). Thus, sex ratio in this population was predicted by the equation $\pi = e^x/(1 + e^x)$ where $x = 1.467 - 5.668r_{WW} - 0.110k$.

We next turned our attention to the question of whether the overall population sex investment ratio (cf. sex ratio above) produced by queenright colonies differed significantly from expectations under worker or queen control of investment. We found the mean relatedness of workers

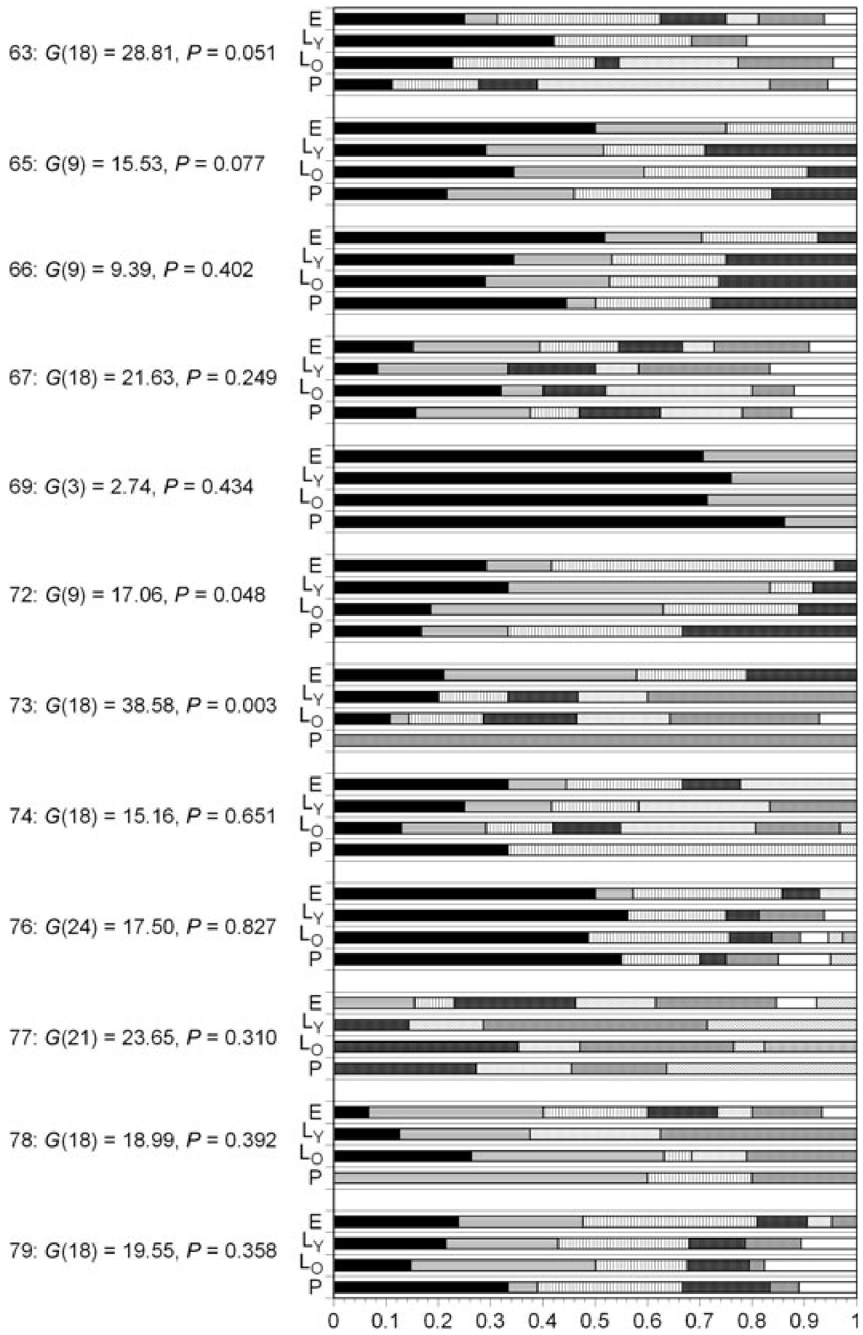

Fig. 2 Distribution of male patrilines among workers in four life stages (E, egg; L_Y, young larva; L_O, old larva; P, pupa) in 12 *Vespula maculifrons* colonies. Different shading represents different patrilines within colonies. *G*-tests of heterogeneity (with degrees of freedom) and associated *P* values provide the statistical significance of variation in contributions of males to workers. Overall, males showed weak but significant variation in sperm use over time.

and mean number of male mates of queens in queenright colonies was $r_{WW} = 0.329$ and 7.00, respectively. The expected proportion of gynes in the population, obtained by fitting the mean values into the regression model, thus equals 0.244 ± 0.088 (± SEM). In contrast, the optimal sex investment in gynes under queen and worker control given the dry mass ratio of $c = 2.39$ and the mean optimal energetic allocation to females ($o = 0.5$ and $o = 0.561$ for queen and worker control) were found to be 0.295 and 0.348, respectively. Neither of these estimates differed from the model predicted values for queen and worker control ($t_4 = -0.59$, $P = 0.592$ and $t_4 = -1.19$, $P = 0.300$, respectively).

Discussion

Allocation to growth and reproduction

Vespula maculifrons colonies displayed at least partially graded control of reproduction. That is, colonies sampled relatively early in the season appeared to be gradually

Table 3 Observed and expected proportions of gynes in seven *Vespula maculifrons* colonies. Expected sex ratios were obtained from logistic regression with significant explanatory parameters of worker relatedness (r_{WW}) and number of male mates of queens (k). The expected proportion of gynes in each colony was calculated as $\pi = e^x/(1 + e^x)$ where $x = 1.467 - 5.668r_{WW} - 0.110k$. The Wald 95% confidence intervals for r_{WW} and k were −6.749 to −4.587 and −0.152 to −0.068, respectively

	Model parameters		Proportion of gynes	
Colony	r_{WW}	k	Observed	Expected
72	0.271	4	0.381	0.376
73	0.297	7	0.252	0.271
74	0.291	7	0.302	0.278
76	0.324	9	0.207	0.205
77	0.349	8	0.208	0.199
78	0.518	7	0.092	0.096

increasing their allocation to reproduction and away from growth, rather than doing so abruptly (Fig. 1). Moreover, colonies collected late in the season also produced a substantial fraction of workers. Thus, colonies do not reproduce according to a strict bang-bang model of reproduction, but instead follow more of a 'bang-sputter' model, whereby allocation to reproduction increases nonmonotonically after a defined switch point away from exclusive allocation to growth.

Theoretical results suggest that graded reproduction is adaptive if the length of the growing season shows substantial variation (King & Roughgarden 1982a; Mitesser *et al*. 2007a). The growing season may indeed be highly variable from the viewpoint of a *V. maculifrons* colony, because the onset of unfavourable conditions likely varies from year to year. In addition, graded control may arise if successful investment in reproduction requires co-investment in growth (Kozlowski 1992; Beekman *et al*. 1998). This seems plausible in *V. maculifrons* and other social insects, because large worker populations may be required to rear developing gynes and males (MacDonald & Matthews 1981; Greene 1984).

In addition to discovering graded reproduction within colonies, we also found that colonies showed unexpected patterns of investment in male production. In particular, colonies collected late in the season showed substantial variation in male production over time and thus did not appear to produce males in a monotonically increasing pattern (Fig. 1). The proportion of worker cells dedicated to male production was particularly low among old larvae in these late colonies. It is possible that these patterns arise because males spend little time in the late larval stage relative to workers. However, nests collected early in the season did not display frequencies of males among old larvae that were out of line with the trends in the other life stages (Fig. 1). Alternatively, the data may indicate that reversions in allocation occur frequently and abruptly in *V. maculifrons*.

We note that we cannot be certain that such putative switches from rearing males to rearing workers reflects an overall change in colony level allocation from reproduction to growth, because we did not monitor allocation to gynes. Thus, it is possible that colonies that transition away from male production actually invest particularly heavily in gyne production at the same time, thereby leading to an overall high level of allocation to reproduction. Regardless, reversions from reproduction back to growth are only expected to be favoured under restrictive conditions (Mitesser *et al*. 2007a). For example, selection may favour reversions if major losses in growth components occur during the season (King & Roughgarden 1982b). In addition, social insect colonies are predicted to change their allocation away from producing males to producing gynes late in the reproductive season if survival and mating opportunities of gynes and males vary over time (Bulmer 1983). The putative overall drop in male production we observed in late colonies may reflect such adaptive changes in sex ratio.

Comprehensive studies of allocation have been conducted in few other annual, social insect taxa. A notable exception is the bumblebee *Bombus*, which has been shown to display graded control of reproduction (Muller *et al*. 1992), as is the case in *Vespula*. In addition, considerable interest has centred on the timing of the switch from worker production to sexual production in *Bombus.* Data suggest that this switch point is dependent on the condition of the queen and is indirectly related to reproductive competition between the queen and her worker offspring (Bourke & Ratnieks 2001; Duchateau *et al*. 2004; Alaux *et al*. 2005). In *V. maculifrons*, however, there is no evidence that there is direct competition between the queen and her workers for reproduction (Kovacs & Goodisman 2007). Thus, it seems unlikely that the timing of switching from growth to reproduction is affected by the same types of selective factors that affect *Bombus*.

Allocation by male mates of the queen

Multiple mating by queens may lead to unequal reproductive success of males either through sperm competition or female choice after sperm storage. Surprisingly, we found evidence for variation in sperm use over time (Fig. 2). The variation within colonies was generally weak, but our large sample sizes allowed us to detect an overall significant trend. Interestingly, a previous study of *V. maculifrons* failed to detect evidence of variation in sperm use over time (Ross 1986). However, fewer colonies were examined in that study, so it is possible that the subtle variation observed here was not detected.

Our study is not the first to document variation in sperm use by queens over time. Such variation has been noted in some studies of *Apis* honeybees (Franck *et al*. 1999, 2002) but not in others (Estoup *et al*. 1994; Tilley & Oldroyd 1997). In addition, some ant queens have been found to vary in how they use sperm over the course of sufficiently long sampling periods (Keller *et al*. 1997; Sundström & Boomsma 2000).

The observed variation in sperm use by *V. maculifrons* males, although statistically significant, would seem to have little effect on colony function. Indeed, we found no evidence of trends in effective paternity over time. Thus, variation in sperm use was subtle and did not lead to major changes in colony genetic structure. In addition, a previous study in this taxon failed to find differences in the success of males contributing to adult gyne and adult worker production (Goodisman *et al*. 2007a). Consequently, modest variation in sperm use by *V. maculifrons* queens may result from minor sperm clumping that occurs in the queen reproductive system (Franck *et al*. 2002). This is unlikely to greatly affect the ecology or evolution of *V. maculifrons*, as may be the case in some other social insect taxa (Sundström & Boomsma 2000).

Allocation to gynes and males

The investment of social insect colonies into gynes and males can be affected by a variety of factors (Bourke & Franks 1995; Crozier & Pamilo 1996; Chapuisat & Keller 1999; Bourke & Ratnieks 2001). However, simple expectations suggest that colonies should invest in gynes and males according to the relationships of individuals within colonies. In particular, colonies are expected to produce female-biased sex investment ratios, with the magnitude of female bias decreasing with increasing queen mate number, if workers control sex allocation. In contrast, sex investment should be equal if queens control investment in sexuals.

We found that the observed population mean investment in gynes did not differ significantly from the queen or worker optimum. This result was not surprising given that the two optima were quite close, because *V. maculifrons* queens sampled in this study mated many times. Nevertheless, our data do suggest that relationships within colonies may partially govern population level sex investment ratios.

We then turned our attention to the more central question of whether sex ratio was associated with relatedness among workers within individual colonies. Remarkably, we found that worker relatedness and queen mate number provided substantial power in predicting sex ratio within colonies (Table 3). Interestingly, the relationships between the predictive parameters to each other and to predicted sex ratio were unexpected. In particular, worker relatedness is generally expected to be negatively correlated with number of mates of the queen. However, these two variables were positively, although nonsignificantly, correlated in our data set ($r_S = 0.532, P = 0.219$). This lack of correlation may have resulted from the fact that the range in queen mate number was rather narrow and males show substantial variation in reproductive success within colonies. Thus, the relationship between worker relatedness and mate number is likely to be noisy.

Regardless, we found that worker relatedness was negatively associated with investment in gynes in opposition to simple expectations based on kin selection theory (Fig. 3). Queen mate number was also negatively associated with investment in gynes (Fig. 3). However, this latter relationship is predicted under simple theoretical expectations. Thus, our results displayed unusual and contrasting signals arising from multiple mating by queens.

We note that much of the signal in our model arose from a single colony (#78) that showed a strongly male-biased sex ratio and unusually high relatedness, even though the colony was headed by a queen that mated many times (Tables 1 and 3). These peculiar characteristics resulted, in part, from the fact that the queen heading the colony was homozygous at three loci. High homozygosity may indicate that this queen was produced by an inbreeding event, thereby leading to high relatedness among her offspring. Regardless, even when we removed this colony from the analysis, a logistic model still provided significant explanatory power ($P < 0.0001$), and both mate number and worker relatedness remained as significant parameters in the model ($P = 0.027$ and $P = 0.001$, respectively). Consequently, our data suggest that relationships within colonies do influence sex ratio.

In addition to directly investigating colony sex ratio, we also tested for the presence of a previously noted positive association between number of queen cells and queen effective mate number in *V. maculifrons* colonies (Goodisman *et al*. 2007b). However, we failed to detect this trend in our current study. Consequently, the association between worker relatedness and queen cells may vary by year or be affected by more complex factors. Regardless, we note that in the 2007 study, colonies in which worker relatedness was relatively low were predicted to produce more gynes than colonies in which worker relatedness was high. Thus, this previously published study also suggested that there might be a negative association between worker relatedness and proportional investment in new gynes.

Determining the causes of the association between colony genetic diversity and sex ratio are somewhat complicated because of the unusual relationship between queen mate number and worker relatedness. Nevertheless, if we assume that the relationship results from cues based on levels of genetic variation within colonies, then the observed trend in investment preference may be derived from some advantage arising from genetic diversity obtained through

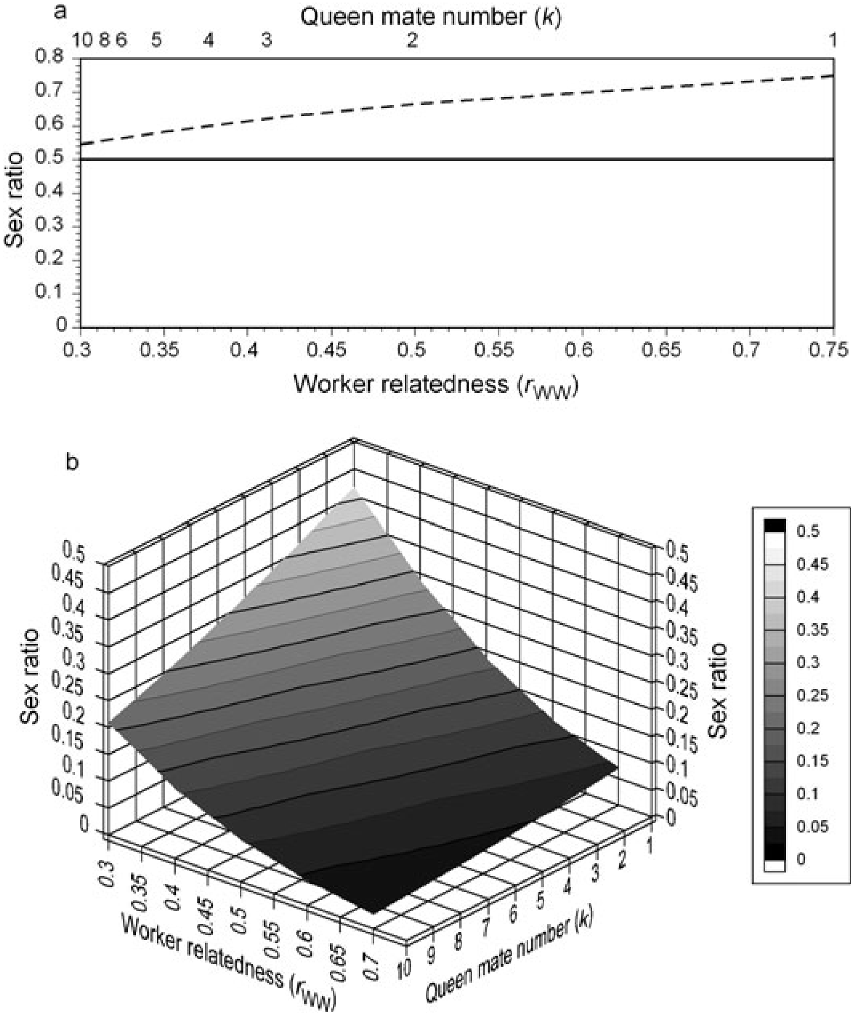

Fig. 3 (a) Optimal colony sex ratio [(number of gynes)/(number of gynes + males)] for queens (solid line) and workers (dashed line) as a function of worker relatedness (r_{WW}) or queen mate number (k). In this theoretical scenario, effective mate number equals actual mate number, the cost of producing both sexes is the same, and $r_{WW} = 0.25 + 0.5(1/k)$. (b) Predicted colony sex ratio in *Vespula maculifrons*, given by $\pi = e^x/(1 + e^x)$ where $x = 1.467 - 5.668r_{WW} - 0.110k$. In contrast to theoretical expectations, r_{WW} is uncorrelated to k and negatively correlated with sex ratio (see text for details).

multiple mating by queens. Indeed, polyandry by social insect queens has been linked to a great variety of potential benefits (Boomsma & Ratnieks 1996; Crozier & Fjerdingstad 2001; Strassmann 2001). In *V. maculifrons*, colonies with high levels of genetic diversity may be able to produce gynes that are more fit than gynes produced by colonies with low levels of genetic diversity. Thus, it is possible that highly genetically diverse colonies may seek to invest heavily in gyne, vs. male, production. This scenario would represent an extraordinary advantage arising from multiple mating, but significant additional testing is needed to confirm this hypothesis.

Conclusions

The reproductive decisions faced by colonies of social insects involve the coordination of individuals with different interests and the consideration of environmental variation that may also affect reproductive options. This investigation provides insight into the ways in which allocation to reproduction are affected by mating behaviours and relationships within colonies. Our research suggests that reproductive decisions of colonies of the social wasp *Vespula maculifrons* involve the suppression of overt reproductive conflict because workers do not compete with their queen mother directly for reproduction or indirectly for control of sex investment ratio. In addition, the male mates of queens apparently do not actively compete for reproductive success after mating has occurred. Instead, colonies apparently alter allocation patterns by readily modifying their investment in growth to augment their reproductive output. Colonies may also modify their investment in the sexes to accommodate potential genetic benefits. Thus, queenright *V. maculifrons* colonies appear to act as highly coordinated groups whose reproductive decisions rely on both environmental cues originating from outside the colony and genetic cues arising from genetic diversity levels of the group.

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Research in the Goodisman laboratory focuses on understanding how evolutionary processes affect social systems and how sociality, in turn, affects the course of evolution. The main subjects of our research are the social insects, which display the most advanced levels of sociality and dominate ecological communities because of their sophisticated cooperative and helping behaviours. E.L.J., T.W.C., S.M.M., and D.B.B. are undergraduate students, and J.L.K. and B.G.H. are PhD students, studying social insect ecology, evolution, behaviour, genetics, and development in the Goodisman laboratory.