

Research article

**Mediators of dominance and reproductive success
among queens in the cyclically polygynous
Neotropical bumble bee *Bombus atratus* Franklin**

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Summary

Bombus atratus, a bumble bee found throughout much of South America, undergoes periods of polygyny during its colony cycle, which is often perennial. This temporary polygynous phase persists as multiple queens compete fiercely to become the sole reproductive. The outcome of these battles is death or expulsion from the nest of all but one of the queens, representing an extreme form of conflict of reproductive interests. We examined various factors which may influence survival and succession of rival *B. atratus* queens during a polygynous phase of a colony found in southern Brasil. Queens exhibiting greater ovarian development and a higher frequency of inter-queen aggression in the early period of polygyny survived significantly longer. Body size did not appear to contribute to the outcome of queen survival.

Introduction

Social Hymenoptera (ants, bees, and wasps) display considerable variation in the number of queens present in a colony. Most Neotropical social wasps and many ants form colonies with multiple reproductive queens (polygyny) which establish a colony together (reviewed in Reeve, 1991 for *Polistes*; Jeanne, 1991 for swarm-founding Polistinae; Keller, 1995 for ants). Queen number is rarely permanent within a colony, fluctuating from low to high or high to low in many ants (Keller, 1995), and it may be cyclical in many of the polistine wasps. In cyclical polygyny, a colony may fluctuate between large and small numbers of reproductive queens within a cycle (Strassmann et al., 1992), or may cyclically return to a single queen state (cyclical or secondary monogyny) (West-Eberhard, 1978). Polygyny may provide a survivorship advantage to colonies during the period when they are most at risk (Strassmann, 1981; Queller and Strassmann, 1988; Reeve, 1991), but once a colony is established,

a shift to one or a few queens can increase worker-to-brood relatedness (Strassmann et al., 1992), thought to be an important factor in the maintenance of sociality in polygynous wasps (Queller et al., 1994).

Social dominance hierarchies often develop in polygynous colonies (Pardi, 1942; Jeanne, 1991), reflected by agonistic interactions among the multiple queens. Social dominance usually leads to reproductive dominance, with individuals high on the dominance scale holding exclusive access to ovipositions, while subordinate queens become the physiological equivalent of workers or are driven from the nest (Pardi, 1948; West-Eberhard, 1969; Packer, 1993). Dominant queens may exhibit greater ovarian development (Pardi, 1948; Queller and Strassmann, 1989) and may be larger than subordinate females (Pardi, 1948; Sullivan and Strassmann, 1984). A reproductive hierarchy of this nature should tend to favor the behavioral and morphological attributes of successful competitors as the colony shifts from polygyny to monogyny.

In contrast to ants and wasps, bees rarely exhibit polygyny. Polygyny has been found in communal or normally solitary species of Xylocopinae (Schwarz, 1986; Sakagami and Maeta, 1984), and some species of eusocial Halictinae initiate multiple-foundress nests governed by size-based dominance hierarchies (Packer, 1993). In the family Apidae (sensu Roig-Alsina and Michener, 1993), which includes the eusocial honey bees, stingless bees, and bumble bees, polygyny is especially rare. Zucchi (1973) was the first to report polygyny in a bumble bee, observed in the Neotropical species *Bombus atratus* Franklin. In southern Brasil where the winter season is mild enough for perennial foraging and nest initiation, *B. atratus* gynes do not enter diapause (Sakagami, 1976). Instead, they mate and initiate new nests alone (Cameron and Jost, pers. obs.), while a fraction of inseminated gynes may return to the natal nest to begin a new colony cycle when the old queen dies (Zucchi, 1973). A nest may continue for many generations in this fashion (Zucchi, 1973). Zucchi observed a single laboratory colony over a ten-year period, recording repeated phases of polygyny and high levels of antagonism among multiple sister queens which ultimately led to colony monogyny after the death or expulsion of all but one of the queens (Zucchi, 1973). Zucchi suggested that emerging queens leave the nest, mate, and return to the colony to compete for oviposition territories.

Because Zucchi's conclusions regarding the polygynous behavior of *B. atratus* were based on a single colony, we feel it worthwhile to communicate our observations resulting from the study of another colony of the same species. We verify Zucchi's observations of cyclical polygyny and queen aggression, and address a question which has not been examined directly – whether variation in queen phenotypes may be related to a young queen's chances of succession within a highly competitive polygynous colony.

Methods

We observed and documented agonistic interactions between nestmate queens during a polygynous phase of a field-collected *B. atratus* colony. We then tested whether a queen's survival time (a potential measure of her own dominance and/or reproductive success) can be predicted by body size, insemination, ovarian development, territoriality, oviposition, or agonistic interactions, factors associated with

reproductive dominance and establishment of dominance hierarchies in other social insects (Pardi, 1948; Queller and Strassmann, 1989; Sullivan and Strassmann, 1984; Packer, 1993). Our study is based on the examination of a single colony and therefore our conclusions may not apply to all colonies of *B. atratus*.

Colony collection

We collected an underground nest of *B. atratus* from its natural location on the University of São Paulo campus in Ribeirão Preto, Brasil (21°09'S, 47°48'W), 14 October 1993, at the onset of the rainy season (usually Sept.–March, with the heaviest rains Dec.–Feb.). The nest entrance was 1.5 m below the top of a 5-m high vertical embankment, hidden under grassy vegetation on a well-drained, shaded northwest-facing slope. The nest cavity was located approximately 1 m below ground. We began excavation at 06.00 hours, and because of the reputed aggressive behavior of *B. atratus* (Sakagami et al., 1967; Sakagami, 1976), we infused the nest with CO₂ to anesthetize the bees prior to digging. We transferred the colony to an observation laboratory that allowed free flight outside, and recorded the total number of adults, their caste, and sex. All adults were kept cool in large glass flasks while we inventoried the brood clump. No wax canopy (involucrum) surrounded the brood clump, which facilitated the counting of brood cells. We counted all pupal cocoons, larvae, egg cells, and food-storage vessels (honey and pollen pots), and discarded old, moldy brood comb after categorizing it and counting the empty cells. We then re-established the brood, workers and queens in a wooden observation box (inner dimensions 30 by 20 by 8 cm), and provided the colony with pollen collected from *Apis* colonies and 50% sucrose solution. The colony was left undisturbed for 44 h before further study.

Observation procedures

To identify individual queens, which in *B. atratus* are approximately two to three times the mass of workers, we tagged each on the thoracic dorsum with differently numbered plastic disks (Opalithplättchen) 24 h after collection. Queens were tagged without chemical anesthetization. Instead, they were placed briefly at 4°C to reduce activity, tagged, returned within 20 min of removal from the nest, and left to recover until the following day. Of a total of 14 queens, we tagged ten (#s 01, 02, 03, 05, 06, 07, 08, 10, 11, 12), the remaining 4 queens stayed hidden beneath the comb and were left untagged. As each untagged queen was later encountered during the course of the study, either dead or, by process of elimination, determined to have abandoned the colony, she was recorded sequentially as untagged (UA-UD).

We began experimental observations on the morning of the second day after colony collection, when the colony appeared to return to normal activity. On the third day the colony was opened to the outside, permitting free traffic to and from the nest. A queen excluder was not used during the experiment, allowing movement of the queens in and out of the nest. We considered that the preservation of relatively natural conditions (i.e., not having an excluder) was more important than

the potential departure of queens. The colony appeared to return to normal activity after its re-establishment. Twice daily (a.m. and p.m.) we observed the colony for 2-hour periods. During each period we recorded dead or missing queens, and the frequency with which queens exhibited the following types of agonistic behavior: (1) lunge (rapid, darting movement in the direction of a queen), (2) retreat (rapid movement away from a lunging queen), (3) grapple (two queens locked in combat, often with one or both attempting to sting its opponent through an intersternal abdominal membrane), (4) sting (penetration of the stinger into an opponent, resulting in death), and (5) abandonment (permanent departure from the nest). It was possible to record all such interactions during an observation period because they took place in the open on the upper surface of the brood comb or observation box, and were infrequent enough so as not to be simultaneous. Queens killed during agonistic encounters were removed and dissected immediately or frozen (-20°C) for later dissection. Throughout the study, following the method of Zucchi (1973), we made daily maps of the relative position, size and shape of each queen's territory by ascertaining the boundaries of the total area each queen patrolled during each day's observations. When aggressive interactions occurred between queens, they usually occurred near the peripheral areas of territories. Often, a territory corresponded to a discrete clump of brood on which a queen remained. We also recorded the presence of egg cells. Twice-daily observations continued until only one queen remained in the colony. Thereafter, we scanned the colony every other day to document new egg cells and the emergence of the first brood of workers.

Queen measurements

We used a dissecting microscope (Wild M5, fitted with an ocular micrometer for scale) to measure relative body size, ovarian development and insemination of each deceased queen. Body size was measured as the distance between the insertion points of the forewings (intertegular width) (Cane, 1987). We examined spermathecae and ovaries by removing tergites 2–6 with optical scissors, and measured three features reflecting reproductive status: (a) *Insemination*: determined by the presence of an opaque, pearl-like spermatophore, encased in the translucent spermatheca; (b) *Number of mature oocytes undergoing resorption*: examination of all ovarioles (4 per ovary, 8 total) for resorbed oocytes, identified by the presence of yellowish, irregular-shaped oval bodies near the proximal end of the ovarioles. We tallied the total number of eggs undergoing resorption and used the sum as the index of resorption (IR) (Duchateau and Velthuis, 1989), and (c) *Stage of maturity of the ovaries*. We assigned a score (1–4) to oocytes in each ovariole using modifications of the method of Duchateau and Velthuis (1989) for *Bombus terrestris* (Table 1). The oocyte scores were used to assess the overall maturity of each ovary. We assigned a score (1–5) to each ovary independently and summed the scores for both ovaries as an index of ovarian development (IO) (Table 1).

Voucher specimens from the *B. atratus* colony are deposited in the Arthropod Museum at the University of Arkansas, Fayetteville.

Table 1. Characterization of oocytes and ovaries used for scoring ovarian development of *B. atratus* queens (after Duchateau and Velthuis, 1989)

<i>Most advanced oocyte score</i>	<i>Oocyte description</i>
4	mature, well-formed, with a visible chorion
3	somewhat smaller than (4) but larger than adjacent trophocytes
2	of proper shape but equal to or smaller than trophocytes
1	anything less than (2) in development
<i>Ovarian score</i>	<i>Ovarian description</i>
5	each ovariole contains at least two eggs of type (4)
4	each ovariole contains at least one egg of type (4) with second eggs of at least type (2)
3	at least 2 separate ovarioles contain an egg of type (4)
2	at least one egg of type (4) exists in the ovary
1	oocytes present but ovarioles clearly underdeveloped

Statistical analyses

We used the LIFEREG procedure implemented in SAS, version 6.09 (SAS Institute 1988) to examine the effects of (1) body size, (2) presence or absence of sperm in the spermatheca, (3) ovarian development (IO), (4) presence or absence of a territory, (5) presence or absence of egg cells on the territory, and (6) frequency of agonistic encounters on queen survival time (the response variable). The LIFEREG procedure is designed to fit a parametric, linear effects model to survival (or failure-time) data. We tested different models for queen survival using different combinations of the above six covariables together with a random error term taken from a Weibull distribution (SAS Institute, 1988). In accordance with the Weibull distribution, survival values were log transformed. For queens that died during the first 48 h of observations (median survivorship time), we compared mean body size, ovarian development score, and frequency of aggression with queens that lived longer than 48 hours, using two-tailed Mann-Whitney U-tests, $\alpha = 0.05$ (Wardlaw, 1985). Short- and long-lived queens were also compared for significant differences in the proportion that were inseminated, had territories, and laid eggs, using one-tailed 2×2 contingency tests for small groups, $\alpha = 0.05$ (Finney et al., 1963).

Results

Phase of colony life cycle

At the time of collection, the polygynous colony was producing only males. This is characteristic of a late or post-reproductive phase typical of many social Hymenoptera (Zucchi, 1973; Reeve, 1991). There was no sign of an old tattered queen, denoting a parental queen, but there were 50 empty gyne cocoons and 14 young, potentially reproductive queens (gynes), determined by their size, brightness and

density of the pubescence (pile), absence of wing wear, and behavior (described below). It is likely that these queens were sisters based on observations by Zucchi (1973), who repeatedly observed that a fraction of sib queens remained in the natal nest of *B. atratus* following a reproductive phase. Furthermore, they were probably full sisters as *B. atratus* queens are reported to mate only once (Garófalo et al., 1986). The colony also contained 120 workers, not including at least 50 that escaped during excavation, and 102 males. From 15–20 additional males emerged daily over the ten-day period following collection, but no new workers emerged from cells present at the time of colony collection. There were 3 egg cells, each containing 8–10 eggs. We estimate that during the extant generation the colony produced a minimum of 550 offspring, 350 of which were reproductives (50 queens and 300 males). This estimate does not account for an indeterminate number of previously emerged worker offspring that could not be counted because some cocoons were in a state of decay from fungi and wax moths (Pylalidae). A significant number of wax moth larvae and adults and accompanying wax moth parasitoids (*Apanteles* sp.) (J.B. Whitfield, pers. comm.; see Whitfield and Cameron, 1993 for biology) were present in the colony at the time of collection.

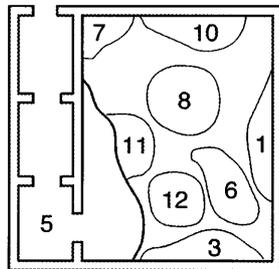
Based on these observations and previous colony size records for *B. atratus* (Sakagami, 1976), this was a medium-size colony which was likely to have been relatively old. However, the large number of active workers indicated that the colony was not in decline; throughout the experimental period, they engaged in the routine activities of foraging, nursing and guarding.

Queen-queen interactions

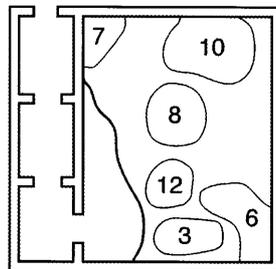
Within ten hours of colony collection and re-establishment in the laboratory, several queens (at least #s 03, 06, 08) were observed guarding distinct portions of the brood clump (territories): Perched on their territories, queens lunged at intruder queens whenever they encroached on their boundaries. Other queens remained on the periphery of the brood comb (#s 01, 11) or were out of sight under the comb.

When experimental observations were initiated thirty-six hours later, individual territories had been established by nine of the ten tagged queens (Fig. 1, Day 1). Each territory comprised a small area of brood comb (5–7 cm diam.), and five of them had one or two egg cells (#s 05, 07, 08, 10, 11). A tenth tagged queen (02) had been stung just prior to the first observation period and was being dragged from the brood comb by queen 10. Untagged queen UA was found dismembered and a second (UD) was never recovered during the experiment, presumably having abandoned the nest. The remaining two untagged queens (UB and UC) were beneath the brood clump and not visible during any interactions.

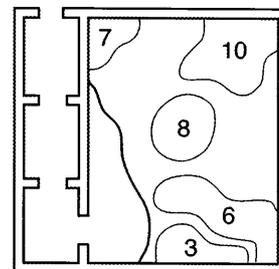
Between days 1 and 15, agonistic interactions among the queens resulted in the death or expulsion of all but a single queen (06). No agonism occurred between queens and workers, or among workers, observations which are in accord with those of Zucchi (1973). A daily account of physical changes in queen territories and behavioral interactions is summarized in Figure 1. Several instances of territorial expansion were observed when a queen extended her territory to absorb that of a deceased neighbor (e.g., days 3 and 6, Fig. 1). Bouts of agonism were most severe



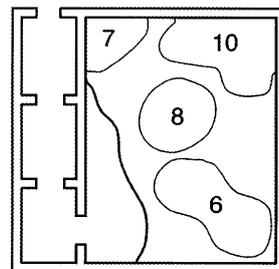
Day 1: Nine of the ten marked queens had established territories. Q-10 was observed dragging the dead Q-02 off the comb, and Q-08 was observed stinging and killing Q-11. Q-05 maintained a territory in the entrance tunnel. Q-01 was missing (found dead the next day).



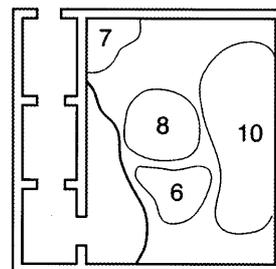
Day 2: The nest was opened to the outside. Q-12 received aggression from queens 03, 06, 07, and 10. Q-12 died. Q-UB was also found dead and Q-05 abandoned the nest.



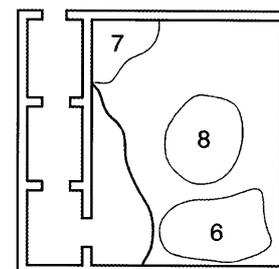
Day 3: Q-06 absorbed the territory of the dead Q-12, and Q-03 was found dead.



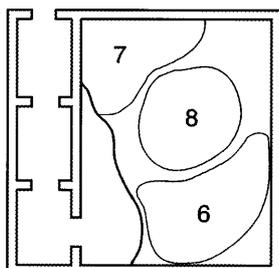
Day 4: Q-10 began to enlarge her territory along the vacant side of the observation box.



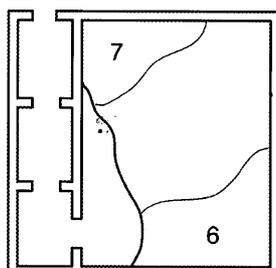
Day 5: Q-10's territory continued its rotation around the nest, forcing Q-06 into a smaller brooding area. Unmarked Q-UC was found dead in the entrance tunnel.



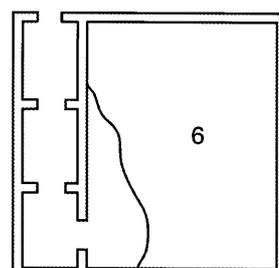
Day 6: Q-10 was found dead in the morning, perhaps from conflict with Q-06, who reclaimed her former territory. The nest continued with three queens until day 13.



Days 7-11: The territories of queens 06, 07, and 08 expanded, dividing the nest into approximate thirds. By day 11, several new egg cells had been constructed.



Day 12: A grappling fight was observed between queens 07 and 08 on their territorial boundaries, lasting over one h. Retreat was simultaneous. On day 13, Q-08 was found dead under the comb.



Day 15: Q-07 abandoned the nest. Q-06 became the single monogynous queen, and produced a first brood of adult workers 20 days later (day 35).

Figure 1. Daily territorial maps denoting physical changes in queen territories and behavioral interactions during the polygynous period

during the first two days of observations: within the first 24 h-period, three agonistic interactions were recorded and three queens were dead, and during the second 24-h period, nine agonistic events were recorded, two more queens found dead and one abandoned the nest. Thus, half of the queens were eliminated within the first two days. Over the succeeding 12.5 days, four more queens were stung (and killed) and another abandoned the nest. Thus by day 14.5 the colony had become monogynous.

Predictors of queen survival

The six variables we measured as potentially significant factors in deciding the outcome of survival among the interacting queens appear in Table 2. Queens are listed in ascending order of survival time, with queen 06 ultimately superseding all other queens to become the sole reproductive in the colony. LIFEREG analysis revealed that frequencies of agonistic interactions (ST, LU and RE) during the first few days, and degree of ovarian development, were significant indicators of queen survival ($p = 0.0001$), respectively; Table 3). Ovarian development alone could not predict queen survival. Specifically, queens that exhibited more stinging and lunging enjoyed greater survival. Those that retreated when encountered had the lowest survivorship. No other variables, including body size, could significantly predict survival time.

Six queens died within the first 48 h of observations (Table 2). These queens did not differ significantly in body size ($\bar{X} \pm SD = 6.8 \pm 0.35$ mm) from the remaining longer-lived queens (6.98 ± 0.26 mm; Mann-Whitney $U = 6.5$, $p > 0.05$), nor in the proportion that were inseminated (4/5 in both groups), proportion that acquired territories (4/5 versus 5/6, contingency test, $p > 0.05$), proportion with egg cells (2/5 versus 5/6, contingency test, $p > 0.05$), or ovarian development ($\bar{X} \pm SD = 4.2 \pm 2.2$ versus 6.97 ± 2.2 mm; Mann-Whitney $U = 5.5$, $p > 0.05$). However, queens that died within the first 48 h exhibited significantly less aggression during that time period ($\bar{X} \pm SD = 0 \pm 0$) than longer surviving queens during the same time period (1.6 ± 0.89 ; Mann-Whitney $U = 3$, $p < 0.05$).

Queens 10, 08, 07, and 06 (Table 2) exhibited (1) a high frequency of aggressive (LU, ST) interactions, including the stinging of other queens, and (2) no submissive behavior (retreat or hide) during the first 48 h, or thereafter (Table 2). These queens also lived longest. Queens 01, 11 and 12 exhibited (1) retreat responses in the vicinity of, or when challenged head on by other queens and (2) no observed aggressive behavior toward other queens. These were among the shortest-lived queens.

Differential reproductive success

Nineteen days after colony succession by queen 06, five first brood workers emerged from the brood clump she had been guarding throughout the study. These offspring exhibited the normal activities of bumble bee workers (Cameron, 1989), and the queen continued to maintain her growing brood. No offspring emerged

Table 2. Summary of data collected for each *B. atratus* queen observed during a fifteen-day period when the colony was polygynous. *No. days survived* (the response variable) is inferred as a measure of queen dominance. The remaining variables were tested for their power to predict queen dominance. The types of agonistic interactions under column (6) are RE (retreat), LU (lunge), and ST (sting). In the body of the table, d = death, a = abandonment, s = survives, m = missing data due to queen abandonment, except queen 06 who was left intact to keep the colony alive, + = yes, and - = no

Queen in order of expiration	No. days survived	Outcome	(1) Body size intertegular width (mm)	(2) Inseminated	(3) Ovarian development (IO) (IR)		(4) Territory	(5) Presence of egg cells	(6) Frequency of agonistic interactions during first 48 h			
									RE	LU	ST	
24 h	02	< 0.5	d	6.6	+	2	0	-	-			
	01	0.5	d	7.0	-	2	0	+	-	1		
	11	1.0	d	7.3	+	7	6	+	+	1		
48 h	12	1.5	d	6.4	+	5	0	+	-	2		
	05	1.5	a	m	m	m	m	+	+			
	UB	2.0	d	6.7	+	5	3	-	-			
> 48	03	3.0	d	6.8	+	8	4	+	+	1		
	UC	5.0	d	6.7	-	4	3	-	-			
	10	5.5	d	7.2	+	9	7	+	+			1
	08	12.5	d	7.2	+	7	5	+	+	1		1
	07	14.5	a	m	m	m	m	+	+			1
06	> 14.5	s	m	+	m	m	+	+	2		1	

Table 3. Summary of LIFEREG analysis for the effects of frequency of agonistic encounters (sting, lunge, retreat) within the first 48 h and ovarian development (IO) on the likelihood of *B. atratus* queen survival. m = missing value

Variable	df	Estimate	Standard error of the estimate	Chi Square	P value
Sting	1	1.5868	0.1111	203.85	0.0001
Lunge	1	1.1009	0.0984	125.03	0.0001
Retreat	1	0.3637	0.0471	59.59	0.0001
IO	1	0.1202	0.0201	35.53	0.0001

Bee No.	Observed (O) survival (days)	Expected (E) survival (days)	Residual (O-E)	Error sum of squares
02	0.5	0.46	0.038	0.00149
01	0.5	0.66	-0.164	0.02684
11	1.0	1.21	-0.211	0.04448
12	1.5	1.37	0.130	0.01697
05	1.5	m		
03	3.0	2.85	0.146	0.02124
10	5.5	5.23	0.267	0.07138
08	12.5	12.37	0.128	0.01642
07	14.5	m		
06	15.0	m		
				0.19881

from brood clumps guarded by any other queens for as queens died, their brood clumps were left untended by workers and quickly deteriorated.

Discussion

The structure of queen interactions

Our observations of the reproductive life-cycle of *B. atratus* are fully concordant with observations made by Zucchi (1973, his Figs. 13, 14) on a single colony over a ten-year period. Zucchi observed that the alternation of monogynous and polygynous phases occurred two to three times a year in his laboratory colony, and that the polygynous phase may persist for several months, although the duration was highly variable (several weeks to six months). In a summary of Zucchi's unpublished (thesis) investigation, Sakagami (1976) illustrated a polygynous phase lasting 56 days, encompassing 17 queens. Several of the queens held conspicuous territories throughout the period of rivalry until one succeeded in taking over the colony. In our study, we witnessed a period of rivalry lasting only two weeks, suggesting that the colony may have been well into a polygynous period when it was collected, or that the polygynous phase was shorter than average.

Our results augment those of Zucchi (1973) by examining likely factors leading to succession of a rival queen. In our study, dominance interactions governed the

survival outcome of young queens. Individuals comparable to Zucchi's persistent queens (Sakagami, 1976) were conspicuous within 10 h after colony collection, exhibiting significantly higher levels of aggression, including lunging at other queens and stinging them. Likewise, other queens could be distinguished by their tendency to remain on the periphery of the comb or hide beneath it, and to draw back when another queen passed nearby. These queens retreated if challenged by a lunging queen, and exhibited no acts of aggression toward other queens. The more aggressive queens lived significantly longer than submissive queens, with one ultimately superseding all others to become the new parental queen of the colony. This is contrary to the statement by Sakagami (1976) that the rivalry among new *B. atratus* queens never leads to social dominance, such as occurs among *Bombus* workers in a queenless colony (van Honk and Hogeweg, 1981) or in a multiple-foundress association of *Polistes* (Pardi, 1942, 1948). In these latter associations, dominant individuals direct their attacks toward individuals immediately beneath them in the hierarchy and monopolize oviposition while subordinates specialize in nonreproductive tasks. However, this model of a linear hierarchy is far from universal even in *Polistes*, which exhibit substantial variation in development of a linear hierarchy and division of labor (Reeve, 1991). Indeed, our results suggest that there are differing degrees of dominance among *B. atratus* queens, which govern their survival and reproduction.

Factors influencing queen dominance

Socially dominant queens combined aggressive behavior with a tendency toward greater ovarian development, independent of body size (but see caveat in the next paragraph). Queens with ovaries above the median size ($IO > 5$) laid eggs on their acquired territories. Queens with ovaries at or below the median ($IO < 5$) did not lay eggs, even though some of them acquired territories (01, 12); others (UB, UC) retreated out of sight beneath the comb. Of the queens that were measured, those with the most mature ovaries were most aggressive, except queen 11 who, in spite of her well-developed ovaries, retreated when attacked. In this case it is noteworthy that queen 11 was resorbing proportionally more oocytes (IR: $IO = 0.86$) (Table 2) than the other queens, an indication that she had no opportunity to lay her eggs (Medler, 1962). Queens without developed eggs were never aggressive. Egg resorption might also explain why subordinate queens (socially dominated) did not leave the colony to initiate new colonies of their own.

Of course, it is possible that the high level of aggression among *B. atratus* queens is caused by factors other than those we have measured, and that high levels of aggression in turn stimulate ovarian development or degeneration. Out of practical necessity in our study, ovaries and agonistic behavior were often measured at different times. Ovarian development was measured at the time of queen death, whereas agonistic scores reflect behavior within the first 48 h. For some of the longer surviving queens (03, UC, 10, 08), ovaries were measured from 1–11 days after the behavior was recorded. This time difference between observed behavior and ovarian measurements, and the fact that surviving queens were dissected later than those that died earlier may have confounded the effect of ovarian development.

The differences in ovarian development may have been enhanced after the association of the queens was formed, as has been reported for *Polistes* (reviewed in Röseler, 1991). An important future study could be designed to examine queens from another polygynous colony, dissecting all of the them at the end of an initial 48 h period of observation to detect whether those with survival-related behavior also had the most developed ovaries.

The positive relationship between fecundity and dominance status has been observed repeatedly in ants (Sommer and Hölldobler, 1995) and wasps (see chapters in Ross and Matthews, 1991; but see Röseler and Röseler, 1989). Interestingly, the presence of ripe eggs in the ovaries of *B. terrestris* workers is not a factor in determining their status in a dominance hierarchy. Van Doorn (1989) showed that ovariectomized workers were able to establish a position among the “elite” (dominant) workers in a colony, those that are typically the first to produce eggs later in colony development. Röseler and Röseler (1989) observed the same result with ovariectomized *Polistes gallicus* (now *dominulus*) queens. This suggests that other mechanisms, independent of ovarian condition, give rise to dominance behavior in these groups. Röseler et al. (1985) have demonstrated an endocrine mediation of dominance behavior in *Polistes dominulus*. It would be especially valuable to know whether hormones, such as juvenile hormone and ecdysteroids, are released during egg maturation and stimulate *B. atratus* queen aggression, as occurs in some *Polistes* (Röseler, 1991) and bumble bee workers (Van Doorn, 1989). In a future study of *B. atratus* it would be possible to estimate the effect of JH by measuring the volumes of queen corpora allata.

Other mediators of dominance?

Queen size appeared to have no influence on dominance in our study, although the generality of this conclusion requires further investigation in view of missing data on two queens with the longest survivorship. We regard our conclusions about the lack of correlation between body size and survivorship as preliminary. In other groups, size has frequently been reported to play a positive role in competitive ability among multiple queens (Packer, 1993; Reeve, 1991; but see Röseler, 1991). With respect to age, we have no evidence that it played a role in dominance. Although we do not have absolute age data, the absence of wing wear and brightness of the pile suggested that all queens were recently emerged and of similar age. We cannot rule out, however, the possibility that order of emergence may have influenced dominance outcomes, as has been found in *B. terrestris* workers (Duchateau, 1989) and suggested in *Polistes* (Hughes and Strassmann, 1988). Insemination was not a useful indicator of dominance in our study because most queens (80%) were inseminated, including subordinate queens. The acquisition of a territory was also a poor dominance indicator because most queens, including some that were uninseminated, claimed territories. The only queens without territories were those that hid beneath the comb; one of these was inseminated (UB), the other uninseminated (UC). Further experimental investigations into factors that mediate dominance and reproductive success among queens of *B. atratus* would extend these preliminary findings and allow comparisons with other polygynous social insects.

Ecological influences on polygyny

B. atratus cyclical polygyny and intracolony conflict should also be assessed within the context of ecological factors and the potential for direct reproduction among queens. Nest-site limitation could lead to philopatry (long-term fidelity to nesting sites) which, in *Polistes*, always results in polygynous foundress associations (Queller and Strassmann, 1988). Reeve (1991) has hypothesized that polygynous associations and the intensity of dominance interactions in *Polistes* co-foundresses is tied to ecological constraints on solitary founding. In species which show cyclical polygyny, the large benefits of inheriting an established nest full of worker "helpers" may greatly outweigh the costs of initiating a new nest alone. Comparable circumstances arise with *Bombus* queens starting nests in temperate regions, where queens may supersede (or attempt to supersede) colonies founded by other queens (Sladen, 1912; Free and Butler, 1959). Just as with *B. atratus*, a ready-made nest full of workers is capital worth fighting for. Sladen (1912) reported a nest of *B. terrestris* that contained the remains of 20 queens, presumably casualties of many competitive contests for the nest. The assumption from many such observations of supersedure is that nest sites are limiting. Although it has not been studied directly in *B. atratus*, there is evidence that nest site availability is limiting (Zucchi, pers. comm.), possibly presenting a severe ecological constraint in some parts of its range, including the site of our study.

Seasonality may also play a role in polygynous associations in *B. atratus*, which is perennial throughout much of its range. In lowland Neotropical areas the queens do not spend a period in diapause and are able to initiate nests throughout the year. However, in the southern extension of its range, which is temperate in climate, queens may spend the winter in diapause, hence would be unable to maintain perennial colonies. In those temperate regions, cyclical polygyny is unlikely to occur.

In summary, one would predict that if nest site limitation is an influencing factor in dominance battles for the natal nest, cyclical polygyny accompanied by intense fighting should increase as ecological constraints (on nest site availability) increase. Moreover, one would predict that cyclical polygyny would disappear from the nesting cycle as the climate becomes more temperate in the southern distribution of its range. *B. atratus*, which occurs widely across different habitats throughout most of South America (Milliron, 1973), provides an excellent opportunity to test hypotheses of ecological constraints on behavior.

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