

The Cost of Queen Loss in the Social Wasp *Polistes dominulus* (Hymenoptera: Vespidae)

JOAN E. STRASSMANN,¹ ANGELO FORTUNATO,² RITA CERVO,³
STEFANO TURILLAZZI,³ JESSE M. DAMON,¹ AND DAVID C. QUELLER¹

ABSTRACT: Loss of the queen is a crisis for a social insect colony. The process of queen succession could cause increased aggression and work inefficiencies, and the new queen, if the colony can produce one, could be slow to develop mature eggs. We evaluated the cost of queen replacement in *Polistes dominulus* by removing the queen from a set of single-foundress colonies while leaving a control set with their queens. At 2 and 11 days after queen removal, we found that the queenless colonies had increased levels of some dominance behaviors, chewing and climbing, but not of the far more common lunging and biting. However, foraging behavior did not decrease on nests without queens as compared to nests with their original queens. Nest growth diminished as compared to control nests, as would be expected if new queens were not as competent at egg laying or if dominance behavior interfered with nurturing activities. Furthermore, replacement queens did not mate in the first 12 days after queen removal and few had mature eggs in their ovaries, though after a month most had mated and had developed ovaries. The degree of ovarian dominance of the top egg-layer over the others was also diminished at 12 days, but by a month the new queen was as dominant as control queens. The high cost of replacing the queen may indicate that workers are kept reproductively suppressed enough not to be a threat to the existing queen.

KEY WORDS: Queen loss, *Polistes dominulus*, caste, orphan, ovary, conflict

A key feature of social insect colonies is that eggs are laid by a single queen or by a few queens, while workers focus on non-reproductive tasks (Wilson, 1971). Because of the queen's unique position as sole supplier of eggs in monogynous colonies, the death of the queen constitutes a crisis for her colony. In some species queen death means the end of the colony. In others the queen can be replaced, but major costs could still ensue from any delays owing to the time required to develop to a fully reproductive state and to find a mate. During this time, individuals die and are not replaced through new egg laying, reducing current reproduction and possibly jeopardizing future success.

Along with this demographic crisis, queen death may provoke a social crisis for two reasons. First, there is a disruption of the status quo in which a particular individual was recognized as queen. Whatever allowed her to hold her position, whether physical force or physiological signals, is removed, potentially opening up the reproductive position to many contenders. Second, the death of the queen often changes relatedness relations in favor of conflict. For example, in a colony with a single once-mated queen, workers have little to lose from letting the queen reproduce, being related by an average of 1/2 to the queen's offspring and 1/2 to their own (though conflict may sometimes occur over male production). When a queen dies, a female's choice is now between a sister's (replacement queen's) offspring ($r = 3/8$) versus her own ($r = 1/2$), so that each worker is expected to prefer being the reproductive.

In highly eusocial insects with morphological castes, queen death may be a rare event because the protection afforded by workers has allowed queens to evolve very long

¹ Department of Ecology and Evolutionary Biology, Rice University, 6100 Main St., Houston, Texas 77005-1892, USA

² The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK.

³ Dipartimento di Biologia Animale e Genetica dell' Università via Romana 17, Firenze, Italia

lifespans (Alexander, 1974; Keller and Genoud, 1997). When queen death does occur, finding a replacement may be made more difficult by the difference in castes. In honey bees, workers can sometimes rear a new queen by transferring a young larva to a queen cell, though the resulting long period before worker production resumes presumably represents a significant cost. Stingless bees may avoid some of this cost by keeping one or more non-laying reserve queens in the colony.

These conditions are likely to differ in primitively eusocial insects that lack morphological castes. The similarity of queen and worker developmental programs has two consequences. First, queens and workers have not evolved widely divergent lifespans, and queen death is likely to come more quickly. Second, there should be no need to rear, or to already have at hand, replacements from a specialized caste. In principle, any individual, including a worker, might be able to develop reproductively and become a new queen. These features suggest that queen replacement may be both more frequent and more uneventful in primitively eusocial species. On the other hand, any individual might compete to fill the vacancy, which could increase the likelihood of conflict.

The social wasp *Polistes* is a cosmopolitan genus that lacks morphological castes and so is particularly good for studies of queen replacement and its cost when the castes are very similar. Colonies are typically begun by one to several mated females. One of these foundresses typically dominates reproduction initially and nearly monopolizes it after the workers emerge (Pardi, 1948; West-Eberhard, 1969; Noonan, 1981; Reeve, 1991; Peters *et al.*, 1995; Field *et al.*, 1998; Seppä *et al.*, 2002). *Polistes* queens are singly mated (Peters *et al.*, 1995; Arévalo *et al.*, 1998; Field *et al.*, 1998; Queller *et al.*, 2000; Strassmann, 2001; Seppä *et al.*, 2002). When colonies have one singly mated queen, on relatedness grounds workers should prevent other workers from producing female eggs, but not male eggs (Ratnieks, 1988). Early in the season most progeny are females who become workers, not future queens, and there should be little conflict over who produces these sterile females. Later in the season, competition to produce males might take the form of reduced working, and worker production of males. If initiating working means that a female will not reproduce, she might delay working to lay eggs.

We studied the cost of queen loss in *Polistes dominulus* Christ, a well-studied circum-Mediterranean species (Pardi, 1948; Reeve, 1991). Colonies are begun by one to several mated, overwintered females in late March or April depending on the year (Pardi, 1942a, b, 1948; Queller *et al.*, 2000). Nests are generally found in protected places, under roof tiles, in man-made structures, or under rocks (Pardi, 1942b). We focus on the question of queen replacement in single-foundress colonies where only workers are available as replacement queens. The first generation of workers and a few males emerge in mid to late May (Turillazzi, 1980). In late summer and fall, males and reproductive females congregate at vertical landmarks and mate, and then females enter hibernacula to wait out the winter (Beani and Turillazzi, 1988, 1990). To evaluate the cost of queen loss, we removed queens during two years, and observed behavior and subsequent worker ovarian development. Does conflict increase as workers compete to become queens, reducing colony productivity? Are all workers physiologically capable of becoming queens?

Materials and Methods

Study Site

Our study site is near San Giovanni Valdarno, Italy, between Florence and Arezzo. The field had been planted with a 2 m × 3 m grid of small trees as part of a reforestation project

on land from which many of tons of dirt had been removed to restore a nearby former lignite strip mine. Most of the site had been planted in early 1999, though a small part of the site had been planted previously. Each small tree was protected by a beige plastic corrugated tube about 12 cm in diameter. *Polistes dominulus* nests freely in the plastic tubes. For example, in 1999 we found 182 nests of *P. dominulus* out of 328 tubes in the 2-hectare field. In 1999 there was bare earth between the tubes, though diverse meadows with many insects were nearby. In 2000 the ground was covered with vegetation.

Field Techniques

In early Spring, we visited the study site and began censusing the tubes, and noting any nascent wasp nests. When we found nests inside the tubes, we cut a door in the tube which could be propped open with a twig so nests could be censused and videotaped easily. We marked *P. dominulus* foundresses with enamel paint and censused at least twice a week so we could determine the natural rate of queen loss. At each census we counted cell and pupa numbers, checked for marked wasps and marked unmarked ones. The foundress was marked with orange and workers were marked with a color indicating their date of emergence. We found 12 multiple-foundress colonies in the area of the field that had tubes the previous year, but not in the remainder of the field.

For queen removal experiments we identified 30 monogynous colonies for this project in 1999 (one was lost early on) and 26 colonies in 2000. We divided nests into experimental and control treatments by pairing nests by number of wasps and randomly assigning one to each class. This pairing was just to be sure the two classes were equivalent; all statistics were done between the two groups unpaired. We removed the queens from the experimental colonies on 10 and 11 June in 1999 and on 20 June in 2000. We left the queens of the remaining colonies as controls. We used only colonies that had been singly founded from the beginning. In 1999 we collected all colonies and their associated females on 23 June, 12 or 13 days after queen removal. In 2000, we lost a number of control nests (see below) before collecting on 20 July, 30 days after queen removal. We collected colonies and wasps before sunrise when all wasps were on the nests.

To assess queen loss frequencies from single-foundress nests, we observed 40 unmanipulated colonies from near initiation in late April to 23 June 1999. We also observed 25 single-foundress colonies for queen loss in 1995 from 20 June to 2 August, the period covered by our experiments.

Measurement of Nest Growth

In the field we counted cells and pupae. We could not accurately distinguish all cells with eggs, larvae or empty in the field so these are not reported. After nest collection, we counted nest cells and recorded their contents (empty, eggs, larvae, pupae).

We lost the 6 largest control nests in 2000 to a vandal that we suspect was a fisherman taking the combs for bait. Another 3 control nests had been taken earlier and rebuilt by the surviving wasps, as was one experimental nest. Because only the smaller control nests remained, we cannot do any quantitative nest size comparisons for 2000.

Behavioral Analyses

In 1999 we watched the behavior of wasps on experimental and control colonies in the 12-day period after queen removal. To quantify observations, we videotaped 9 experimental and 9 control nests 2 days after queen removal and 11 days after queen removal. At each videotaping period we simultaneously videotaped two experimental and two control

colonies using 4 Hi8 Sony TRV66 camcorders. We were interested in determining whether queen loss was associated with increased aggression and slowed foraging on the experimental colonies. To determine this we analyzed a videotape from each date that averaged 120 minutes per nest per time period for a total of 73 hours 55 minutes. From the videotapes we noted aggressive behaviors directed toward victims, including chewing, climbing, biting, and lunging. Chewing and climbing are classic dominance interactions of *Polistes*, first described for this species by Pardi, and later extended to other species (Pardi, 1948; West-Eberhard, 1969; Strassmann, 1981a). Biting and lunging are behaviors involved in keeping others off an area of the nest, often the area with imminent egg-laying opportunities, as was first described by West-Eberhard (1986). Both begin with a lunge. Biting adds a bite or a fast nip at the end of the lunge.

As indicators of foraging, we counted mandible-to-mandible exchanges and arrivals at the nest with solid food comprised of parts of insect bodies. Solid food is essential to larval growth and is more reliably recorded than nectar foraging.

We counted the total numbers of wasps in these colonies and scaled all behavioral observations on a per-wasp per-hour basis. Queens were marked in orange and were present only on control nests and we noted their individual acts. Workers were marked by date of emergence. We did not identify which workers performed which behavior because we were interested in whole colony patterns. Identifying which worker performed each behavior would have meant we could process far fewer tapes in the over 500 person hours we spent watching videotapes.

For comparisons of control versus experimental nests, we included all wasps, including the orange marked queen on control nests and the replacement queen on experimental nests. We could not break this down between queens and workers behaviors because the workers, including the replacement queen on experimental nests, were not individually marked. This is more likely to influence comparisons of aggressive behaviors than foraging behaviors since queens do not forage for caterpillars.

On control nests, where the queen was individually marked, we compared the behavior of queens versus workers using paired tests to control for nest.

Comparing experimental to control colonies, we predicted aggressive behavior to be higher and foraging to be lower on queenless nests, so we used one-tailed tests. We use a two-tailed test only for exchanges since less food brought to the colony could decrease exchanges because there is less to be exchanged, or increase apparent exchanges because of increased demand for food by some individuals.

Measurement of Ovaries and Insemination Status

We dissected all collected females and measured their ovaries. We counted all mature and nearly mature eggs in their ovaries, and measured the length of the longest egg or oocyte. A mature egg has the size and appearance of an egg laid in the nest. A nearly mature egg has yolk and is at least 2/3 the size of a mature egg. We also examined the spermatheca to see if it contained sperm. This is particularly difficult in this species because spermathecae sometimes contain very few sperm, meaning that sometimes inseminated females are scored as uninseminated (Strassmann, Arévalo, Queller, unpubl. data).

DNA Microsatellite Genotyping of Eggs for Sex Determination

Because of difficulties with visualizing sperm in spermathecae, we also genotyped 53 eggs that had been laid in 14 experimental nests in 1999 using techniques of Strassmann *et al.* (1996). Since eggs take only a few days to hatch into larvae, all these eggs were

laid after the original queens were removed, and so should all be haploid if replacement queens had not mated on experimental nests. Because of the very small amounts of template DNA in eggs, PCR amplification is often problematic. We dealt with this by attempting to amplify 6 loci designed for this species, Pdom2, Pdom7, Pdom20, Pdom117, Pdom122, and Pdom140 (Henshaw, 2000). These highly polymorphic loci had observed heterozygosities of 0.75, 0.75, 0.88, 1.0, 1.0 and 0.88 respectively in females of our population. We assigned a sex to any egg with genotypes from at least three loci, calling individuals female if they had at least one heterozygous locus, and male if they had only one allele at every locus.

Results

Natural Frequency of Queen loss

Between late April and 23 June 1999, about a month after worker emergence, the queen had disappeared from 20% of single-foundress nests ($N = 40$). This period covers both pre-emergence and post-emergence periods. We checked the ground under the tubes to see if queens had died on the nest and fallen, but did not find them there. Aggression on the nests leading to death has been found in multiple-foundress colonies (Cervo, Turillazzi, Queller, Strassmann, unpubl. data). Queens in this population regularly leave the nest to forage for pulp and water, particularly in the late afternoon when temperatures at the nests are very high, even after worker emergence. Queen loss is probably due to predation during foraging trips.

In 1995 we checked 25 post-emergence single-foundress colonies for the presence of the queen and found that 40% of them had lost the queen between 20 June and 2 August.

During our experimental study, queens were not lost from any of the 15 control nests during the 2 weeks of the study in 1999 or from the 6 non-vandalized control nests in the month of our study in 2000.

Colony Characteristics

At the time of queen removal from experimental nests, colonies had 3–18 females, averaging 8.6 (SE 0.6, $N = 29$) in 1999 and 3–9 females averaging 5.7 (SE 0.4, $N = 26$) in 2000. The nests had 33–144 cells, averaging 79 (SE 5) in 1999 and 26–74 averaging 44 (SE 2) in 2000. The nests had 0–37 pupae, averaging 12.6 (SE 2.0) in 1999 and 0–16 pupae averaging 5.6 (SE 1.2) in 2000. No colonies had any adult males associated with them.

Nest Growth of Colonies with Replacement Queens

In 1999 control nests were larger two weeks after queen removal than were experimental nests (Fig. 1). The 15 control nests had gained double the number of cells as compared with the 14 experimental nests (Mann-Whitney U , $P < 0.007$), and had larger absolute numbers of eggs (Mann-Whitney U , $P < 0.006$), but not more empty cells (Mann-Whitney U , $P > 0.78$) or larvae (controls: $47.8 \pm \text{SE } 4.4$; experimental 37.9 ± 3.2 SE; Mann-Whitney U , $P > 0.07$). This suggests that the workers add cells in proportion to their reduced ability to fill them with eggs (Fig. 1).

Since the control nests had one more wasp on them than did the experimental nests who had the queen removed, we looked at the impact of wasp number on nest growth over the two week period, using just the control nests. This will indicate whether or not the difference between control and experimental nests is simply due to the single wasp removed from control nests. The regression of increase in cell number on wasp number at

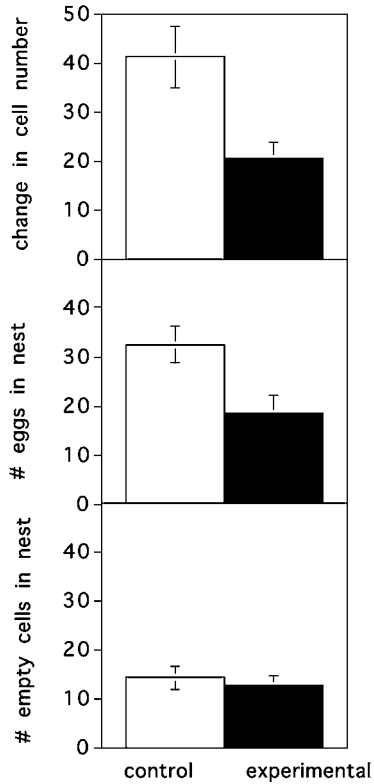


Fig. 1. Means and standard errors of nest condition variables 12 days after queen removal in 1999. Control nests added significantly more cells than experimental nests. Absolute numbers of eggs was significantly lower in experimental nests after 12 days. Absolute numbers of empty cells did not differ after 12 days between experimental and control nests. Open bars are control colonies, and solid bars are experimental colonies whose queens were removed.

the beginning of the experiment was highly significant ($F = 12.9, P < 0.002, N = 15$), and explained 41% of the variance. Each wasp adds on average 5.2 cells to the nest size over this period. The control colonies gained 41 cells on average, while the experimental colonies gained only 21 cells on average. This 20 cell difference is nearly 4 times the difference that could be accounted for by losing a single wasp, so clearly losing the queen has other impacts than just losing a single individual.

Behavioral Conflict and Foraging in Colonies with Replacement Queens

In all, during our 74 hours of videotapes on 9 control and 9 experimental colonies we observed 17 incidents of one female climbing on another, 232 chewing attacks, 445 bites, 7958 lunges, 2285 mandible-to-mandible exchanges, and 384 arrivals with a caterpillar. On the control nests we observed 5 times that the queen climbed on another, 34 times the queen chewed on another, 51 times the queen bit another, 637 times the queen lunged, and 180 times the queen initiated a mandible-to-mandible exchange. On the control nests, directed to the queen were no chews or climbs, 12 bites, 395 lunges, and 14 mandible-to-mandible exchanges. Queens did not forage for caterpillars.

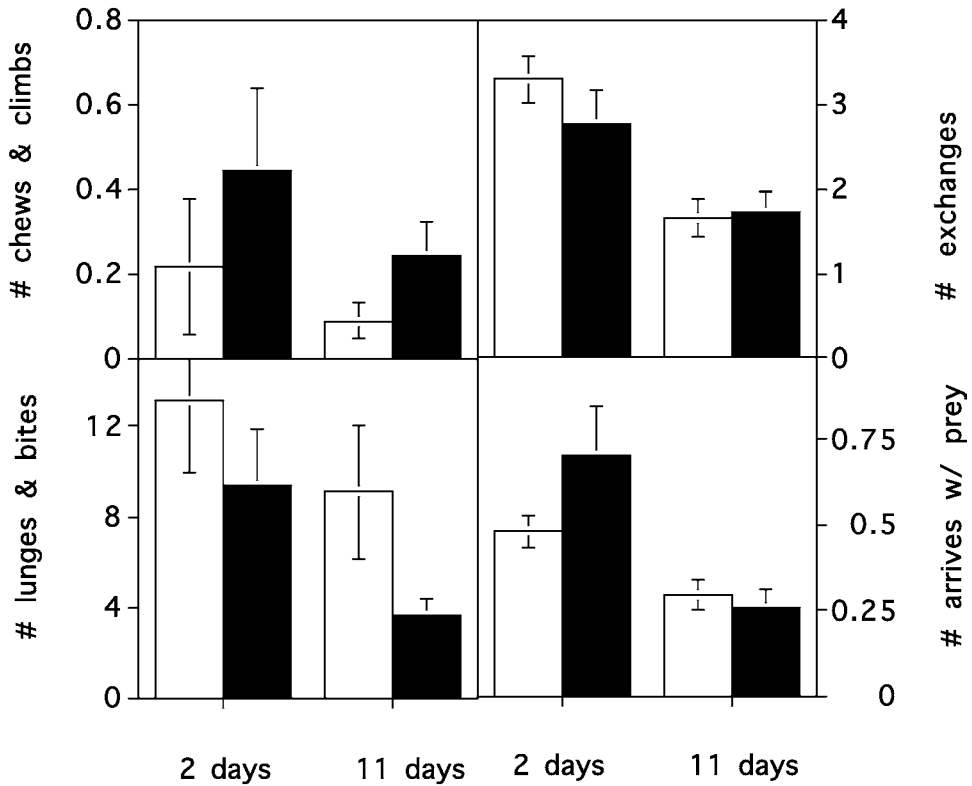


Fig. 2. Means and standard errors of behavioral acts 2 and 11 days after queen removal. All measures are per wasp per hour. Open bars are control colonies, and solid bars are experimental colonies whose queens were removed. Chews and climbs increase significantly 2 and 11 days after queen removal. No other differences are significant.

On control colonies, queens did not differ from the average worker in the same colony in frequency of climbs and chews on 13 June ($N = 8$) or on 22 June ($N = 9$; Wilcoxon Matched Pairs Signed Ranks Test, $P > 0.68$ each date). Queens performed more lunges and bites than did the average worker at each date (Wilcoxon Matched Pairs Signed Ranks Test, $P < 0.025$ and $P = 0.0506$ respectively).

Females climbed on and chewed on others significantly more often on the experimental nests than they did on the controls, both 2 days (Mann Whitney U , $P = 0.035$) and 11 days (Mann Whitney U , $P = 0.045$) after queen removal (Fig. 2). We found no differences between treatments in lunges and bites, both 2 days (Mann Whitney U , $P > 0.88$) and 11 days (Mann Whitney U , $P > 0.95$) after queen removal. In fact, there was a trend towards lower levels of these behaviors, opposite to our prediction. These behaviors serve to keep other wasps off a certain area of the nest. Females on nests lacking the original queens did not forage for prey less than those on nests with original queens, either 2 days (Mann Whitney U , $P > 0.83$) or 11 days (Mann Whitney U , $P > 0.34$) after queen removal (Fig. 2). Mandible-to-mandible exchanges did not differ between experimental and control colonies either 2 days (Mann Whitney U , $P > 0.20$) or 11 days after queen removal (Mann Whitney U , $P > 0.57$; Fig. 2).

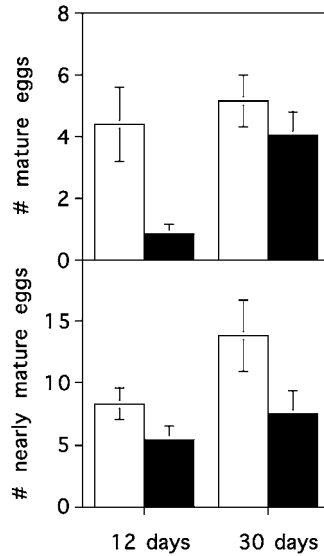


Fig. 3. Means and standard errors of ovarian development of the most developed female. Open bars are control colonies, and solid bars are experimental colonies whose queens were removed. After 12 days queens had fewer mature eggs on experimental colonies. No other differences are significant.

Ovarian Characteristics of Replacement Queens

We considered as replacement queen the worker with the most developed ovaries on each experimental nest, whether or not she was mated. We then compared these replacement queens to the foundress queens on the control nests. The foundress queens had emerged the previous year, mated, overwintered, and begun a nest. After two weeks, in 1999, replacement queens on experimental colonies had fewer mature eggs in their ovaries than foundress queens had on control colonies (Mann-Whitney U , $P = 0.016$, Fig. 3). The replacement queens, however, did not differ significantly from the foundress queens in number of nearly mature eggs in their ovaries (Mann-Whitney U , $P = 0.126$, Fig. 3). After a month, in 2000, replacement queens had no fewer mature eggs in their ovaries on experimental colonies than did foundress queens on control colonies (Mann-Whitney U , $P = 0.587$, Fig. 3). Likewise, the replacement queens did not differ from the foundress queens in number of nearly mature eggs in their ovaries (Mann-Whitney U , $P = 0.104$, Fig. 3). These results show that workers have the ability to develop their ovaries, but that two weeks is not long enough to develop them fully.

Mating Status of Replacement Queens

We found no females with sperm in their spermathecae in the experimental colonies after two weeks. None of 14 replacement queens had sperm evident, while 11 of 12 foundress queens did (Fisher Exact Test, $P < 0.001$). The foundress that apparently had no sperm in her spermatheca probably did, since the colony had not had a queen turnover and had female workers. After a month in 2000 we found that some experimental colonies had replacement queens with spermathecae containing sperm. Four of 13 experimental colonies had queens with sperm while 5 of 6 control colonies did (Fisher Exact Test, $P < 0.057$).

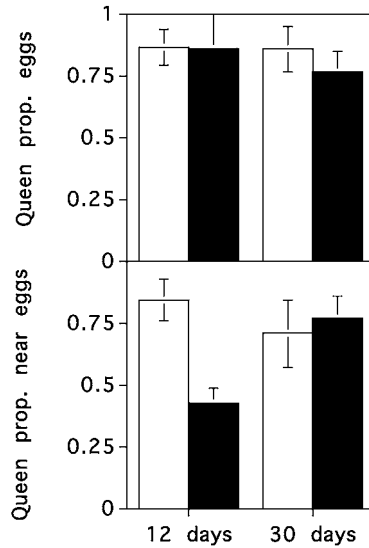


Fig. 4. Means and standard errors of the share of eggs in the most developed female compared with all other females in the nest. The top panel shows mature eggs and the bottom panel nearly mature eggs. Open bars are control colonies, and solid bars are experimental colonies whose queens were removed. After 12 days queens had a significantly lower share of nearly mature eggs on experimental colonies. Other differences are not significant.

Three of the experimental colonies had 1 to 3 other (worker) females with sperm in their spermathecae as did 2 of the control colonies (Fisher Exact Test, N. S.).

We confirmed the lack of mated queens after 12 days in 1999 by genotyping a sample of eggs laid in the experimental nests after queen removal. These eggs could not have been laid by the old queen because the eggs she laid before she was removed would have all hatched by the time we collected the nests. All 53 of the eggs from 14 nests were haploid at all loci we genotyped, indicating that they were males. The probability that any one of these eggs was diploid is very low. If we assume that an egg's genotype comes from the three least polymorphic loci we used, the probability that it was a diploid female and homozygous at all three loci is only 0.0076. This supports the finding from dissections that replacement queens in 1999 were unmated. At this time the colonies would normally be producing mostly female workers.

We occasionally saw adult males in the field near the nests in previous years, but could not quantify their numbers, or their frequency. Therefore we cannot say if replacement queens are easily able to find males or not.

Reproductive Conflict in Colonies with Replacement Queens

Colonies whose queens had been removed might have more ovarian development among workers as they vie to become queens. We examined reproductive effort in workers by determining the fraction of mature and nearly mature eggs in the queen's ovaries as compared to the fraction of mature and nearly mature eggs in worker ovaries in the whole colony. In 1999 there was no difference between replacement queens and foundress queens in the proportion of mature eggs that were in the queen's ovaries; in both cases it was over 75% (Mann-Whitney U , $P = 0.288$, Fig. 4). However, the share of nearly mature eggs in the queen's ovaries was smaller in experimental colonies (Mann-Whitney U ,

$P = 0.001$, Fig. 4). In 2000 there was no difference between replacement queens and foundress queens in proportion of mature eggs that were in the queen's ovaries; in both cases it was over 75% (Mann-Whitney U , $P = 0.500$, Fig. 4). The share of nearly mature eggs in the queen's ovaries did not differ between experimental and control colonies (Mann-Whitney U , $P = 0.405$, Fig. 4).

There was no particular indication that the females on experimental nests had opted to become future reproductives in greater numbers than on control nests. In 1999 when colonies were collected on 23 June, no colonies had females with large fat bodies typical of females that will overwinter. In 2000 when the colonies were collected a little later, on 20 July, one of them contained 4 females likely to overwinter (indicated by the abundance of the fat body) and one other colony produced one such female. One control colony had also produced 4 females likely to overwinter.

Discussion

Queen loss can cause a demographic and social crisis for the colony. The social crisis involves a transition to a new queen. In *P. dominulus*, the old queen is replaced by a worker who eventually becomes a queen similar to the original queen. The transition involves a return to the kinds of dominance behaviors that characterize these colonies very early in the season. When we videotaped, 2 and 11 days after queen removal, aggression in the form of climbing and chewing was higher in the queen removal treatment. This most likely means that a single female had assumed the queen's role, but her position was shaky, requiring more dominance behavior. Lunging and biting, by contrast, are behaviors that an active queen performs more often than do workers on natural nests at this time of the year. Thus, removing the queen caused the early season dominance behaviors to return, but did not change lunging and biting relative to control nests, perhaps because natural queens still use these to dominate the front of the nest.

Though there was more aggression in the form of chewing and climbing, we did not find any significant reduction in food intake, perhaps indicating that the social crisis (competition for queenship) does not seriously retard nest growth. But there is a different kind of cost which does reduce nest growth. New queens were lacking in one of their most essential functions: the ability to produce male and female eggs. After 12 days replacement queens have less developed ovaries and are less dominant in ovarian development over their nestmates than are control queens. By 30 days, replacement queens have caught up with original queens in ovarian development and in dominance in ovarian development. Females had not mated at 12 days though they largely had by 30 days after queen removal. Lack of mating may have been due to the rarity of males in the population in mid June. Though we have seen Spring males in *P. dominulus*, they are not common, and there is no obvious way to quantify their numbers. It is harder to explain delays in ovarian development. Perhaps the delay is because the workers' ovaries were quite undeveloped when we removed the queens, so they took a long time to develop. This lack of egg laying by the new replacement queens is the most probable cause of a reduction in nest growth.

There was clearly an appreciable demographic cost to replacing the queen in *P. dominulus*. Nest growth of experimental colonies was behind at 12 days, when we could measure it in 1999, and appeared to have been even more behind in 2000 after 30 days when we couldn't quantify it because the largest nests were stolen. Replacement queens do eventually mate and catch up with original queens in ovarian development and degree of domination over the other workers, but in the meantime workers are lost to predators, and are not replaced as the new queens produce no eggs.

Colonies are most likely to have adaptations to recover quickly from queen loss if it is a common event. Our estimates of natural queen loss on single-foundress colonies of *P. dominulus* were quite variable, ranging 0% of the 15 control colonies over 2 weeks to 40% from the end of June to early August. The higher estimate included more colonies over a longer time period and establishes that queen loss is at least sometimes common. This makes it puzzling that the colonies seem so ill-equipped to respond, particularly since queen replacement is common in other species of *Polistes* (see below). Perhaps single-foundress colonies are comparatively rare in this species, and multiple foundress colonies seldom lose all queens. Perhaps queens keep workers deeply suppressed to prevent them from posing being reproductive competitors to the queen while she is alive, with the cost being delayed queen replacement. Alternatively, workers may not develop their ovaries because this may make them less functional as workers.

Our study was limited to a particular time window involving queen loss in mid-June. Workers first emerge towards the end of May, and by mid to late July the final batch of eggs are usually laid, so queen loss with workers present could occur a couple of weeks before our study and up to a month afterwards. Earlier workers seem unlikely to be more adept at taking over, though we have no data to support this. Later workers could be in better condition, but our ovarian data from control colonies collected on July 20 suggest that queens at that time remain just as reproductively dominant over workers (Fig. 4). It is unlikely that workers are much better at becoming replacement queens earlier or later.

Queen replacement has been investigated in naturally occurring colonies of other *Polistes*. Queen loss is common in *Polistes chinensis antennalis* Pérez (Miyano, 1986). By early August 24.7% of colonies had lost their original queens. Workers began to lay eggs 2 to 5 days after queen loss, but overall orphaned colonies attained only 3/4 the size of colonies with original queens (Miyano, 1986). In *P. c. antennalis*, the number of workers laying eggs increased over time but this is also found in colonies with original queens. This species has early males and worker mating has been reported (Suzuki, 1981). In *Polistes jadvigae* Dalla Torre, behavior following queen loss was observed in two colonies (Miyano, 1991). A dominance hierarchy was established and the top-ranking worker laid the most eggs. Growth of these colonies was similar to two with queens. *Polistes nipponensis* Pérez colonies lose their queens 46% of the time before worker emergence (Hagiwara and Kojima, 2002). Workers initiated egg laying 10 days after their emergence as adults. These workers laid male eggs since there were few males in the population for them to mate with—only 2 of 75 colonies had early males.

Queen loss has been thoroughly studied in *Polistes exclamans* Viereck (Strassmann, 1981b). In this species 58% of colonies lose their queens before July, and many of the remaining colonies lose them subsequently. Early males emerging with the first generation of workers are common, coming from the larger 22% of colonies. Workers that take over queenship mate with early males and their colonies suffer no apparent cost to colony growth. The new replacement queen, who is one of the oldest females in the colony, mates and lays eggs within a day of taking over the queen role. The high frequency of queen loss and the commonness of early males probably explains why queen replacement proceeds so much more easily in this species as compared to *P. dominulus*.

In *P. instabilis* de Saussure, one of the oldest workers becomes the new queen after queen removal (Hughes and Strassmann, 1988). The day after queen removal colonies were collected so ovaries reflect pre-removal conditions; at that time the most dominant worker had close to 10 oocytes in her ovaries, half the number queens had.

In Texas a few males are produced in May–July in *P. metricus* Say, *P. bellicosus* Cresson, and *P. dorsalis* Fabricius, but not in *P. carolina* Linnaeus (Strassmann and Hughes, 1986). This indicates that loss of all foundress queens in *P. carolina* is likely to be very rare. In *P. carolina*, there are multiple foundresses in each colony, providing another source for a new queen, at least early on (Seppä *et al.*, 2002). Unlike the other three species, but like *P. dominulus*, *P. carolina* nests in sheltered places.

Clearly queen loss is sometimes common in *Polistes*, and workers commonly replace the queen. There is not a general breakdown in the hierarchy; many workers do not begin laying eggs. Spring males are common in many but not all species, allowing replacement queens to mate where they occur. Having a single reproductive female has costs that appear to vary depending on the likelihood of queen loss. This may indicate that the cost of having a ready replacement queen is high. Whether this cost is high because of potential conflict that it causes, or because specialization is more efficient, is hard to determine.

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Dedicated to the memory of Howard Evans whose deep love of wasps inspired us.

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