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Behavioral transitions with the evolution of cooperative nest founding by harvester ant queens

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Abstract Research on the evolution of cooperative groups
tends to explore the costs and benefits of cooperation, with
less focus on the proximate behavioral changes necessary for
the transition from solitary to cooperative living. However,
understanding what proximate changes must occur, as well as
those pre-conditions already in place, is critical to understand-
ing the origins and evolution of sociality. The California
harvester ant *Pogonomyrmex californicus* demonstrates
population-level variation in colony founding over a close
geographic range. In adjacent populations, queens either
found nests as single individuals (haplometrosis) or form
cooperative groups of nonrelatives (pleometrosis). We com-
pared aggregation, aggression, and tolerance of queens from
one pleometrotic and two haplometrotic populations during
nest initiation, to determine which behaviors show an evolu-
tionary shift and which are present at the transition to
pleometrosis. Surprisingly, within-nest aggregative behavior
was equally present among all populations. In nesting boxes
with multiple available brood-rearing sites, both queen types
readily formed and clustered around a single common brood
pile, suggesting that innate attraction to brood (offspring)
facilitates the transition to social aggregation. In contrast,
queens from the three populations differed in their probabili-
ties of attraction on the ground to nest sites occupied by other
queens and in levels of aggression. Our results suggest that
some key behavioral mechanisms facilitating cooperation in *P.
californicus* are in place prior to the evolution of pleometrosis
and that the switch from aggression to tolerance is critical for
the evolution of stable cooperative associations.

Introduction

The evolutionary transition from solitary to social living is one
of a series of fundamental shifts in biological scale and com-
plexity (Wilson 1975; Szathmáry and Smith 1995; Hölldobler
and Wilson 2009). There is a robust body of work on costs and
benefits of the transition to cooperative sociality (Aviles 2002;
Baker et al. 1998; Bono and Crespi 2006; Costa and Ross 2003;
Cowan 1987; Jakob 1991; Macdonald 1983; Rosengaus et al.
1998; Sachs et al. 2004; Uetz and Hieber 1997), but less
investigation of the proximate behaviors driving and/or accom-
panying group formation (Jeanson et al. 2005; Tschinkel 1998).
However, these proximate mechanisms are important to under-
standing social evolution. Stable cooperative systems cannot
evolve until individuals aggregate, tolerate, and interact with
one another, regardless of benefits they ultimately receive
through social living.

One way to explore the mechanistic underpinnings of social
transitions is to compare the interactions of individuals that form
cooperative associations with those of others who are solitary.
After mating, new ant queens typically excavate and establish
nests by themselves, living alone until their first worker off-
spring emerge (haplometrosis; Hölldobler and Wilson 1977,
1990). However, in some populations, unrelated queens form
associations (pleometrosis) where they cooperatively construct a
nest, rear brood, and in some cases forage (Hölldobler and
Wilson 1977; Keller 1995; Bernasconi and Strassmann 1999;
Cahan and Fewell 2004; Johnson 2004; Kellner et al. 2007;
Helms and Helms Cahan 2012). Queens of both types ultimately
produce eusocial colonies, meaning they already have some
capacity to behave as social entities in the context of family

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groups. Despite this, the formation of pleometrotic groups is rare (Bernasconi and Strassmann 1999; Balas and Adams 1996; Aron et al. 2009; Rissing and Pollock 1987).

In the species *Pogonomyrmex californicus*, populations exhibit striking variation in whether queens form cooperative groups versus solitary colony founding. Throughout most of their range in the deserts of western North America, queens primarily found nests solitarily, and this is likely the ancestral state (Johnson 2004; Overson 2011). However, at least one population in San Diego County, California exhibits pleometrosis (Rissing et al. 2000). Genetic sampling from mature colonies has shown that foundresses remain together beyond worker emergence and jointly produce both workers and sexual offspring in mature colonies (Overson 2011). This contrasts with most cases of pleometrosis, where queens found nests together but become agonistic after the founding stage until only one queen remains (Bernasconi and Strassmann 1999; Helms and Helms Cahan 2012).

Haplometrotic and pleometrotic queens vary in their task behaviors when placed into laboratory associations (Cahan and Fewell 2004; Jeanson and Fewell 2008), supporting the assertion that these are genetically distinct types and that selection operates on behaviors relevant to solitary versus cooperative nest founding in this taxon. In fact, selection during this phase of their life history is likely to be strong because levels of mortality during nest establishment and early colony growth are extremely high (Cole 2009). Thus, variation in colony founding behavior provides a particularly useful context to compare behavioral changes with the transition to group living.

To form stable associations, queens must aggregate with other queens and continue to tolerate their presence. Organisms can aggregate in two ways: (1) when they come together via attraction to external factors such as patches of resources, or (2) when they exhibit mutual attraction to conspecifics, the latter of which has been termed congregating (Stamps 1988; Parrish and Hamner 1997; Fletcher 2006). To be a true social group, individuals must congregate independently of external conditions, but the transition to congregation could be mediated through factors that induce aggregation as preconditions to sociality (Seeley and Morse 1978; Visscher et al. 1985; Beauchamp et al. 1997; Muller 1998; Jeanson et al. 2005; Jeanson and Deneubourg 2007).

Although not always a negative social phenotype (e.g., in cases of social regulation), aggression that produces significant fitness costs could be a barrier to cooperative sociality (Cahan et al. 1998). Ant colonies defend territories, and their workers correspondingly exhibit avoidance and/or aggression, especially at nest sites (Hölldobler and Wilson 1990). Our expectation is that the evolution of cooperation within foundress associations, as in cooperative groups more generally, requires a shift from avoidance to attraction and from aggression to tolerance of conspecifics. To test these expectations, we created groups of

queens from two haplometrotic and one pleometrotic population. We compared their behavior in nesting boxes and in arenas with nest sites, to determine whether queen–queen interactions vary in the contexts of choosing nest sites on the ground versus within-nest behavior, both contexts being relevant to aggregation and social behavior.

Methods

Study populations and queen collection

We performed two experiments (A and B) across different years to compare social interactions of queens from haplometrotic versus pleometrotic populations. We collected newly mated queens directly following mating flights from one pleometrotic and two haplometrotic populations. One haplometrotic population is contiguous with the pleometrotic site, and the other is geographically removed (populations described by Rissing et al. 2000; Cahan and Fewell 2004; Overson 2011). Queens were collected on the ground after they had mated and removed their wings. They were placed into individual plastic tubes with a moist piece of paper towel and brought to the lab for experimentation within 1–3 days. All queens were weighed and individually marked on the abdomen with enamel paint (*Testors* brand) before being placed into treatment groups.

Collections for experiment A were made in June and July, 2006. On June 6–9, we collected 267 foundresses from a haplometrotic population (H-A$_{rizona}$) at the Salt River recreation area northeast of Phoenix, AZ (33°32.870′N, 111°38.617′W; 409 m). On July 2–4, we collected queens from haplometrotic (H-C$_{alifornia}$) and pleometrotic populations (P-C$_{alifornia}$), approximately 50 km apart in San Diego County, CA. Ninety foundresses were collected from the H-C population at Lake Henshaw (33°13.928′N, 116° 45.381′W; 824 m) and another 90 foundresses from two P-C sites 9 km apart: Pine Valley, CA (32°49.420′N 116°31.680′W 1,133 m) and Cibbet Flat Campground (32°46.614′N, 116°26.819′W; 1,265 m).

Experiment B was performed in 2010 to examine variation in behavior when queens select nest sites at the soil surface, as a follow-up to the within-nest results of experiment A. Foundresses were collected after mating flights from the same sites as in experiment A, with the exception that, due to lack of sufficient queens at Cameron Valley, queens from a nearby site at Pine Valley, CA (13 km away) were used instead (32°49.420′N, 116°31.680′W; 1,132 m). This site has a confirmed pleometrotic population with a high majority of incipient, multi-queen nests (Johnson 2004).

Experiment A: within-nest behavior

To measure population-level variation in within-nest behavior, including clustering and aggression, we created groups of

three queens from each population (H-A, H-C, P-C). This group size equals the median size of natural foundress associations in the pleometrotic population (Overson 2011). Queens were placed together with two other individuals selected haphazardly from the same population. Each group of three was placed into a nest box consisting of a transparent plastic container (11×11×3.5 cm), which served as a central chamber, with four glass test tubes (7.5×1 cm) fused to each wall and extending externally from the box. This positioned tubes at right angles to neighbors, with the box as a common central chamber. The central chamber contained Kentucky bluegrass seed, provided ad libitum. The tubes served as independent brood-rearing chambers. Each tube contained water behind a cotton plug and was kept covered with aluminum foil to maintain darkness. In laboratory colonies, queens use these water tubes to lay eggs and rear brood. The colonies were maintained in a temperature-controlled incubation room at a constant temperature of 28 °C with 12-h day/night cycles and ceiling lighting. Nest boxes were distributed haphazardly relative to the room space. The arrangement of tubes within nest boxes (circular, rather than linear with end and middle tubes) and distribution of boxes within the room (random as to treatment) helped control for possible positional effects on queens' choice of water tubes.

Each trio of queens was placed simultaneously into the central chamber of the nest box. Observations began after an initial 24-h "settling-in" period, during which queens explored the foraging area, frequently antennated one another, and explored the water tubes. To record behavior, we scan sampled nest boxes (Altmann 1974), recording observations twice daily for the first week (morning and afternoon) and then once daily over a total of 80 days. During each observation, we noted queen mortality, the location of each queen (whether she was in one of the four nest tubes or the foraging area), and presence/absence and location of brood. We recorded any incidents of overt aggression and, whenever possible, noted the identity of the aggressor and target. An aggressive incident was defined as either one queen grasping another with her mandibles or a live queen exhibiting damaged or missing body parts. Damage serves as strong indirect evidence of aggression, as observed aggression almost always resulted in damaged or missing body parts.

Analysis of survival and aggression

We compared queen survivorship across the three populations using a log rank multiple comparison survival analysis. To test for an effect of queen weight on survival, we ranked queens within a nest box as heaviest, middle-weight, and lightest. We then tested for survival differences between the weight classes for each population separately within the survival analysis.

We also compared populations for mortality, including mortality linked to aggression. We used a χ^2 contingency analysis to compare differences among populations in the frequency of nest boxes with or without aggression and of aggression-related deaths. A queen's death was considered to co-occur with aggression if she died within 48 h of being observed as the target of aggression and/or her body was severed in some way.

Analysis of clustering behavior

We scored each nest box daily over the first 40 days of the study for presence of clustering, which we defined as occurring when either two or three queens simultaneously occupied a single nest tube. A 40-day cutoff was chosen for the clustering analysis because beyond this time the cumulative mortality of queens in the haplometrotic treatments made statistical comparisons across populations difficult. During the first week, when two observations were made per day, the analysis included only one of the two observations, chosen at random. Mortality occurred throughout the study, and when nest boxes no longer contained at least two living queens, they were removed from the clustering analysis.

To determine whether queens actively clustered together, we performed randomization tests (Manly 2006) on the proportion of nest boxes containing clusters. Specifically, we tested the null hypothesis that the clustering proportion did not differ from that expected if queens chose a nest tube without regard to the presence of other queens. To estimate the probability distribution of this proportion under the null hypothesis, we simulated a data set with the same number of nest boxes and observation days as the data. Each simulated nest box had the same number of live queens in tubes per day as the corresponding real nest box. Queens in the foraging area were not counted for that observation period in either the real or simulated data sets (6.7 % of all queen observations). The full data set was simulated 1,000 times, using MatLab (The MathWorks, Inc., Natick, MA, USA). The average expected proportion of nest boxes with clusters per day was then calculated across the 1,000 simulations. To estimate the 95 % confidence interval of the mean proportion under the null hypothesis, we sorted the 1,000 simulated proportions and excluded the highest and lowest 2.5 % of values. For each day, we then determined whether the observed proportion lay within the confidence interval. If it did not, we rejected the null hypothesis of no clustering with a significance level of 5 %.

We next tested for differences in clustering between the simulated and real populations by generating three separate bootstrapped versions of the observed data, one for each population. Each bootstrapped version was produced by resampling the real data with replacement to create a new data set of the same size. The sampling unit was the entire series of observations for an individual nest box. We calculated the proportion of nest boxes with clusters for each day of each bootstrapped data set, as described above for the real data. We

then determined the mean and 84 % confidence interval of this proportion on each day across the 1,000 bootstrapped samples. We used 84 % confidence intervals because these are expected to yield a true significance level of 5 % for this test (Payton et al. 2003). We judged populations as differing in degree of clustering whenever their confidence intervals did not overlap. Over the 40 days, we were able to generate 32 pairwise comparisons for the H-A/P-C and the H-C/P-C populations and 30 comparisons between H-A and H-C. Copies of both MatLab programs are available from the authors upon request.

Experiment B: nest site choice behavior

For the second experiment, we again created three-queen groups from each population, but this time placed queens in open containers of soil rather than in nest boxes. This design did not permit observation of queen behavior below ground, but captured queen behaviors on the surface as they chose nesting sites, either by excavating a single nest or establishing multiple separate nests. Queens were marked as described above, grouped haphazardly, and placed into $36 \times 43 \times 17$-cm plastic containers filled with 10 cm of soil brought back from the mating sites and sifted for uniform consistency. We constructed and observed 20 replicate trays for each population. Kentucky bluegrass seed was provided as food ad libitum throughout the experiment by sprinkling it lightly across the container's surface.

Analysis of nest choice behavior

The groups of three queens were placed into the center of each container, starting at 7:00 am. Over the next 8 hours, we scanned each container approximately every six minutes (1,500 total observations per population). During scanning observations, we recorded any instance of aggression (i.e., biting with mandibles) and excavating behavior (i.e., digging a hole in a series of dirt removal bouts with mandibles). When an excavated tunnel was large enough for a queen to fit fully inside, we assigned it a unique identifying number and recorded the queen or queens associated with its initial excavation. By the second day, most queens had settled underground. Observations were continued twice daily for one week and then once daily for the following week.

After 3 weeks, containers were censused for individual queen survival and location and for the presence of brood. This allowed us to compare the nest where a queen was observed during excavation to that where she finally resided. Desiccation in the containers of the H-A population caused almost all individuals to die before the 3-week census; therefore, no mortality data were collected for this population. The H-A population protocol was run slightly earlier than the other populations, so the water protocol was altered to avoid this problem for the California populations.

We compared behavioral interactions, including aggression and displacement, which generally took the form of one queen carrying the other away from the nest entrance. We also measured instances of cohabitation, more than one queen sharing a nest, across the three populations. Differences in the mean number of surviving queens per container across populations were tested with ANOVA. We used χ^2 contingency analyses for all other comparisons. In the case of aggression, we compared the number of groups per population that either did or did not exhibit the focal behavior at any time during the experiment, to avoid problems of pseudo-replication caused by queens of a given group repeatedly exhibiting a specific behavior. For comparison of cohabitation, we compared both the number of groups per population in which queens jointly founding a single nest after 24 h (when the majority of queens had settled underground) with the number of groups with at least one instance of queens sharing nests at the end of the experiment.

Results: experiment A

Survival

Individual queen survival differed significantly among the three populations (overall log-rank: $\chi^2=42.7$, $df=2$, $P=5.5\times10^{-10}$; Fig. 1). Individual P-C queens had higher survival over the course of the experiment than queens from either the H-A (log-rank: $\chi^2=24.8$, $df=1$, $P=6.3\times10^{-7}$) or H-C populations (log-rank: 35.4, $df=1$, $P=2.6\times10^{-9}$, Bonferroni corrected $\alpha=0.017$). The two haplometrotic populations did not differ in queen survival (log-rank: $\chi^2=3.8$, $df=1$, $P=0.051$, Bonferroni corrected $\alpha=0.017$). Survival was also not associated with relative queen weights within groups for any of the three populations (P-C overall log-rank: $\chi^2=0.1$, $df=2$, $P=0.95$; H-C overall log-rank: $\chi^2=2.8$, $df=2$, $P=0.24$; H-A overall log-rank: $\chi^2=4.4$, $df=2$, $P=0.1$).

Aggression

Haplometrotic and pleometrotic groups varied in aggression, as measured by the number of nest boxes in which aggression was observed (overall $\chi^2=22.7$, $df=2$, $P=1.2\times10^{-5}$; Fig. 2). This variation was largely due to much lower aggression in the pleometrotic groups, as the two haplometrotic populations did not differ from each other ($\chi^2=0.6$, $df=1$, $P=0.45$). A similar pattern was seen for aggression-linked mortality, with significant variation overall ($\chi^2=30.2$, $df=2$, $P=2.7\times10^{-7}$; Fig. 2), but no difference between the haplometrotic groups ($\chi^2=0.1$, $df=1$, $P=0.77$). Differences in aggression were not absolute: Some haplometrotic groups showed no aggression and some pleometrotic groups experienced significant aggression. Specifically, in three of the 117 haplometrotic nest boxes
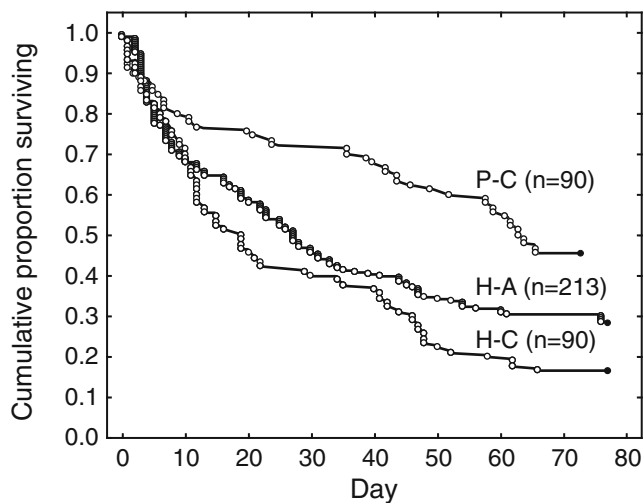
Fig. 1 Cumulative proportions of surviving queens per day over 77 days in experiment A. Queens were placed as groups of three into nests on day 1; nests were observed twice daily for the first week and once daily through the end of the experiment and the number of living and dead queens noted. Data are shown for pleometrotic queens from the California site (*P-C*), haplometrotic queens from the California site (*H-C*), and haplometrotic queens from the Arizona site (*H-A*)

(2.6 %), all three queens associated together in the same nest tube beyond first worker emergence. Likewise, six of 30 pleometrotic groups (20 %) lost at least one foundress during the experiment due to aggression-related mortality (indicated by removal of head or gaster).
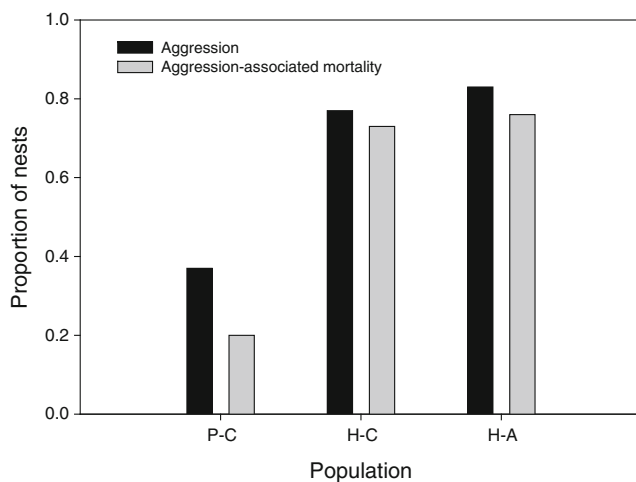

Fig. 2 The proportion of nest boxes in which aggression and aggression-related mortality were observed across 77 days in experiment A. Queens were placed as groups of three into nests on day 1; nests were observed twice daily for the first week and once daily through the end of the experiment. Aggression was scored if one or more queen was directly observed biting another queen, or if queens displayed evidence of prior aggression, generally from missing body parts. Data are shown for pleometrotic queens from the California site (*P-C*; *N*=89), haplometrotic queens from the California site (*H-C*; *N*=30), and haplometrotic queens from the Arizona site (*H-A*; *N*=30). Almost all incidences of aggression in haplometrotic groups were followed by the death of one or more queens

Clustering behavior

Queens from all three populations showed a strong tendency to gather together within a single nest tube, as indicated by the significantly higher occurrence of clustering in observed data compared to simulations (Fig. 3). Both haplometrotic and pleometrotic queen sets showed similar clustering propensities (Fig. 4). Queens from the H-A population clustered at the same frequencies as queens from the P-C population and even exceeded them on seven of 32 observation days. H-C did not differ from P-C queens in clustering on any of the 32 days, although H-C queens were significantly less clustered than H-A queens for 6 of the 30 days available for comparison.

For the majority of observations across queen types, queens grouped together in a single nest tube; in fact, 98 % of groups
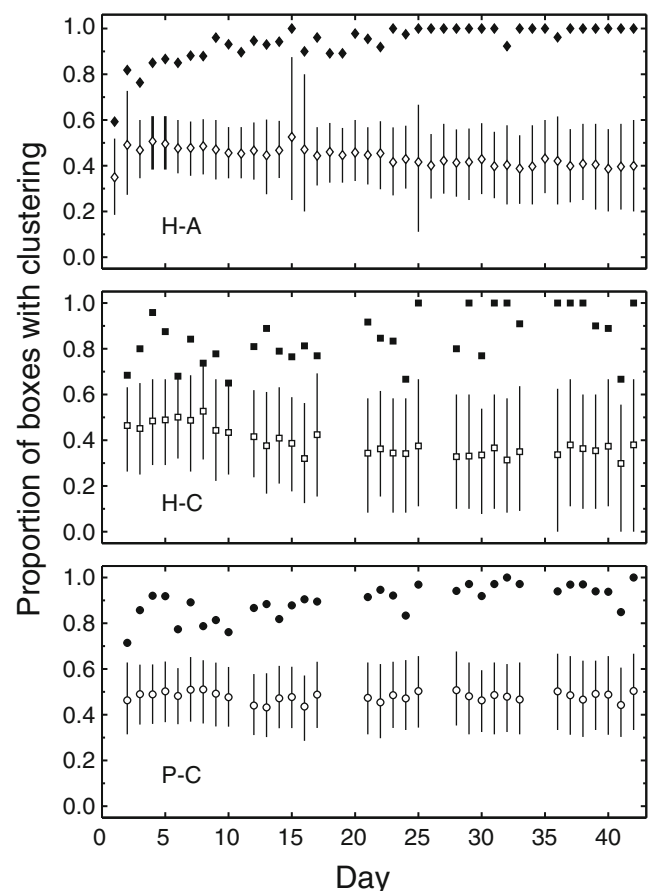

Fig. 3 Comparison of observed instances of two or more queens clustering (*closed symbols*) with that of simulated queens (*open symbols*) choosing nest tubes without regard for the presence of another queen (experiment A). Clustering is defined as two or more queens occupying the same chamber (of four possible) within a nest box. Shown are the proportions of nest boxes per day exhibiting clustering for each population: pleometrotic queens from the California site (*P-C*; *N*=30), haplometrotic queens from the California site (*H-C*; *N*=30), and haplometrotic queens from the Arizona site (*H-A*; *N*=71). *Error bars* show 95 % confidence intervals for simulated data (based on 1,000 bootstraps). All three populations differed significantly from the simulated values, indicating that all queens actively cluster
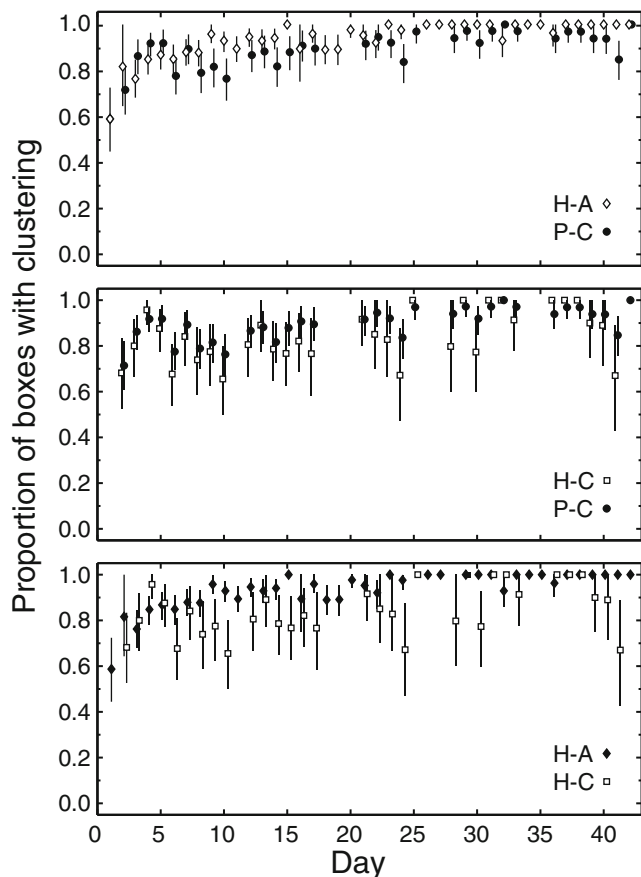
Fig. 4 Comparison of clustering within nest boxes by queens of the three populations: pleometrotic queens from the California site (*P-C*; *N*=30), haplometrotic queens from the California site (*H-C*; *N*=30), and haplometrotic queens from the Arizona site (*H-A*; *N*=71). Shown are the 3-day running means of the proportion of nest boxes in which at least two queens clustered within a single chamber (of four possible). *Error bars* show 84 % confidence intervals. There was no significant difference in the clustering behavior of queens coming from haplometrotic or pleometrotic populations

with brood clustered around a single brood pile. We made 17,525 total queen-observations, or 7,056 observations per nest box across the duration of the experiment (84 days). Of these, queens were observed in the foraging area 1,176 times (6.7 % of all queen-observations) and in separate nest tubes 366 times (5.19 % of all queen-observations). When queens were observed in different nest tubes, the behavior lasted at most for 1–2 days, after which queens were again found clustered. There were only six occurrences where queens were observed in separate nest tubes for three or more consecutive observation days.

Results: experiment B

Contact rates, aggression, and survival

Pleometrotic queens were observed contacting each other on the soil surface a total of 1,021 times, compared to 518 times for H-C

and 361 for H-C and H-A queens, respectively. Despite higher contact rates, P-C queens had lower levels of aggression (zero aggressive acts) on the soil surface than the other populations (χ^2=17.9, df=2, P=0.0001). H-A groups showed somewhat higher levels of aggression than H-C groups (χ^2=5.0, df=1, P=0.025); 16 and 20 aggressive interactions occurred in the H-C and H-A populations, respectively, out of 1,280 total observations per population. Survival data from the H-A population were not compared due to high mortality from desiccation. However, similarly to the within-nest experiments, queen survival was again higher for P-C (queens collected from Pine Valley) than for H-C queens (*t* test; t=−5.2, df=38, P=3.5×10^{-6}).

Carrying behavior and joint-founding

When queens came into contact with one another on the soil surface they: (1) ignored one another, (2) antennated one another without aggression, (3) behaved aggressively, or (4) engaged in queen-carrying behavior, in which one queen picked up the other and relocated her. The queen being carried assumed a pupal position with legs tucked without struggling. Carrying behavior was often associated with nest construction, when a queen entered a hole that another queen was excavating. Queen-carrying was observed only once in the P-C population, but was seen 10 (six of 20 groups) and 15 times (11 of 20 groups) in the H-C and H-A populations, respectively (overall χ^2=11.9, df=2, P=0.003). The two haplometrotic populations did not differ in queen-carrying (χ^2=2.6, df=1, P=0.11), but removal of queens from excavated tunnels was only seen in the H-A population (five replicates; χ^2=10.9, df=1, P=0.001).

Queens in a subset of all populations constructed nests together (joint nest founding); however, the frequency differed among populations and over time. After the initial 24 h (when all queens were still alive), 11 of 20 pleometrotic containers had two or more queens sharing one nest. At the same stage, queens in 15 of 20 H-C containers shared nests, compared to only two of 20 H-A replicates (overall χ^2=17.8, P=1.4×10^{-4}). In contrast, at the end of the experiment (21 days), all 20 P-C replicates included nest sharing, with all three queens together in 15 cases. Of the H-C replicates, nine had two or more queens by the end of the experiment. Of these, queens in only three continued to share nests, a lower proportion than for the P-C population (χ^2=16.8, df=1, P=4.1×10^{-5}). The reduction in joint founding in the H-C population was due in all cases to queen mortality rather than because of queens cohabitating and then later separating.

Discussion

The evolution of cooperative sociality requires a series of proximate behavioral changes in addition to ultimate selective

benefit. We explored this issue by asking which proximate behavioral changes occur at the transition to cooperative sociality and which may already be in place. To answer this, we compared the behavioral interactions of queens from one pleometrotic and two haplometrotic populations of the harvester ant, *P. californicus*. Queens from the haplometrotic populations displayed a combination of behaviors that potentially function as preconditions for sociality, in particular clustering together within nests, usually around a common brood pile. Aggregation behavior was context dependent, however. Queens from one of the two haplometrotic populations avoided other queens on the soil surface and at nest entrances, but the other showed a strong attraction to nest entrances being constructed by other queens; this population is geographically closer to and behaviorally more similar to the pleometrotic population. These behaviors suggest that the initial aggregation necessary for the transition to sociality is easily achieved in this system.

In contrast, haplometrotic and pleometrotic queens showed clear differences in the degree to which they tolerated and/or behaved agonistically toward other queens. Queens of both haplometrotic populations were much more aggressive than pleometrotic queens, with associated effects on queen mortality. This aggression generally destroyed the social groups in which they were placed. Even so, haplometrotic queens showed considerable variation in aggressiveness. Given a genetic basis, the variation in attraction, avoidance, and aggression displayed by haplometrotic queens (summarized in Table 1) suggests a pathway for selection to act on behaviors relevant to cooperative sociality.

Attraction to offspring as a precursor for sociality

Because ant colonies face competition from conspecifics, we expected the solitary behavioral conditions for ant queens to be avoidance and aggression. Surprisingly, haplometrotic queens clustered together within nests, despite being offered multiple separate compartments. Almost all queens, whether haplometrotic or pleometrotic, aggregated around a single brood pile through the duration of the experiment. This suggests that lack of opportunity to interact within nests is unlikely to be a barrier to social evolution.

Queens of this species lay eggs within a few days of nest construction, and all queens in an association (whether haplometrotic or pleometrotic) generally produce brood (Clark and Fewell, submitted for publication; Fewell, unpublished data). The fact that queens almost universally clustered around one brood pile suggests that they actually combined their brood and that brood may serve as a focal attractant for social aggregation. Brood attraction in the context of maternal behavior has been suggested as an important proximate mechanism for the transition from solitary to eusocial living in wasps. In the wasp case, eusociality is achieved when adult female offspring delay dispersal, remaining instead in the natal nest to perform brood care (Hunt 2007, 2012). Attraction to brood within the natal nest, a proximate behavior already present in the solitary context, sets the stage for the evolution of a novel social phenotype. Maternally directed wasp workers show similar brain gene expression patterns to queens, suggesting a co-opting of the physiological mechanisms underlying maternal care as a driver of eusociality (Toth et al. 2007). We did not directly test the contribution of brood to foundress aggregation, but attraction to brood is a fundamental component of maternal care in ants (Mas et al. 2009; Mas and Kölliker 2008; Morel and Vander Meer 1988). Brood attraction is therefore a plausible evolutionary developmental foundation for aggregation by ant queens once in the same nest.

Aggression as a barrier to social evolution

Although queens generally aggregated within nests, they did not universally tolerate each other. Our data suggest that aggression serves as perhaps the most important proximate barrier to cooperative group formation by normally haplometrotic queens. Although queens were not immediately aggressive, haplometrotic groups showed a much higher incidence of aggression over time than did pleometrotic associations. In fact, aggression-related mortality effectively destroyed most haplometrotic associations, indicating that aggression-related conflict can generate a high fitness cost at the transition to social living.

Studies show that haplometrotic queens have much lower levels of aggression and mortality in mixed haplometrotic/pleometrotic associations (Jeanson and Fewell 2008; Clark and Fewell, submitted for publication). Data also suggest that

Table 1 Summary of observed behaviors relevant to cooperative nest founding and polygyny in both experiments A (within nest boxes) and B (soil surface)

	Aggression		Queen removal		Clustering/joint founding		Tolerance	
	Exp A	Exp B	Exp A	Exp B	Exp A	Exp B	Exp A	Exp B
Pine Valley (pleometrotic)	Low	None	N/a	Low	High	High	High	High
Lake Henshaw (haplometrotic)	High	High	N/a	High	High	High	Low	Low
Salt River (haplometrotic)	High	High	N/a	High	High	Low	Low	N/a

the cumulatively high levels of mortality in haplometrotic associations may be due to conflict escalation, in that haplometrotic queens are more likely to initiate aggression and also more likely to escalate to agonistic interactions into mutual aggression (Clark and Fewell, submitted for publication). The differential outcomes for queens in different social groups illustrate that fitness outcomes of social phenotypes, including cooperation and conflict, are highly social context dependent. Expectations for the role of selection in the evolution of cooperative sociality must take such social dynamics into consideration.

Attraction to nest sites

For cooperative associations to evolve, queens must first establish nests together. Unsurprisingly, pleometrotic queens routinely constructed shared nests when placed together on the soil surface. However, the haplometrotic populations showed distinct variation in attraction during nest site selection. Queens from the Arizona population avoided other nest entrances and physically removed other queens trying to enter nests under construction. But queens from the California population often constructed joint nests, even though the groups later collapsed from aggression-related effects. This population is only 50 km from the pleometrotic population and genetically close to it, with an F_{ST} of 0.144 (Overson 2011). Thus, it may represent an intermediate stage between cooperative and solitary nest foundation. Some variation for this behavior was seen even in the Arizona population, in that a small subset of queens initiated nests together, although mortality in this population did not allow confirmation of whether these few associations persisted.

The attractiveness of newly constructed nests is not surprising. Queens commonly explore small holes, including nest entrances, as they search for nest sites. Because *P. californicus* queens are obligate foragers, they do not seal nest entrances during colony founding (Johnson 2004). New nests can be a potentially important resource for possible usurpation because they minimize the time spent on the surface and costs of construction (Tschinkel 1998; Johnson 2004). Thus, queens constructing nests likely encounter foreign queens with some regularity, especially under crowded conditions. Indeed, the ants *Solenopsis invicta* and *Lasius niger* show facultative pleometrosis in which frequency of cooperative founding is related to queen density after mating flights, and queen number per nest is a proximate response to local conditions (Nonacs 1992; Sommer and Holldobler 1995; Tschinkel and Howard 1983; Tschinkel 1998). This does not seem to be the case in *P. californicus* because queens of the different populations show consistent differences in attraction, avoidance, and aggression under common laboratory conditions. However, nest density may be an ecological factor in the evolution of pleometrosis by *P. californicus*.

Avoidance and queen removal

The two haplometrotic populations also differed qualitatively in avoidance behavior, as evidenced by queens removing (but not attacking) foreign queens around nest entrances. Variation in queen removal, coupled with the low propensity to enter other nests by the Arizona population again, suggests a series of steps from haplometrosis to pleometrosis, with the California haplometrotic population as an intermediate state, being less likely to avoid queens during nest establishment, but with higher levels of aggression than truly pleometrotic queens. The levels of inter-population variation across the behaviors of clustering, aggression, and avoidance also suggest that these social traits can be acted upon separately by selection.

Queen removal was an interesting and unexpected way to assert nest ownership. Haplometrotic queens of both populations engaged in queen removal, although only queens from the Arizona population were observed removing queens who had actually entered nests. Often the invading queen would be removed multiple times during nest construction. A similar pattern of queen removal during excavation is performed by *Pogonomyrmex barbatus* queens, who are completely haplometrotic (Fewell, personal observation). However, the behavior has never been noted in our lab to be performed by pleometrotic queens. This behavior, which must have been co-opted from its normal colony contexts of transporting brood, workers, and queens, provides a useful alternative to aggressive nest defense, especially given the high costs of aggression during nest founding.

Conclusions

Ant foundress associations provide a useful context in which to explore the mechanisms and consequences of cooperation among non-kin (Clutton-Brock 2002; Sachs et al. 2004). Our results suggest that a relatively small number of key mechanistic differences separate solitary founding queens from closely related populations in which queens behave as cooperative units with non-relatives. Certain behaviors already in place, including attraction to conspecific brood, take on new meaning in a cooperative context, by contributing to the stabilization of the group as a social unit. Others, particularly aggression and associated conflict escalation, can generate significant fitness costs to sociality.

Queens with cooperative nest founding showed clear differences in aggression and avoidance in comparison to haplometrotic queens, allowing us to dissect the specific behavioral changes at the transition between cooperative sociality and solitary living. However, all three populations showed variation in social behaviors. Tolerant individuals (those not engaging in aggressive behaviors or queen-removal) were present in haplometrotic populations, and likewise, aggressive

individuals were present in pleometrotic associations, albeit at low frequencies. Likewise, all three populations showed some variation in queens' propensities to form associations during nest construction. Thus, the transition to cooperative sociality should be relatively easily achieved under the correct environmental conditions. However, cooperative colony founding remains rare across ant taxa (Hölldobler and Wilson 1977; Keller 1995; Bernasconi and Strassmann 1999). The question thus becomes: what are the costs and benefits imposed by the ecological and social environments that limit cooperation in this system?

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