Surface hydrocarbons of queen eggs regulate worker reproduction in a social insect

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A hitherto largely unresolved problem in behavioral biology is how workers are prevented from reproducing in large insect societies with high relatedness. Signals of the queen are assumed to inform the nestmates about her presence in the colony, which leads to indirect fitness benefits for workers. In the ant *Camponotus floridanus*, we found such a signal located on queen-laid eggs. In groups of workers that were regularly provided with queen-laid eggs, larvae, and cocoons, with larvae and cocoons alone, or with no brood, only in the groups with queen-laid eggs did workers not lay eggs. Thus, the eggs seem to inform the nestmates about the queen's presence, which induces workers to refrain from reproducing. The signal on queen-laid eggs is presumably the same that enables workers to distinguish between queen- and worker-laid eggs. Despite their viability, the latter are destroyed by workers when given a choice between both types. Queen- and worker-laid eggs differ in their surface hydrocarbons in a way similar to the way fertile queens differ from workers in the composition of their cuticular hydrocarbons. When we transferred hydrocarbons from the queen cuticle to worker-laid eggs, the destruction of those eggs was significantly mitigated. We conclude that queen-derived hydrocarbon labels inform workers about the presence of a fertile queen and thereby regulate worker reproduction.

The fundamental difference between solitary and highly social insects is reproductive division of labor between one or a few breeders and their nonbreeding helpers (1–3). It is assumed that in large insect societies reproduction is regulated by pheromones (4). One hypothesis suggests that these pheromones may be coercive tools of the breeder (the queen) to prevent its helpers (the workers) from reproducing against their own fitness interests (5, 6) as a form of parental manipulation (7). According to an alternative hypothesis, they may represent cooperative signals that inform workers of how they can realize their fitness interests (8–10) in line with kin selection theory (11). In the presence of a fertile queen worker reproduction may impose costs on colony productivity, which reduces the indirect fitness gains of workers (12–14). Therefore, they should either refrain from reproducing (self-policing) or control each other's reproduction (worker policing) (13).

So far, the presence of such a cooperative signal and its compounds has been shown only in the honey bee, *Apis mellifera.* Here, the queen mandibular gland pheromone with its main component, 9 oxodecenoic acid, causes workers to refrain from reproducing (15–17). However, workers seem not always to respond to an artificial pheromone or to queen presence in *A. mellifera* (18, 19). Other factors such as brood pheromones play an important role as well in this species (19–23). Although there exists some evidence that queen pheromones affect reproduction in ant workers (24, 25), it is difficult to understand how such a queen signal can be transmitted in large colonies to reach worker groups that do not have direct contact to the queen.

Thus, there must be alternative ways of indirect communication. One way has become manifest in the honey bee, *A. mellifera*, in which messenger bees distribute the queen mandibular pheromone throughout the colony (26, 27). Another possibility of indirect communication is the use of eggs as a vehicle to distribute a queen signal throughout the colony, which has been suggested for the ant *Myrmica rubra*, where queen-produced egg clusters had some inhibitory effect on worker ovarian development (28) and for large, monogynous, and polydomous colonies of *Aphaenogaster cockerelli* or *Oecophylla* weaver ant, in which the queen remains in one restricted nest zone but her eggs are distributed by workers all over the large nest area (29). However, no experimental proof exists so far for this hypothesis. We tested the presence of queen signals on queen-laid eggs in the ant *Camponotus floridanus*.

In this species a single queen lays eggs while the majority of workers (presumably up to 10,000 per colony) remain infertile. Even in subcolonies workers do not lay eggs, although the queen is not present. However, brood items including eggs are usually carried into these subnests, which suggests an indirect communication of a queen signal by means of eggs. The experiments reported in this paper demonstrate that the presence of queen-laid eggs induces workers to refrain from reproducing. Furthermore, we show that workers differentiate between queen- and worker-laid eggs. The pattern of discrimination corresponds to differences in the composition of the egg surface hydrocarbons, which are qualitatively similar to the cuticular hydrocarbon profiles of the adults. Finally, worker-laid eggs onto which we transferred cuticular hydrocarbons of the queen were largely protected from destruction by the workers. This strongly supports our hypothesis that these hydrocarbons represent the queen signal.

Methods

Animals. Queens of *C. floridanus* ($n = 75$) were collected at the Florida Keys after the mating flight in August 2001 and transferred to the laboratory. They were cultured at 25°C (12-h day and 12-h night). Subsequently, these incipient colonies grew populations of 1,000–2,000 individuals within the next year. Experimental worker groups were provided with honey water and 1.5 cockroaches (*Nauphoeta* sp.) twice a week.

Egg Inhibition Experiment. The brood composition of queenless worker groups ($n = 19$; for each treatment 19 worker groups of 19 colonies) was varied in three different ways. Group a received 250 workers without any brood, group b received 200 workers with 50 larvae and 50 pupae, and group c received 200 workers with 35 ± 5 queen-laid eggs, 35 larvae, and 35 pupae. The groups were controlled for the presence of eggs twice a week. Whenever the number of eggs present in group c had dropped below 6, another 35 ± 5 queen-laid eggs were added. Brood from parental colonies was regularly added to approximately maintain the brood composition (group b, 50 larvae; group c, 35 ± 5 eggs).

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The groups were regularly controlled for the presence of eggs. The beginning of worker egg laying is very conspicuous, because up to 700 eggs are produced within a week. Sporadic egg laying by workers in group c cannot be excluded. However, we have no evidence that this occurred. No males were ever produced in these groups, which would be expected if workers perceive the absence of the queen.

Discrimination of Queen- and Worker-Laid Eggs. In the first experiment, worker groups each containing 150 individuals that had been orphaned <2 h ago were provided with eggs of different origin: they received either 30–35 eggs from sister workers, 30–35 eggs from their own queen, or 30–35 eggs from a foreign queen. Paired worker groups were used; i.e., nine queen colonies were used from which three worker groups were isolated each time. During the next 5 days the surviving eggs were counted daily. In the second experiment, four worker groups were isolated from queen colonies. In this case they received either 30–35 eggs from their mother queen or from sister workers (n = 9 queenright colonies for each treatment) or 30–35 eggs from sister workers treated either with the cuticular hydrocarbons of foreign queens (n = 9 queenright colonies) or with cuticular hydrocarbons from sister workers (n = 5). The sample size of the last group is smaller due to an insufficient number of eggs available at that time. Remaining eggs were counted 1, 2, and 24 h after the transfer. It is natural that worker groups remain without contact of the queen for some time, because they often form subcolonies. Therefore, we assume that the workers of the freshly orphaned groups behaved as if they were queenright.

Extraction and Transfer of Compounds. Single queens or two workers, respectively, were extracted for 15 min in 1 ml of hexane for each experiment. The extracts were fractionated on conditioned SiOH glass columns (CHROMABOND, 500 mg, Macherey-Nagel, Düren, Germany) with 4 ml of hexane, the nonpolar hydrocarbon fraction was transferred onto clean glass slides, and the solvent was evaporated. Thirty worker-laid eggs were then swiftly rolled on the extract for 5 min. A solid-phase microextraction fiber (Supelco) coated with a 7-μm polydimethylsiloxane film was used to roll the eggs. This allowed simultaneous sampling of the hydrocarbon profiles of the manipulated eggs. The extracted profiles were directly injected into the gas chromatograph. Programming of the gas chromatograph was the same as for the cuticular extraction (see *Chemical Analysis*). The hexane used had been distilled to the highest possible purity.

Chemical Analysis. Cuticular hydrocarbons from queens and workers and from eggs were extracted with solid-phase microextraction (see *Extraction and Transfer of Compounds*). The fiber was swiftly rubbed on the tergites of queens and workers for 3 min and on eggs for 2 min. Then the fiber was directly injected into the injection port of a ThermoQuest Trace GC with a split/splitless injector. We used a nonpolar capillary column [DB 1 (J&W Scientific, Folsom, CA), 20 m × 0.18 mm, 0.18-μm film thickness] with H_2 as carrier gas. The temperature was kept at 60° for 2 min with the split closed for the same time. Then temperature was raised at 60°C/min to 200°C. Temperature subsequently increased at 4°C/min to 320°C and then held constant. The injector port was kept at 260°C, and the flame ionization detector was kept at 340°C. Peak areas were computed with Chrom-Card 1.19 (CE Instruments, Milan).

One part of the GC/MS analysis was carried out with a Hewlett–Packard 5890 GC directly coupled to a 5970B mass selective detector (quadrupole mass spectrometer with 70-eV electron impact ionization). The system was controlled by a Hewlett–Packard Series 300 computer with an HP 5972/5971 ChemStation. Chromatography was performed by using a nonpolar capillary column (Restek, Bellefonte, PA; RTX-5, 15 m × 0.25 mm, 0.25-μm thickness) using helium as the carrier gas at 1 μl/min. Samples were injected in splitless mode, the split valve being closed before the sample was injected and reopened 45 seconds later. The solvent delay was set at 3 min, and the injector port was set at 250°C. The oven temperature was programmed to increase from 50°C (3 min) at 5°C/min to a final temperature of 300°C (10 min). Structures were determined by equivalent chain length and the use of standard MS databases (the National Institute of Standards and Technology/Environmental Protection Agency/National Institutes of Health mass spectral library and J. Wiley and Sons).

The other part of the GC/MS analysis was performed with a GC 8000 Series gas chromatograph (Fisons Instruments, Egelsbach, Germany) coupled to a Fisons Instruments MD 800 quadrupole mass detector. The GC was equipped either with a J&W Scientific DB-5 fused silica capillary column (30 m × 0.25

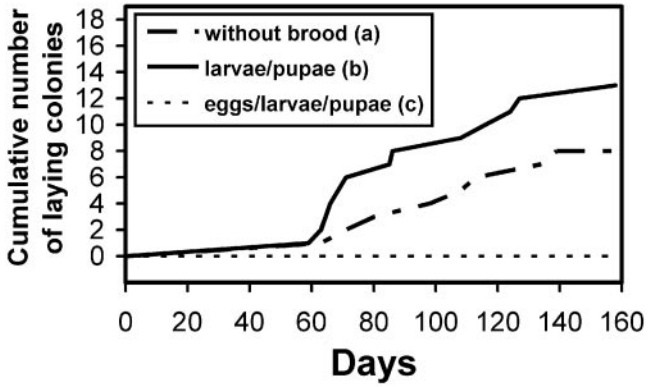

Fig. 1. Inhibition of worker egg laying by the presence of queen-laid eggs. After 160 days of separation from the parental colonies, the difference in worker egg laying among the groups was significant. Overall comparison: Cochran Q test, n = 19, Q = 17.64, $P < 0.0001$. Post hoc comparison: one-tailed Fisher's exact test, group b versus group c, $P < 0.0001$; group a versus group c, $P < 0.002$; group a versus group b, not significant.

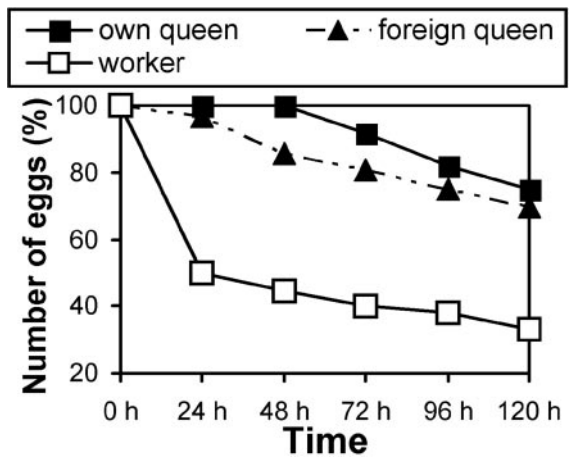

Fig. 2. Discrimination of untreated queen- and worker-laid eggs. Only medians are presented. Already after 24 h >62% of the worker-laid eggs disappeared on average whereas the queen-laid eggs remained almost untouched. The differences between the queen-laid eggs and the worker-laid eggs were statistically significant after 120 h [N_{groups} = 9; Friedman's ANOVA, $P < 0.001$, Wilcoxon-Wilcox test for multiple comparisons, $P < 0.01$ (own queen-laid eggs versus worker-laid eggs) and $P < 0.05$ (foreign queen-laid eggs versus worker-laid eggs); the difference was not significant between queen-laid eggs]. The decrease after 24 h may be partly due to accidental egg destruction during egg counting.

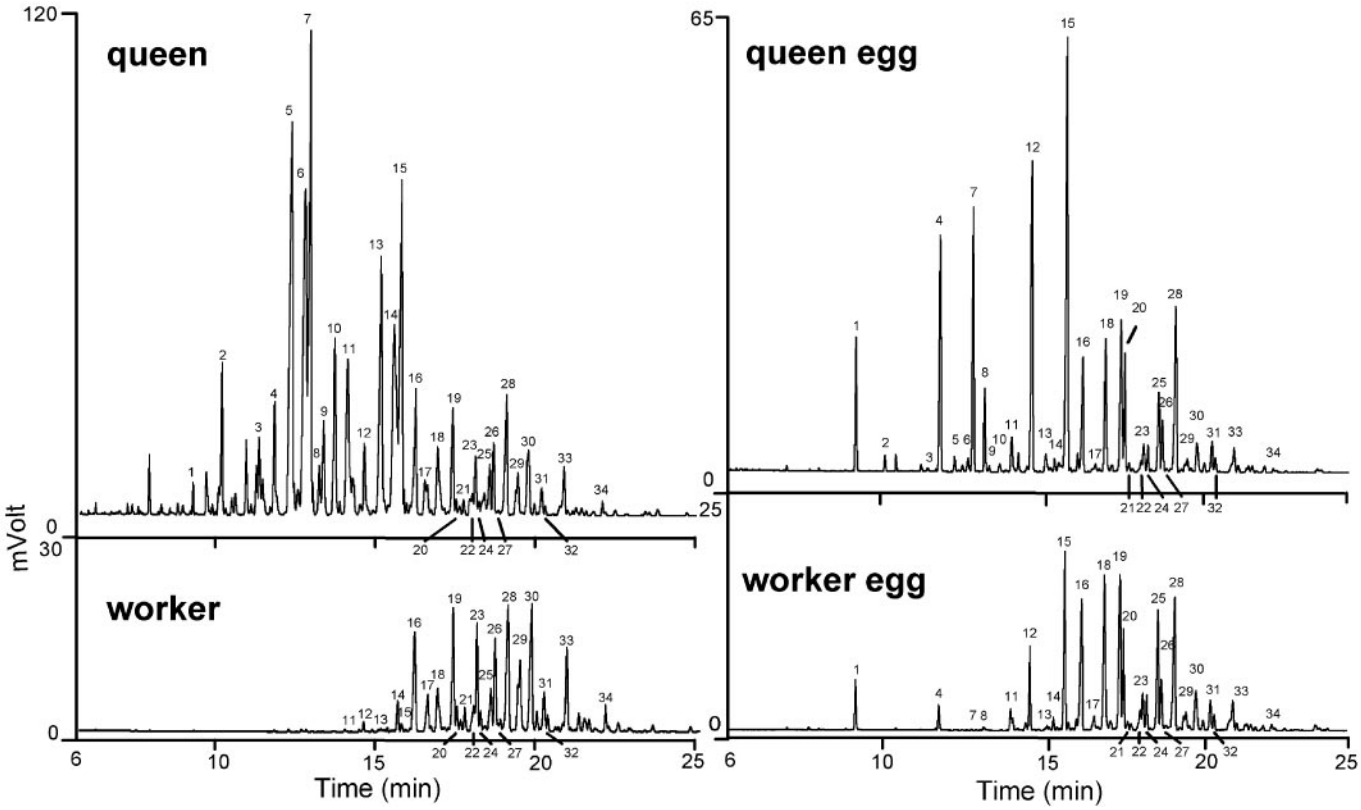

Fig. 3. Chromatograms of the surface hydrocarbons of eggs and the cuticular hydrocarbons of fertile queens and workers. The compounds have been identified on the basis of retention times (in reference to GC/MS analysis). 1, *N*-pentacosane; 2, 3-methylpentacosane; 3, 10,14-dimethylhexacosane; 4, *N*-heptacosane; 5, 9-methyl-, 11-methyl-, and 13-methylheptacosane; 6, 11,15-dimethylheptacosane; 7, 3-methylheptacosane and 7,11-dimethylheptacosane; 8, *N*-octacosane; 9, 3,7-dimethyl-, 3,9-dimethyl-, 3,11-dimethyl-, and 3,13-dimethylheptacosane; 10, 10-methyl-, 12-methyl-, and 14-methyloctacosane; 11, 12,16-dimethyloctacosane; 12, *N*-nonacosane; 13, 9-methyl-, 11-methyl-, 13-methyl-, and 15-methylnonacosane; 14, 13,17-dimethyl-, 11,15-dimethyl-, and 9,13-dimethylnonacosane; 15, 3-methylnonacosane; 16, 3,7-dimethyl- and 3,9-dimethylnonacosane; 17, 10-methyl-, 12-methyl-, and 14-methyltriacontane; 18, 4-methyltriacontane and 12,16-dimethyltriacontane; 19, 4,8-dimethyl-, 4,10-dimethyl-, 4,12-dimethyl-, and 4,14-dimethyltriacontane; 20, *N*-hentriacontane; 21, 4,8,12-trimethyltriacontane; 22, 11-methyl-, 13-methyl-, and 15-methylhentriacontane; 23, 7-methyl- and 9-methylhentriacontane and 13,17-dimethylhentriacontane; 24, 11,15-dimethylhentriacontane; 25, 3-methylhentriacontane; 26, 5,9-dimethyl-, 5,11-dimethyl-, and 5,13-dimethylhentriacontane; 27, 7,11,15-trimethylhentriacontane; 28, *N*-dotriacontane, 3,7-dimethyl-, and 3,9-dimethylhentriacontane; 29, 3,7,11-trimethylhentriacontane; 30, 3,9,15,21-tetramethyl- and 3,7,11,15-tetramethylhentriacontane; 31, 4,8-dimethyl-, 4,10-dimethyl-, 4,12-dimethyl-, and 4,14-dimethyldotriacontane; 32, *N*-tritriacontane; 33, 4,8,12,16-tetramethyldotriacontane; 34, 5,9,13,17-tetramethyltritriacontane.

mm; df = 0.25 μm; temperature program, from 60°C to 310°C at 5°C/min and held for 10 min at 310°C) or with a J&W Scientific DB-1 fused silica capillary column (30 m × 0.25 mm; df = 0.25 μm; temperature program, from 60°C to 150°C at 10°C/min, from 150°C to 310°C at 1.5°C/min, and held for 10 min at 310°C). Helium was used as carrier gas at a constant pressure of 90 kPa. Injection was carried out at 250°C in the splitless mode for 60 sec. The electron impact mass spectra were recorded with an ionization voltage of 70 eV and a source temperature of 220°C. The software XCALIBUR (ThermoFinnigan, Egelsbach, Germany) for Windows was used for data acquisition. Methylalkanes were characterized by the use of standard MS databases and diagnostic ions and by determining Kovats indices by the method of Carlson *et al.* (30).

Results

When worker groups were isolated from the queen for 160 days, some workers started laying eggs provided no brood or only larvae and pupae were present (Fig. 1). All those groups produced male brood. In 21 of the 23 experimental groups we observed the completed development of the brood until the eclosion of males, which occurred, on average, 64 days (±13.5 days SD) after the onset of worker egg laying. In contrast to these groups, isolated worker groups exposed to queen laid eggs regularly refrained from reproduction. No males were produced in these groups.

If the queen-laid eggs carry a specific queen signal that causes inhibition of worker reproduction, worker-laid eggs should not elicit this response and workers should be able to discriminate between worker- and queen-laid eggs. When eggs from a queen or from sister workers were given to freshly orphaned worker groups, the eggs from sisters were destroyed, whereas eggs from their mother or from a foreign queen were tolerated (Fig. 2). In fact, in several colonies instant destruction of worker-laid eggs was observed.

But what makes the eggs different? Chemical analysis revealed that queen-laid eggs differ from worker-laid eggs in the composition of their surface hydrocarbons (Figs. 3 and 4). We found qualitative as well as quantitative differences (Fig. 4). The profiles of the egg surface compounds show qualitative similarities to the cuticular hydrocarbon profiles of either queens or workers (Fig. 4). Therefore, we tested whether the surface hydrocarbons of the queen-laid eggs may represent the hypothesized queen signal. Because of the similarity of the cuticular hydrocarbon profiles of adults and the surface profiles of their eggs (Fig. 4), we simulated queen-laid eggs by extracting and transferring hydrocarbon blends of the cuticle of foreign queens onto worker-laid eggs. Successful manipulation was confirmed

EVOLUTION

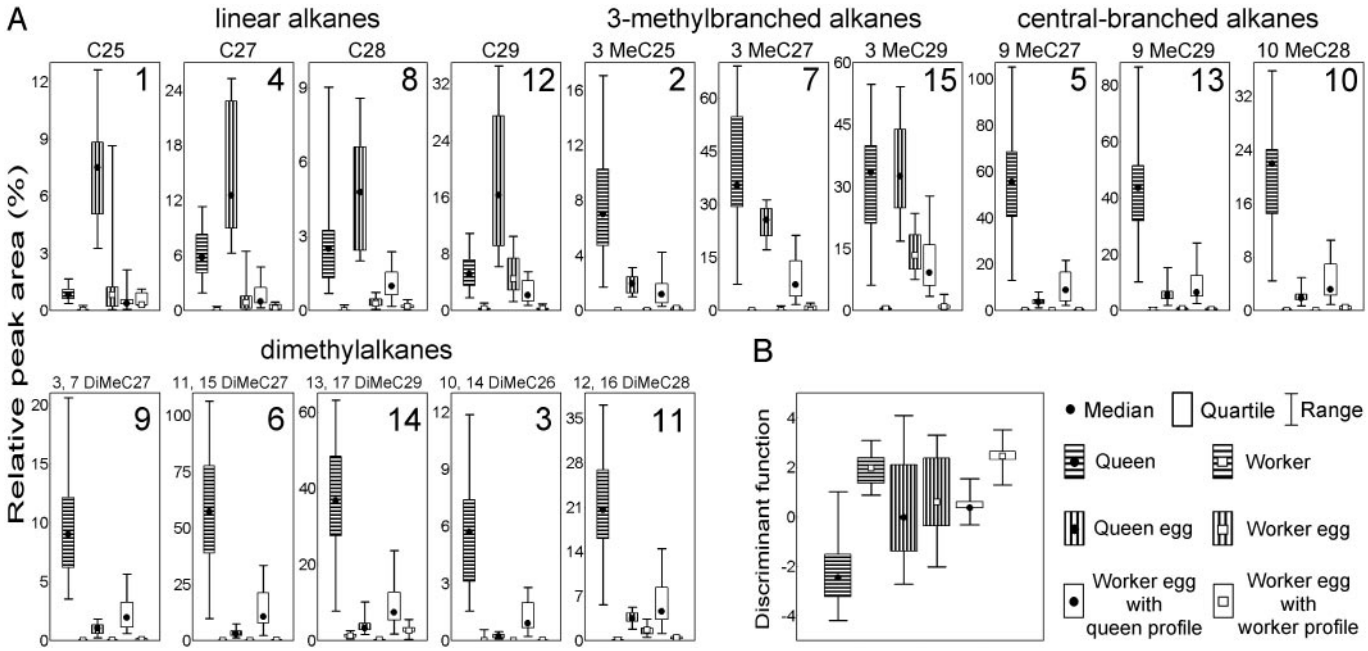

Fig. 4. Differences in surface hydrocarbons between adult queens and workers, between queen- and worker-laid eggs, and between worker-laid eggs treated with either queen or worker extracts of cuticular hydrocarbons. $n = 28$ (queens), 33 (workers), 16 (queen-laid eggs), 13 (worker-laid eggs), 9 (worker-laid eggs treated with queen extracts), and 5 (worker-laid eggs treated with worker extracts). Within a group all samples originated from different colonies to obtain independent data points. (*A*) Major differences exist between queens and workers in 15 compounds. The proportions were calculated in relation to the total amount of the other 19 compounds, leading sometimes to proportions >100%. The differences in the medians of each compound between queens and workers and between queen-laid eggs and worker-laid eggs are significant (Wilcoxon test for paired samples, $P < 0.001$). The manipulation of the worker-laid eggs simulated either queen origin or worker origin as before, because the direction of the differences in the medians between untreated eggs and between treated eggs were not different (sign test, $P > 0.6$). Single variations are described by abbreviation of compound names. The numbers in the upper right corners of the panels correspond to the compound names in Fig. 3. (*B*) Differences in the profile of the remaining compounds of adult queens and workers were determined by a stepwise discriminant analysis. The resulting discriminant function was used to determine the similarity of the profiles of the untreated and treated eggs. Four compounds were excluded from the analysis. Compounds 20 and 34 were not normally distributed, and compounds 16 and 17 did not show variance homogeneity according to Levene's test. In both cases the significance levels were corrected for multiple comparison according to Bonferroni. The stepwise procedure selected the compounds 31, 24, 23, and 27 (see compound names in Fig. 3). Only one discriminant function was extracted. The differences between the queens and workers are statistically significant (Wilk's lambda = 0.181 and $P < 0.001$). The discriminant function correctly assigned queens and workers, with the exception of three misclassifications of queens (leave-one-out criterion used). The plot of the egg hydrocarbon profiles employing this discriminant function shows that the profiles of the selected compounds of the treated eggs are within the range of the natural profiles.

by gas chromatography of hydrocarbons extracted from the treated eggs (Fig. 4). Subsequently, the reaction of worker ants toward eggs carrying a transferred queen hydrocarbon profile was compared with that exhibited toward unmanipulated eggs from queen and workers and worker-laid eggs carrying a transferred worker hydrocarbon profile. The result was unequivocal: significantly fewer worker-laid eggs carrying the transferred queen hydrocarbon profile were destroyed than worker-laid eggs although the manipulated eggs did not have the full protection of queen-laid eggs (Fig. 5).

Discussion

Our results show that queen-laid eggs induce workers to refrain from reproducing in the ant *C. floridanus*. This newly documented indirect way of queen signaling helps us to understand the mechanisms that regulate reproduction in social insects. The experimental transfer of hydrocarbon labels demonstrates that they represent the cue that enables workers to differentiate between queen- and worker-laid eggs. These hydrocarbon blends reliably signal the origin of the eggs, because their pattern closely matches that of queens or workers, respectively.

Egg Inhibition. To our knowledge this study showed for the first time the inhibitory effect of queen-laid eggs. Nevertheless, larvae have been shown to affect worker reproduction in at least two species. In the honey bee, *A. mellifera*, larvae inhibit worker ovarian activation (19–23), and in the ant *Pachycondyla apicalis* larvae affect worker reproduction in queenless groups (31). However, in these cases larvae do not directly signal queen presence; therefore, this regulation mechanism clearly differs from the mechanism described in this paper.

Egg Identification. Workers destroy worker-laid eggs but do not attack queen-laid eggs. We suggest that this discrimination is based on differences in the surface hydrocarbons of the eggs. Our manipulation experiments exclude two alternative explanations for the loss of worker-laid eggs. First, worker-laid eggs may posses a lower viability, which could elicit egg destruction in workers. Second, workers may identify the sex/ploidy of the eggs and destroy haploid, male-destined eggs preferentially. However, worker-laid eggs were not destroyed when they carried cuticular hydrocarbons of the queen. Therefore, workers primarily destroy eggs based on specific hydrocarbon profiles and not as a consequence of different viability or male determination. Actually, in those groups that produced male brood many males emerged from pupae, demonstrating the viability of worker-laid eggs. Furthermore, there is no evidence so far that workers can recognize the sex of eggs (32).

Surface Hydrocarbons of Eggs and Reproductive Physiology. Our data are further supported by a study in the queenless ant *Dinoponera quadriceps* (33). Here, the amount of one compound of the

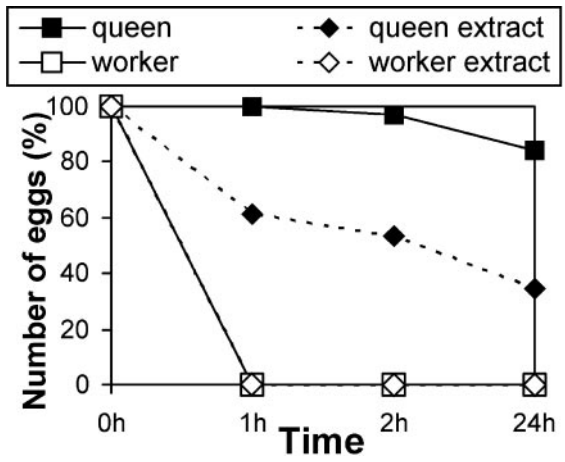

Fig. 5. The survival of eggs treated with cuticular hydrocarbons in comparison to untreated eggs. The difference between the untreated eggs from queens and workers and the eggs treated with queen profile is statistically significant after 24 h (Friedman's ANOVA; $N_{groups} = 9$; $P < 0.0005$). The important difference is between the worker-laid eggs treated with queen cuticular hydrocarbons and the untreated worker-laid eggs (Wilcoxon test for paired samples, $P < 0.02$). The sample size of the last group is smaller due to an insufficient number of eggs available at that time. However, the difference among all groups remains statistically significant with only the five samples including the control with manipulated worker-laid eggs (Friedman's ANOVA, $P < 0.005$). The important differences between worker-laid eggs treated with either queen extracts versus worker extracts are each significant (Wilcoxon test for paired samples, $P < 0.05$). The medians of workers and worker profiles overlap.

cuticular hydrocarbons of reproductive workers correlates with the amount of this substance on their eggs. Whereas in *D. quadriceps* the difference relates to one compound, eggs of workers in *C. floridanus* differ from queen-laid eggs in many compounds.

The close linkage between cuticular hydrocarbons and surface hydrocarbons of eggs is based on specific transport mechanisms in the hemolymph (34). Hydrocarbons are transported by lipoproteins to different tissues in the insect body, including the ovaries and the cuticle. In the ovaries they are incorporated in developing oocytes (34). Differences between the profiles of the eggs and the cuticle in *C. floridanus* may be due to a different transport mechanism of hydrocarbons or to changes after oviposition.

In several ant species, the hydrocarbon profiles of adults correlate with the fertility of individuals, which suggests that hydrocarbons represent a signal regulating reproduction (35–45). In fact, workers can identify gradual differences in the fertility of nestmates in some of these species (42, 46) as well as in others (47–49). In *Myrmecia gulosa*, it has been experimentally shown that workers can differentiate between the hydrocarbon profiles of reproductives and infertile workers (44). However, in these species it had not been demonstrated that cuticular hydrocarbons regulate worker reproduction.

Egg Marking. The transfer of queen cuticular hydrocarbons on worker-laid eggs had the effect that these eggs were largely not destroyed. This finding indicates that the hydrocarbons represent a queen signal. This kind of destruction of worker-laid eggs is actually a case of worker policing, i.e., the mutual control of the workers' reproduction (13, 32). Because *C. floridanus* is monogynous with a singly mated queen (50), workers should be selected to lay eggs even in the presence of their mother to maximize their inclusive fitness (13, 51). On the other hand, if worker reproduction reduces colony efficiency they should police each other's reproduction despite their greater relatedness to their sons and nephews than to their brothers (13); this seems to be the case in *C. floridanus*.

Egg marking is known from several other species. In the honey bee *A. mellifera*, worker policing of eggs occurs as well (52); this has led to a number of studies of the compounds found on queen- and worker-laid eggs and the compounds present in the queen's Dufour's gland (53) and in egg-laying workers (54–56), which appear to mimic queen specific profiles. Although there is experimental evidence that the egg identification cue originates from Dufour's gland secretions (57), behavioral experiments have failed so far to show which of the many compounds are active (58–60), and it appears that in this case hydrocarbons are not involved (58).

Egg marking also occurs in the fire ant, *Solenopsis invicta*. Here, fertile queens apply poison gland contents on their eggs (61). However, in this species workers do not lay eggs due to their lack of ovaries (4). Interestingly, the poison gland contents of the reproductive queen delay dealation and subsequently ovarian activation in winged *S. invicta* queens (62). In the queenless ant *D. quadriceps* the eggs of the reproductive workers are marked with a compound that is also found on their cuticle (33). However, no function of this compound has been shown so far. Nevertheless, the dominant worker identifies eggs from subordinates and eats them, which is a case of "queen" and not of worker policing.

Regulation of Reproduction. Our results in *C. floridanus* strongly suggest that components of the queen's hydrocarbon profile serve as a signal that regulates reproduction in a dual way: (*i*) it causes workers to refrain from reproduction (self-policing) (ref. 13 and Fig. 1) and (*ii*) it enables workers to discriminate between queen- and worker-laid eggs and to destroy the latter if necessary (worker policing) (ref. 13 and Fig. 2). Although it is not known whether it is a single compound that is the active signal, we know that the active signal is not colony-specific but is common to all *C. floridanus* queens and can be detected by all *C. floridanus* workers and hence is acting as a true queen signal present on the surface of the queen and her eggs. This finding also suggests that the workers are particularly sensitive to the signal, because they can detect it within a mixture that contains a large number of other like molecules. This could be achieved by a specific pheromone binding protein that selectively transports the signal molecules from the surface of the antennae to the receptors on sensory neurons. Krieger and Ross (63) have recently reported such a pheromone binding protein in the fire ant *S. invicta*, which, in this case, allows workers to distinguish between queens of different genotypes. On the other hand, the differences between queens and workers and their eggs in *C. floridanus* are largely linked to compound classes that are structurally different (Figs. 3 and 4*a*). Therefore, high receptor specificity would not be required to detect these differences.

In *C. floridanus*, hydrocarbons are reliable indicators of the presence of the queen and presumably also of her fertility due to the close linkage between hydrocarbon production and physiological processes. These processes are very basal and widespread, as indicated by the close correlation of variations in the cuticular hydrocarbon profile and reproductive activity in many ant species (35, 37, 41, 42). Besides their function in protecting eggs and cuticle from desiccation (64–66) and contributing to nestmate recognition (67–69), hydrocarbon profiles additionally represent a queen signal that regulates reproduction in *C. floridanus* and possibly in many other social insects as well. Workers can perceive the queen signal either directly from the queen or indirectly by means of her eggs. These two ways of signaling efficiently provide the workers with the information they rely on to adjust their reproductive activities.

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