antagonize opiate-mediated analgesia suggests that blocking the endogenous release of CCK may potentiate opiate action. Conversely, elevated levels of CCK may account for the absence of analgesia in response to morphine administration, as in morphine tolerance. Therefore blockade of CCK action may be an effective supplement to morphine administration in the treatment of chronic pain.

It is likely that CCK, in addition to modulating opiate involvement in analgesia, antagonizes other opiate-mediated behaviors, such as feeding. This idea is supported by developmental studies of the functional onset of endogenous satiety mechanisms. For instance, naloxone suppresses milk intake in suckling rat pups on day 14 of age but not earlier (*24*), and CCK first induces satiety around day 15 (*25*). However, empirical data supporting an antiopiate action of CCK for behaviors other than pain responsiveness are yet to be obtained.

Note added in proof: Itoh *et al.* (*26*) recently reported that CCK-8 suppresses β-endorphin–induced analgesia.

PATRICIA L. FARIS*
BARRY R. KOMISARUK
Institute of Animal Behavior, Rutgers University, Newark, New Jersey 07102
LINDA R. WATKINS
DAVID J. MAYER
Department of Physiology, Medical College of Virginia, Richmond 23298

References and Notes

1. E. Straus and R. Yalow, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38**, 2320 (1979).
2. M. A. Della-Fera and C. A. Baile, *Science* **206**, 471 (1979).
3. K. Fuxe, K. Anderson, V. Locatelli, D. F. Agnati, T. Hokfelt, L. Skirboll, V. Mutt, *Eur. J. Pharmacol.* **67**, 329 (1980).
4. S. Itoh, R. Hirota, G. Katsurra, K. Odaguchi, *Life Sci.* **25**, 1725 (1979).
5. N. J. Kenny, L. D. McKay, S. C. Woods, R. H. Williams, *Soc. Neurosci. (Abstr.)* **4**, 176 (1978); R. Burckhardt and G. Peters, paper presented at the Seventh International Congress of Pharmacology, Paris (1978).
6. F. Karoum, R. J. Wyatt, E. Costa, *J. Pharmacol. Exp. Ther.* **216**, 321 (1981).
7. J. E. Morley and A. S. Levine, *Science* **209**, 1259 (1980).
8. C. B. Nemeroff, A. J. Osbahr III, G. Bissette, G. Jahnke, M. A. Lipton, A. J. Prange, Jr., *ibid.* **200**, 793 (1978).
9. D. L. Margules *et al.*, *ibid.* **202**, 988 (1978).
10. E. Straus and R. S. Yalow, *ibid.* **203**, 68 (1979).
11. L. R. Watkins, D. A. Cobelli, P. Faris, M. D. Aceto, D. J. Mayer, *Brain Res.* **242**, 299 (1982).
12. L. R. Watkins, D. A. Cobelli, D. J. Mayer, *ibid.* **243**, 119 (1982).
13. Adult male Sprague-Dawley rats (350 to 500 g) were used in these experiments. In the shock procedure, a soft nylon loop was placed around the chest or hips of the rat. The loop was raised so that the shock (90 seconds at 60 Hz) could be delivered selectively to the hind or front paws. Current intensities were 1.2 mA (root-mean-square) for the hind paws and 1.6 mA (root-mean-square) for the front paws. [A detailed description of the procedure is given by Watkins *et al.* (*11*).]
14. F. E. D'Amour and D. L. Smith, *J. Pharmacol. Exp. Ther.* **72**, (1941). The tail flick test measures the latency between the onset of a radiant heat source focused on the tail and the occurrence of a spinally mediated tail flexion.
15. If a tail flick did not occur within 8 seconds the radiant heat was terminated to prevent tissue damage. The degree of analgesia was expressed as a percentage of the maximum by applying the following equation: [(EL − BL)/(8 − BL)]100, where EL is experimental tail-flick latency and BL is baseline latency (3.5 to 4.0 seconds).
16. When FSIA values for experimental animals were compared to values for saline-treated controls, the following P values were obtained by analysis of variance: at 0.75 μg of CCK-8 per kilogram, $P > .05$; at 1.5 μg/kg, $P < .0001$; at 3.0 μg/kg, $P < .0001$; and at 6.0 μg/kg, $P < .0001$. Desulfated CCK-8 (1.5 μg/kg, intraperitoneally) failed to attenuate front paw FSIA. Therefore, the antagonism by CCK-8 is a specific effect of the sulfated variant of CCK rather than a general gastrin-mediated effect.
17. T. Hokfelt, O. Johansson, A. Ljungdahl, J. M. Lundberg, M. Schutlzberg, *Nature (London)* **284**, 515 (1980).
18. L. R. Watkins, D. A. Cobelli, D. J. Mayer, *Brain Res.* **242**, 309 (1982).
19. T. L. Yaksh and T. A. Rudy, *Physiol. Behav.* **17**, 1031 (1976).
20. Demonstration of naloxone reversibility and morphine cross-tolerance is generally considered sufficient grounds for inferring opiate involvement [L. R. Watkins and D. J. Mayer, *Science* **216**, 1185 (1982)]. However, naloxone also affects nonopiate systems [*ibid.*; (*21*)], and morphine tolerance is accompanied by alterations in the concentration of several brain peptides [J. E. Morley *et al.*, *Life Sci.* **26**, 2239 (1980)]. Therefore we cannot rule out the possibility that CCK-8 is acting on other neural or hormonal components that may be unique to electrical stimulation of the front paws.
21. J. Sawynok, C. Pinsky, F. S. LaBella, *Life Sci.* **25**, 1621 (1979); G. A. Olson, R. D. Olson, A. J. Kastin, D. H. Coy, *Peptides* **2**, 349 (1981); W. A. Krivoy, D. C. Kroeger, E. Zimmermann, *Psychoneuroendocrinology* **2**, 43 (1977); L. L. Iversen, J. Nagy, P. C. Emson, C. M. Lee, M. Hanley, B. Sandberg, M. Ninkovic, S. Hunt, in *Chemical Neurotransmission*, L. Stjarne, P. Hedqvist, H. Lagercrantz, A. Wennmalm, Eds. (Academic Press, London, 1981), pp. 501–512.
22. I. Jurna and G. Zetler, *Eur. J. Pharmacol.* **73**, 323 (1981).
23. P. W. Schiller, A. Lipton, D. F. Horrobin, M. Bodanszky, *Biochem. Biophys. Res. Commun.* **85**, 1331 (1978).
24. O. Aroyewun and G. A. Barr, paper presented at the 1981 Annual Meeting of the International Society for Developmental Psychobiology, New Orleans.
25. E. M. Blass, W. Beardsley, W. G. Hall, *Am. J. Physiol.* **5**, 567 (1979).
26. S. Itoh, G. Katsuura, Y. Maeda, *Eur. J. Pharmacol.* **80**, 421 (1982).
27. Supported by PHS grants DA-00576 (D.J.M.) and BNS 78-24504 (B.R.K.). The advice and encouragement of J. Panksepp, R. Conner, D. Margules, J. Walker, and R. Hayes are greatly appreciated. We also thank I. Kinscheck and C. Banas for excellent technical assistance. Desulfated CCK-8 was generously provided by C. A. Baile. Portions of this research were presented at the 1982 Annual Meeting of the American Association for the Advancement of Science, Washington, D.C., and at the 1982 Annual Meeting of the Society for Neuroscience (Minneapolis). Correspondence should be addressed to P.L.F. Contribution No. 357 from the Institute of Animal Behavior.
* Present address: Department of Psychiatry, Washington University Medical School, 4940 Audubon Avenue, St. Louis, Mo. 63110.

28 September 1982

Regulation of Queen Number by Workers in Colonies of Social Insects

Abstract. *Experiments with fire ants suggest that queen pheromones act quantitatively in the regulation of queen number in colonies of social insects. Specific mechanisms probably include recognition by workers of unique quantitative blends of pheromones produced by queens, and quantitative effects of pheromones acting at the level of the colony on workers and at the level of the individual on queens. Several aspects of this quantitative hypothesis of pheromone action were tested.*

A major question concerning sociality among the insects is how the single queen status of most colonies is maintained. Wilson (*1*) has argued that the simplest explanation of monogyny is that it evolved through competition between queens—that is, unless queens are closely related, it is ultimately advantageous for them to avoid sharing the same nest. Although his hypothesis is supported by the common occurrence of animosity between queens (*2*), in many social insects the workers participate in the elimination of supernumerary queens, and this seemed to Wilson (*1*) to constitute a difficulty for his hypothesis. He therefore suggested that "the queen-worker complex could evolve so as to remove queens with the least familiar odor, if some of the odor differences were genetic in origin" (*3*). We have developed a hypothesis to explain the maintenance of monogyny by workers using the fire ant, *Solenopsis invicta* Buren.

Because polygynous colonies sometimes occur (*4*) in the North American population of *S. invicta*, we were able t test the responses of workers from mc nogynous and polygynous colonies t queens from both types of colony (*5* Workers that we made queenless usual accepted the unfamiliar queens mo readily than did those that were quee right, and workers from monogyno colonies tended to be more discrimin ing than those from polygynous coloni Queens from polygynous colonies w distinctly less acceptable to work from monogynous colonies, even w the colonies were queenless (Fig. 1a

In another experiment, we introdu multiple (25) foreign queens into qu less colonies; we predicted that wor from monogynous colonies would ki but one queen, whereas workers polygynous colonies would retain r than one. With few exceptions ou perimental results were in agree with the prediction (Fig. 1b) (*6*) weeks after we introduced the qu we subjected the surviving ones oviposition test (*4*). The mean num

eggs laid in 5 hours by the queens from seven colonies that became monogynous was 103 [standard deviation (S.D.), 43.6], whereas the mean number laid by the 93 queens from nine colonies that became polygynous was 36 (S.D., 19.8). The difference was significant [$P < .001$, $t(98) = 7.61$]. Previously we had shown that the queens of polygynous field colonies also have a substantially lower fecundity than do those of monogynous colonies, and that they are correspondingly less physogastric (*4*).

The hypothesis that we developed from these experiments to explain the maintenance of monogyny by workers in colonies of social insects has four main components. (i) Workers recognize queens by means of pheromones produced only by female reproductives. The exocrine secretion (or secretions) consists of a number of compounds forming a mixture that is characteristic of a species. Hence, queenless workers are able to recognize and accept foreign queens of their own species. (ii) The various constituents of this pheromonal complex are produced in different proportions by different queens, thereby providing each queen with a unique odor (*3*). Workers recognize the singular pheromonal blend of their own queen and can therefore distinguish her from all other queens of the same species. Hence, the same pheromonal mixture permits both species and individual recognition of queens by workers. (iii) Queens also produce other pheromones, such as the inhibitory pheromone (*7*), and the quantity of queen pheromones circulating in a colony is maintained within some optimal range. Wide deviations in the amount of these pheromones cause changes in worker behavior that tend to restore the level to within the optimal limits. Queenlessness causes a pheromonal deficit, resulting in the production or acceptance of a replacement queen (*8*), whereas the presence of supernumerary queens raises the level above a tolerance threshold and causes workers to behave aggressively toward some queens. (iv) Queens produce different amounts of a pheromone complex, and there is a positive correlation between amount and fecundity. Selection of queens depends on their position in this pheromonal hierarchy; the poorest queen is destroyed first and the most productive queen is left unharmed.

Components (iii) and (iv) of this hypothesis hold that a key factor in the maintenance of monogyny is the effect of pheromone quantity, which acts at the level of the colony on workers and at the level of the individual on queens. As Wilson (*1*) suggested, monogyny results from competition between queens, but they compete by means of quantitative, as well as qualitative, pheromonal differences. This increase in complexity is compounded by component (ii) of our hypothesis, because if the quantity of one or more pheromonal constituents reaches zero in the natural range of variation, a qualitative difference results. To test the quantitative effect we conducted four experiments.

1) Twelve monogynous colonies were orphaned by dividing each into two parts and discarding the queenright half (*9*). After 72 hours pairs of queens, one physogastric and the other relatively nonphysogastric (that is, of high and low fecundity, respectively), were introduced together to the queenless workers, and responses were recorded. To eliminate familiar odors, only queens that were all foreign to the workers were used, and to eliminate any possible effects of age differences on pheromone production, only queens from a single mating flight 28 months earlier were used. To obtain relatively nonphysogastric queens, food supply was reduced; the mean weights of the queens in the two categories were 24.2 mg and 16.1 mg (*10*). The workers killed the relatively nonphysogastric queen in 10 of 12 trials, in one they killed the physogastric queen, and in the other they killed both queens.

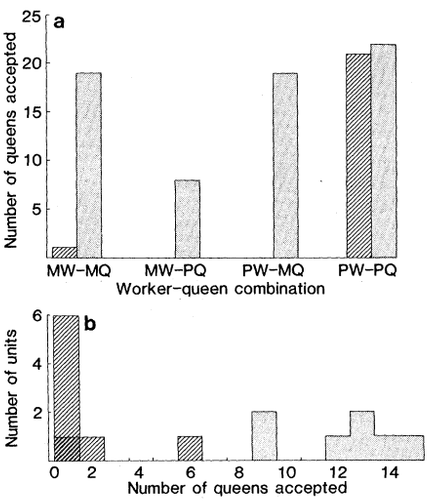

Fig. 1. Responses of fire ant workers to (a) single foreign queens and to (b) multiple foreign queens. In (a), experimental units each had about 5000 workers (*W*) and 5 cm^3 of worker brood. There were 22 units from monogynous (*M*) and 22 from polygynous (*P*) field colonies. *P* units were given only one queen from their parent colony, and all queens were marked on the thorax with Tech-Pen inks. Marked foreign queens (*Q*) from similar colonies were introduced directly into these units and responses of the workers were assessed after 24 hours, when it was clear whether or not they had been accepted. Trials were conducted in a fixed sequence to avoid the introduction of unwanted variables. While the units were queenright, an *M* queen was introduced to each. After 24 hours the units were dequeened by transferring the queens to their own parent colonies, and after 48 hours of queenlessness *M* queens were again introduced. After another 24 hours the queen of each unit was returned for 2 days. The procedure was repeated with *P* queens. Diagonal lines represent queenright units and shaded columns, queenless units. In (b), experimental units each had about 10,000 workers and 10 cm^3 of worker brood. *M* colonies ($N = 8$) and *P* colonies ($N = 8$) each received 25 queens after being queenless for 48 hours. The queens were from a mating flight that had occurred 4 weeks before. The 25 were placed in a culture tube (13 by 1.5 cm) one-third filled with water and a cotton plug and covered with wire screen through which only the smallest workers could pass to ensure that contact between the queens and workers was gradual. The tube was placed next to the nest occupied by the workers and after 48 hours the queens were released directly into it. Diagonal lines, units from *M* colonies, and shaded columns, units from *P* colonies.

2) The first experiment was repeated but with queens of unknown age and mean weights of 23.2 and 15.4 mg for physogastric and nonphysogastric queens, respectively. The relatively nonphysogastric queen was killed in all 12 trials.

3) Two foreign physogastric queens (minimum weight, 21.7 mg) were introduced simultaneously into queenless half-colonies in a similar experimental arrangement to that used in the first two experiments. This served both as a control to experiments 1 and 2 and as a test of whether the workers would retain both queens, or whether the hypothesized quantitative pheromonal effect at the level of the colony would cause them to kill one queen. The workers killed one queen in 11 trials and both queens in the twelfth. Queens were weighed every 24 hours and their weights compared. The mean weight of the surviving queens was 22.4 mg (S.D., 2.1), whereas that of the executed queens declined to only 15.3 mg (S.D., 5.1). The difference was statistically significant [$P < .01$; $t(20) = 3.9$, paired data].

4) To determine whether fecundity and amount of pheromone produced are correlated, we tested the inhibitory pheromone content of physogastric and nonphysogastric queens. Newly mated queens from a single mating flight were kept in artificial nests in which they founded colonies. After 10 weeks, 14 of these incipient colonies were united with large queenless colonies (this was done in stages to avoid execution of the nonphysogastric queens) to encourage the development of physogastry in the young queens (group 1). Another 14 colonies were left to develop at their own

rate with their nonphysogastric queens (group 2). After five more weeks, the queens in the two groups were weighed, killed by freezing, and tested for the inhibitory pheromone (*11*).

The mean weight of the queens of group 1 was 20.1 mg (S.D., 1.17) and of group 2, 10.7 mg (S.D., 1.52). The mean times taken by virgin queens to dealate were controls, 1.2 days ($N = 9$); group 1 queens, 8.6 days; and group 2 queens, 1.6 days. The difference between the queens of groups 1 and 2 was significant [$P < .001$, $t(26) = 5.3$], but group 2 queens were not significantly different from the controls. This result showed that queens produce different amounts of inhibitory pheromone and that the amount is positively correlated with fecundity. However, we do not yet know whether this pheromone is causally involved in the selection of queens for execution by workers. There is a distinct possibility that it is, but we believe that fire ant queens (and the queens of other social insects) produce a number of pheromones (*12*) that interact with each other and that the first step in understanding their effects should be to define their integrated functions and develop bioassays for these.

Although we developed our quantitative pheromonal hypothesis to explain the role of workers in maintaining monogyny in colonies of social insects, it may also explain the occasional occurrence of polygyny in essentially monogynous species. A lower fecundity of queens in polygynous colonies is evidently a general feature in the social insects (*13*) and is indicative of a lower pheromone production. More queens may therefore be present in a colony before the tolerance threshold of the workers is exceeded. Our experimental results suggest that this threshold is, in any case, higher among the workers of polygynous colonies.

The hypothesis also suggests explanations for other well-known social phenomena. For example, it seems probable that the behavioral dominance hierarchies formed in colonies of primitively eusocial Hymenoptera, such as paper wasps, *Polistes* spp., reflect quantitative pheromonal hierarchies, since position in the dominance order is positively correlated with the degree of ovarian development, with the queen occupying the alpha position (*14*). Further, temporary social parasitism occurs in a variety of ant genera, for example, *Formica* and *Lasius* (*1*), and acceptance of the parasitic queen by the workers of the host colony may well depend on her being pheromonally superior to the host queen. Since host and parasite are closely related taxonomically, their pheromones are probably very similar or even identical, and quantitative superiority would be a relatively minor evolutionary adaptation.

Our hypothesis may well have implications for the management of both beneficial and harmful species of social insects.

DAVID J. C. FLETCHER
MURRAY S. BLUM
Department of Entomology, University of Georgia, Athens 30602

References and Notes

1. E. O. Wilson, *The Insect Societies* (Harvard Univ. Press, Cambridge, Mass., 1971).
2. Examples are queens of the honey bee, *Apis mellifera* L. [C. R. Ribbands, *The Behaviour and Social Life of Honeybees* (Bee Research Association, London, 1953)], and of the ant, *Camponotus ligniperda* Latr. [B. Hölldobler, *Z. Angew. Entomol.* **49**, 337 (1962)].
3. The assumption of Wilson that workers recognize queens by their odor is reasonable, although workers may have to touch a queen in order to receive the complete pheromonal signal, as is evidently the case in the honey bee [J. Simpson, *J. Apic. Res.* **18**, 233 (1979)]. The probability that audio, visual, or other signals are involved in recognition seems negligible. Evidence for the existence of genetically controlled "odor" differences between individuals has been obtained for both honey bee queens [M. D. Breed, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2635 (1981)] and workers (M. D. Breed, *Anim. Behav.*, in press) and also for primitively social bees of the species *Lasioglossum zephyrum* [L. Greenberg, *Science* **206**, 1095 (1979)].
4. D. J. C. Fletcher, M. S. Blum, T. V. Whitt, N. Temple, *Ann. Entomol. Soc. Am.* **73**, 658 (1980).
5. Colonies were maintained in the laboratory as described in (*4*).
6. E. O. Wilson [in *Symp. R. Entomol. Soc. London* **3**, 81 (1966)] introduced several foreign queens to queenless (evidently formerly monogynous) colonies of *S. invicta* by chilling the entire colony, with similar results.
7. D. J. C. Fletcher and M. S. Blum, *Science* **212**, 73 (1981).
8. C. G. Butler [*Proc. R. Entomol. Soc. London A* **35**, 129 (1960)] suggested that a deficit of queen pheromones might be the cause of queen rearing by worker honey bees during swarming and queen supersedure.
9. Each half-colony consisted of an estimated 15,000 to 20,000 workers and abundant worker brood, but no adult or immature sexuals.
10. The fecundity of a queen may be conveniently measured by weighing her, since physogastry of mated queens in established colonies is caused almost exclusively by the greatly enlarged ovaries.
11. D. J. C. Fletcher and M. S. Blum *J. Ga. Entomol. Soc.* **16**, 352 (1981).
12. Queen fire ants are known to produce an attractant pheromone [R. K. Vander Meer, B. M. Glancey, C. S. Lofgren, A. Glover, J. H. Tumlinson, J. Rocca, *Ann. Entomol. Soc. Am.* **73**, 609 (1980)] as well as an inhibitory pheromone.
13. C. D. Michener, *Insectes Soc.* **11**, 317 (1964).
14. L. Pardi, *Boll. Ist. Dei Entomol. Univ. Studi Bologna* **15**, 25 (1946).
15. We thank D. F. Howard for helpful criticism of the manuscript. Supported by grants from the American Farm Bureau Federation and the University of Georgia.

14 May 1982; revised 12 October 1982

An Unusual Lepidopteran Sex Pheromone System in the Bagworm Moth

Abstract. *The female sex pheromone of the bagworm moth is* (R)*-1-methylbutyl decanoate. The antipode is biologically inactive and it neither enhances nor detracts from the potency of the* R *enantiomer. Unlike other moths for which female pheromones have been identified, the female secretes the pheromone from glands on her thorax and it is disseminated from hair that is shed from her body.*

We report a novel sexual communication system in the bagworm moth, *Thyridopteryx ephemeraeformis* (Haworth). Unlike other moths that secrete sex pheromones (*1*) from glandular tissue on the tips of their abdomens (*2*), the wingless adult female bagworm produces pheromone from glands situated on its thorax. The cryptic female dispenses the chemical from morphologically specialized, deciduous hairs that are cast from her body. We have identified the pheromone as (*R*)-1-methylbutyl decanoate; the males are ostensibly anosmatic to the antipode. The pheromone is among the first long-chain fatty acid esters to be identified as a sex pheromone in the Lepidoptera (*3*), and it is one of the rare chiral pheromones discovered in the order.

The herbivorous bagworm is a biological curiosity and a serious defoliator of

Table 1. Male bagworm responses to the enantiomers and racemate of 1-methylbutyl decanoate on cotton rolls or to a virgin female placed in Pherocon 1C (Zoecon) insect traps positioned 20 m apart, 1.5 m from the ground, and baited daily. The test was replicated five times on each of 5 days in mid-September 1981, near Beltsville, Maryland; a randomized complete-block design was used. Means followed by the same letter are not significantly different from each other according to Duncan's New Multiple Range Test. Male responses to *S* enantiomer treatments was due to 2 percent *R* in the *S* enantiomer (*9*).

Treatment	Capture (males per trap per day)
R (0.5 mg)	27.3 a
Racemate (1.0 mg)	24.6 a
Racemate (0.1 mg)	7.3 b
R (0.05 mg)	4.1 b
Virgin female (one)	3.0 c
S (0.5 mg)	2.1 cd
S (0.05 mg)	0.2 e