

were treated with pI-pC, and cells were analyzed by flow cytometry. The Ly5.2⁺ peripheral lymphocytes from MxCreMcl-1^{fl} mice were lost within 2 weeks (Fig. 3B); however, the Ly5.1⁺ wild-type BM promoted the survival of the chimeric mice for more than 14 weeks after MxCre-mediated deletion. Liver lysates from pI-pC-treated MxCreMcl-1^{fl} chimeric mice contained no detectable MCL-1 expression (Fig. 3C). Thus, deletion of *Mcl-1* in the liver is efficient, but nonhematopoietic effects of deletion do not appear to be responsible for the failure of the animals to survive.

Expression of MCL-1 is controlled by growth factor signaling pathways. Both mature lymphocytes and lymphoid progenitors increase expression of MCL-1 in response to interleukin (IL)-7 signaling (13). Stem cell factor (SCF) induces expression of MCL-1 in a human BM-derived cell line (23). To determine whether *Mcl-1* is expressed in response to growth factors in HSCs, we used real-time PCR. The amount of *Mcl-1* mRNA was greater 30 min after exposure of purified HSCs to SCF. IL-6 had a smaller effect, whereas culture with IL-11 did not induce expression (Fig. 4A) (24).

To assess whether *Mcl-1* is required for the survival of cultured BM progenitor populations exposed to growth factors, we used retroviral transduction of Cre into purified BM progenitor populations from *Mcl-1*^{fl} or wild-type mice (25). The purified BM progenitor populations (HSC, CMP, CLP, and GMP) were cultured in appropriate growth factors (26). By 48 hours after retroviral transduction, more than 90% of *Mcl-1*^{fl} Cre-expressing [enhanced green fluorescent protein positive (EGFP⁺)] progenitor cells (HSCs, CMP, and CLPs) were apoptotic (Fig. 4B). Expression of Cre in wild-type BM progenitor populations did not induce apoptosis (Fig. 4B). Therefore, survival of BM progenitors in vitro requires the expression of *Mcl-1* induced by early-acting cytokine signals.

Although previous studies had implicated anti-apoptotic BCL-2 family members in regulating the homeostasis of hematopoietic progenitors (3), our studies indicate that a single anti-apoptotic BCL-2 family member, MCL-1, is essential for promoting the survival of HSC and other hematopoietic progenitors.

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Identification of the Sex Pheromone of the German Cockroach, *Blattella germanica*

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The sex pheromone of the German cockroach, *Blattella germanica*, has been characterized as gentisyl quinone isovalerate. This cockroach is a major cause of allergic disease and serves as a mechanical vector of pathogens, making it one of the most important residential and food-associated pests worldwide. The sex pheromone-producing gland in adult females was identified in 1993, but thermal instability of the pheromone made characterization difficult. Now, using a new preparative gas chromatography approach coupled with electroantennographic detection, we have isolated and characterized the pheromone, which we term blattellaquinone, and confirmed the identification by chemical synthesis. The synthetic pheromone was active in behavioral assays and highly effective in field trapping tests, which suggest that it may provide a new tool in cockroach population detection, monitoring, and control.

A sex pheromone that eluded natural product chemists for several decades has been characterized for the German cockroach, *Blattella*

germanica, one of the most important residential and food-associated pests worldwide. Movement of these cockroaches between human and animal waste and food materials allows them to acquire, carry, and mechanically transfer pathogens (1, 2). Moreover, exposure to cockroach-derived allergenic proteins in homes is associated with allergic disease and asthma, particularly in inner-city children (3). Cockroach control, coupled with extensive cleaning, can result in large reductions in cockroach allergens in settled household dust (4-6), but a fundamental constraint in abatement programs has been a lack of effective attractants to lure cock-

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roaches into traps and insecticide baits (7). This deficiency is probably the most important single factor contributing to a continued reliance on scheduled applications of broad-spectrum insecticides to control cockroaches.

A nonvolatile, courtship-eliciting sex pheromone on the female's cuticular surface was previously identified (8), but this pheromone does not have any obvious value in pest control. Based on behavioral and electrophysiological assays, a female sex pheromone that attracts males over some distance, and hence of potential utility in pest control, was discovered in 1993 (9). The pheromone-producing gland was anatomically localized to the pygidium, the last abdominal segment (10), and we observed that virgin females, but not mated females, engage in a characteristic behavior ("calling behavior"), during which they become exceptionally attractive to males (11, 12). Females at this physiological stage are also highly receptive to courting males, and organic solvent extracts of these females specifically attract males, but not adult females or nymphs (9). However, the minute quantity of attractant produced by each female and the thermal instability of the pheromone have hampered efforts to isolate it. Here we report the purification and identification of the pheromone compound, which we accomplished by using a highly sensitive gas-chromatographic-electroantennogram detector (GC-EAD), in which the male cockroach antenna served as a biological detector, and development of a technique for GC purification of a small, thermally unstable sample for nuclear magnetic resonance (NMR) analysis (13).

The pygidia of ~15,000 virgin females were carefully dissected, extracted in dichloromethane, and assayed for attractiveness to males, females, and nymphs in a two-choice olfactometer device (14). We then followed a behaviorally guided chromatographic fractionation of the extract to purify the active fraction. First, the total lipid extract of the pygidia was separated on a silica gel column by eluting successively with mixtures of pentane-ether. The 40% ether fraction, which elicited the highest behavioral responses in males, was subjected to preparative high-performance liquid chromatography (HPLC) purification, and the active fraction was subjected to coupled GC-EAD analysis and GC-mass spectrometry (GC-MS) analysis and further purification using preparative GC (14). GC-EAD analyses of the behaviorally active fractions from preparative HPLC consistently revealed a single EAD-active compound (fig. S1).

The behaviorally active and EAD-active compound was subjected to GC-MS analysis in electron impact (EI) mode (14). It showed a base peak at a mass/charge ratio (m/z) of 57 (100%) and characteristic ions at m/z = 60 (68%), 122 (24%), 138 (16%), 150 (1.7%),

152 (1.3%), 162 (2.7%), 176 (2.0%), 180 (7.1%), 222 (0.9%), and 224 (1.1%). In chemical ionization (CI) GC-MS, the EAD-active compound showed a set of characteristic ions at m/z = 223 (51%), 251 (27%), and 263 (8.9%). Both the EI and CI mass spectra of the natural pheromone indicated a molecular mass of 222 g/mol (EI m/z = 222 and CI m/z = 223), suggesting a number of likely molecular formulas, including $C_{12}H_{14}O_4$, $C_{13}H_{18}O_3$, $C_{14}H_{22}O_2$, $C_{15}H_{26}O$, $C_{15}H_{10}O_2$, $C_{16}H_{14}O$, $C_{16}H_{30}$, and $C_{17}H_{18}$. Fragmentation in the EI mass spectrum gave little

useful information to pare down the number of formulas.

The 600-MHz 1H -NMR spectral data (Fig. 1) supported a $C_{12}H_{14}O_4$ molecular formula. Analysis of chemical shift and coupling data suggested a structure consistent with an isovalerate ester and a *para*-benzoquinone (14). The primary alcohol of *p*-benzoquinone is gentisyl alcohol, and the corresponding quinone is gentisyl quinone (fig. S2). Thus, gentisyl quinone isovalerate was proposed as the structure for the pheromone compound (Fig. 1, inset). Because this is a

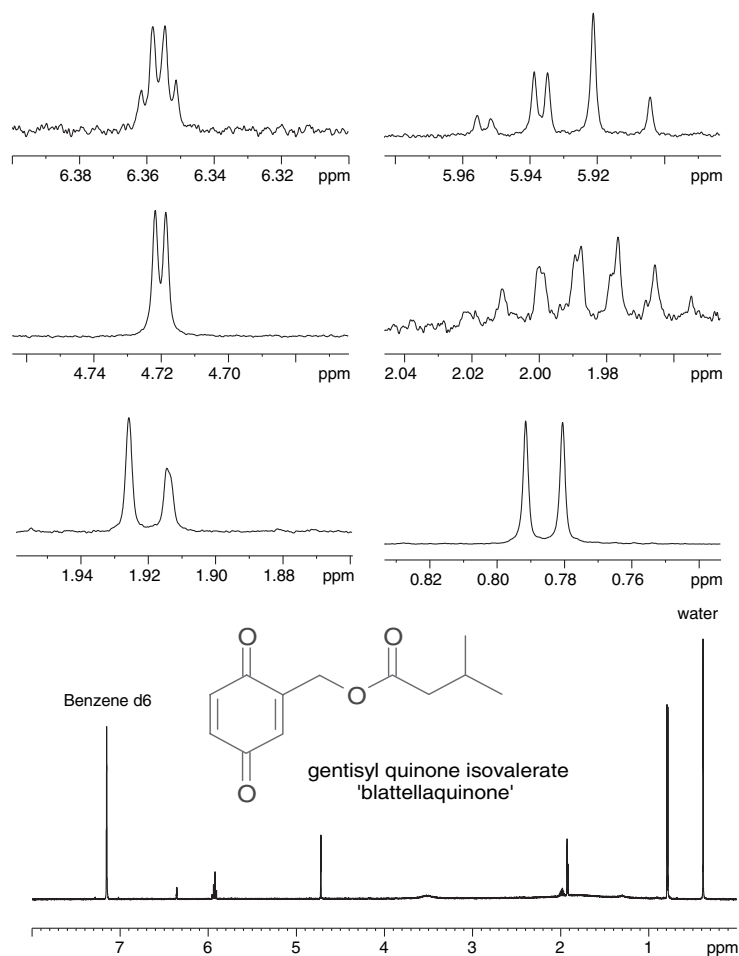


Fig. 1. The 600-MHz 1H -NMR spectrum of the natural pheromone purified by preparative GC. The inset shows the chemical structure of blattellaquinone.

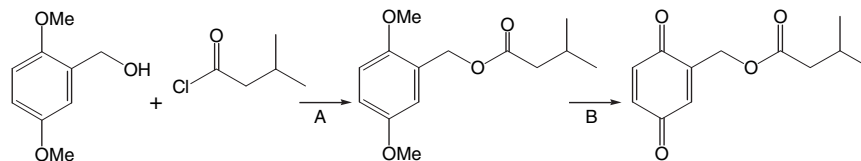


Fig. 2. Scheme for the synthesis of blattellaquinone. Acylation (A) involved addition of isovaleryl chloride to a solution of 2,5-dimethoxybenzyl alcohol, pyridine, and DMAP in CH_2Cl_2 . Excess acid chloride was removed with saturated sodium bicarbonate, and the mixture was extracted with ether, washed with brine, and dried over anhydrous sodium sulfate. The crude ester in acetonitrile was oxidized (B) by adding a solution of $Ce(NH_4)_2(NO_3)_6$ in water. The mixture was extracted with CH_2Cl_2 and redissolved in ether; aqueous sodium bicarbonate was then added, and the ether extract was washed with brine and dried over anhydrous sodium sulfate. See (14) for more details.

previously unknown compound, and there are no recorded chemical shifts of gentisyl quinone esters in deuterobenzene, proof of structure was dependent on chemical synthesis (Fig. 2). The NMR spectrum of the synthetic compound (fig. S3) was found to be identical to that of the natural pheromone (fig. S2). We propose the common name “blattellaquinone” for this pheromone.

The biological activity of synthetic blattellaquinone was compared to that of a crude extract of virgin females in behavioral assays using two-choice olfactometers (14). Males exhibited a clear dose-response to the synthetic pheromone (Fig. 3). More than 60% of the males responded within 1 min by running toward 10 to 100 ng of the pheromone loaded on a filter paper disk. Responding males ran up the olfactometer within 16.4 ± 2.7 s (10 ng) and 8.9 ± 2.2 s (100 ng) of the introduction of the pheromone. When making a choice between 100 pg of synthetic blattellaquinone and a solvent control, $93.8 \pm 6.2\%$ of 53 responding males chose the pheromone. This is similar to the percentage of males ($92.5 \pm$

2.1% of 244 males) that chose the crude dichloromethane extract of one virgin female over a solvent control. However, at high doses (10 and 100 μg), we observed that many males became disoriented as they approached the pheromone, and only $52.3 \pm 7.2\%$ (of 55 responsive males) and $68.6 \pm 9.6\%$ (of 53 responsive males), respectively, chose the pheromone. These observations suggest that precise doses and careful formulations will be required to optimize the efficiency of this pheromone in pest control.

Field tests of blattellaquinone were performed in a cockroach-infested pig farm. Whereas nymphs and adult females did not respond at any dose of the pheromone between 0 and 1 mg, adult males exhibited a clear dose-response in their behavioral attraction to pheromone-baited traps (Fig. 4). These results confirm that blattellaquinone is a female sex pheromone of *B. germanica* that specifically attracts conspecific males.

Substituted benzoquinone compounds are ubiquitous animal and plant excretions and are most commonly used as defensive secretions

and feeding deterrents (15). Quinone-containing defensive compounds have been identified in some cockroaches (16). It is possible, therefore, that blattellaquinone also served a defensive function in *B. germanica* and was co-opted to play a role in sexual communication, as have some other multifunctional semiochemicals (e.g., cuticular hydrocarbons serve in waterproofing as well as in sexual and nestmate recognition in some insects). Our preliminary observation that only nanogram amounts of this compound are stored by females suggests, however, that blattellaquinone probably no longer functions in defense. It will be important to determine whether this compound occurs in immature cockroaches and in adult males, although organic solvent extracts of these stages fail to attract males (9).

The identification of blattellaquinone as the sex pheromone of *B. germanica* culminates a long and arduous search for a volatile attractant in this important pest species. It now offers new options in cockroach population control and allergen mitigation.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/307/5712/1104/DC1
 Materials and Methods
 Figs. S1 to S3
 References and Notes

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Fig. 3. Behavioral responses of male *B. germanica* to one female equivalent of a crude dichloromethane extract and to various doses of blattellaquinone. The pheromone was applied in 5 μl of dichloromethane to a filter paper disk and presented to single males acclimated in the two-choice olfactometer versus a dichloromethane-treated control filter paper disk. Behavioral response is defined as quiescent males that were stimulated to run out of their cages within 60 s. Each assay block consisted of testing 10 to 16 males in individual olfactometers and means \pm SEM were calculated from 3 to 18 blocks of assays. The inset shows a male German cockroach responding to blattellaquinone emanating from a filter paper disk.

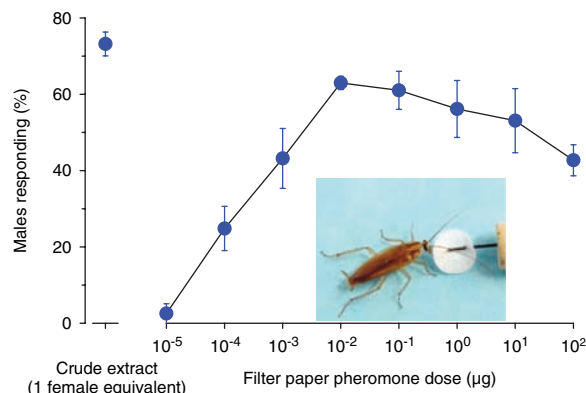


Fig. 4. Trap catches of *B. germanica* using various doses of the synthetic pheromone, blattellaquinone. The pheromone was applied in 50 μl of dichloromethane to a 5-mm sleeve-type rubber septum, which was hung inside a 1-pint (~470 ml) wide-mouth mason jar whose inside wall was treated with a thin layer of petroleum jelly (to prevent trapped cockroaches from escaping). Seven treatments were placed in a completely randomized Latin square design along five walls of a cockroach-infested pig farm, and treatments were rotated daily so that all positions along the wall received all seven treatments over seven consecutive nights (i.e., 35 replicates per dose). Data were log-transformed and analyzed by multiway analysis of variance, followed by Fisher’s least significant difference mean separation (17). An independent trial (not shown) extended the dose-response using three treatments (0, 1, and 10 mg) along seven walls (21 replicates per dose); there were no significant differences between 1- and 10-mg doses.

