were treated with pI-pC, and cells were analyzed by flow cytometry. The Ly5.2$^+$ peripheral lymphocytes from MxCre*Mcl-1*$^{f/null}$ 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 MxCre*Mcl-1*$^{f/null}$ 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*$^{f/f}$ 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*$^{f/f}$ 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.

References and Notes

1. A. J. Wagers, J. L. Christensen, I. L. Weissman, *Gene Ther.* **9**, 606 (2002).
2. M. Kondo *et al.*, *Annu. Rev. Immunol.* **21**, 759 (2003).
3. J. Domen, S. H. Cheshier, I. L. Weissman, *J. Exp. Med.* **191**, 253 (2000).
4. J. Domen, *Apoptosis* **6**, 239 (2001).
5. M. C. Wei *et al.*, *Science* **292**, 727 (2001).
6. L. Scorrano *et al.*, *Science* **300**, 135 (2003); published online 6 March 2003 (10.1126/science.1081208).
7. E. H. Cheng *et al.*, *Mol. Cell* **8**, 705 (2001).
8. D. J. Veis, C. M. Sorenson, J. R. Shutter, S. J. Korsmeyer, *Cell* **75**, 229 (1993).
9. N. Motoyama *et al.*, *Science* **267**, 1506 (1995).
10. A. Ma *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4763 (1995).
11. N. Motoyama, T. Kimura, T. Takahashi, T. Watanabe, T. Nakano, *J. Exp. Med.* **189**, 1691 (1999).
12. J. L. Rinkenberger, S. Horning, B. Klocke, K. Roth, S. J. Korsmeyer, *Genes Dev.* **14**, 23 (2000).
13. J. T. Opferman *et al.*, *Nature* **426**, 671 (2003).
14. K. Akashi, D. Traver, T. Miyamoto, I. L. Weissman, *Nature* **404**, 193 (2002).
15. M. Kondo, I. L. Weissman, K. Akashi, *Cell* **91**, 661 (1997).
16. K. Akashi *et al.*, *Blood* **101**, 383 (2003).
17. RNA was extracted (Trizol, Invitrogen, Carlsbad, CA) and cDNA synthesized (Promega, Madison, WI) for Sybr-green TaqMan real-time PCR analysis (Applied Biosystems Incorporated, Forest City, CA). Primers were Mcl-1 (forward, AGAGCGCTGGAGACCCTG; reverse, CTATCTTATTAGATATGCCAGACC) and hypoxanthine-guanine phosphoribosyl transferase (HPRT) (forward, GTTGGATACAGGCCAGACTTTGTTG; reverse, GAGGGTAGGCTGGCCTATAGGCT).
18. R. Kuhn, F. Schwenk, M. Aguet, K. Rajewsky, *Science* **269**, 1427 (1995).
19. BM was cultured in M3434 Methocult (Stem Cell Technologies, Vancouver, Canada) containing insulin, transferrin, SCF, IL-3, IL-6, and erythropoietin for 7 days.
20. Stained BM was analyzed on a FACSCalibur (Becton-Dickinson), and antibodies were purchased from BD-Pharmingen (San Diego, CA), except for anti-CD127 (eBioscience, San Diego, CA).
21. Cells were lysed in radioimmunoprecipitation assay buffer immunoblotted by using anti-MCL-1 (Rockland Immunochemical, Gilbertsville, PA) and anti-β-Actin (Chemicon, Temecula, CA).
22. J. T. Opferman, S. J. Korsmeyer, unpublished data.
23. H. M. Huang, C. J. Huang, J. J. Yen, *Blood* **96**, 1764 (2000).
24. Lineage-negative BM was positive-selected with the use of magnetic beads (Dynal, Oslo, Norway). HSCs (lineage$^-$c-Kit$^+$Sca-1$^+$) purified with the use of a MoFlow (Cytomation, Ft. Collins, CO) cell sorter were starved in cRPMI-10% fetal calf serum for 4 hours. SCF, IL-6, or IL-11 were administered to the cultures, and cells were harvested at 30, 120, and 160 min. *Mcl-1* expression levels were determined by real-time PCR.
25. H. Iwasaki *et al.*, *Immunity* **19**, 451 (2003).
26. Fluorescence-activated cell sorter (FACS)–purified progenitors were enriched from wild-type or *Mcl-1*$^{f/f}$ mice, transduced with retroviral MSCV-Cre-IRES-EGFP, and cultured in growth factors (10 ng/ml). HSC growth factors were SCF, IL-11, IL-6, and leukemia inhibitor factor. CMP growth factors were SCF, IL-11, IL-6, and IL-3. CLP factors were SCF, IL-7, and Flt3-ligand; for GMP, they were SCF, IL-11, IL-6, and IL-3. After 48 hours, the cells were stained with annexin-V and analyzed by FACS.

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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 ^{1}H-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

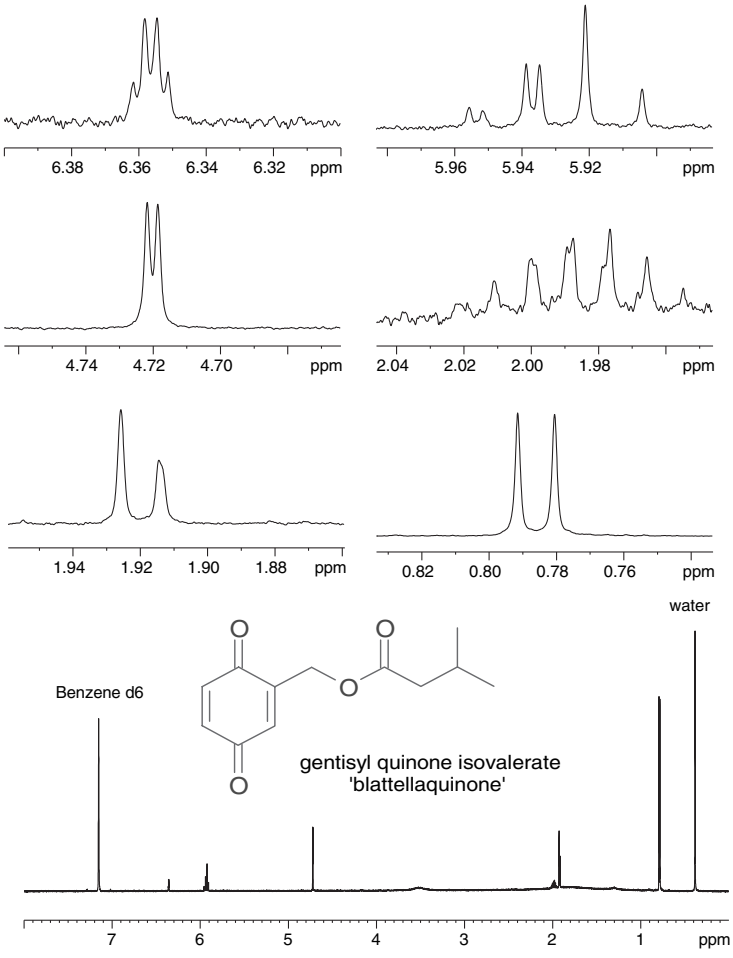

Fig. 1. The 600-MHz ^{1}H-NMR spectrum of the natural pheromone purified by preparative GC. The inset shows the chemical structure of blattellaquinone.

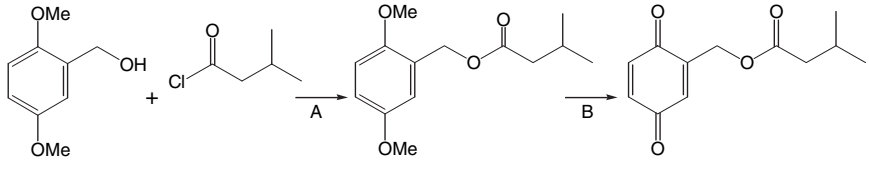

Fig. 2. Scheme for the synthesis of blattellaquinone. Acylation (A) involved addition of isovaleryl chloride to a solution of 2,5-dimethoxybenzyl alcohol, pyridine, and DMAP in CH_2Cl_2. Excess acid chloride was removed with saturated sodium bicarbonate, and the mixture was extracted with ether, washed 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 ± 6.2% of 53 responding males chose the pheromone. This is similar to the percentage of males (92.5 ± 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 ± 7.2% (of 55 responsive males) and 68.6 ± 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.

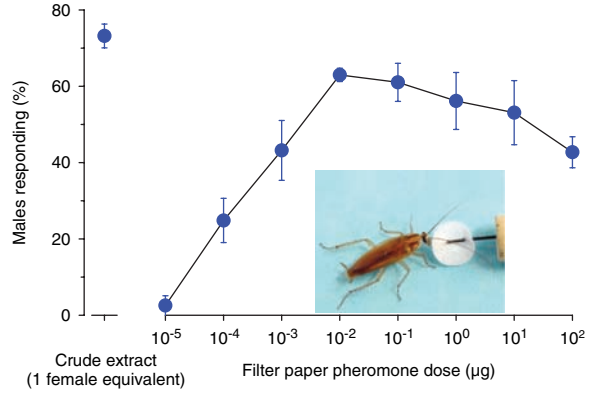

Fig. 3. Behavioral responses of male *B. germanica* to one female equivalent of a crude dichloromethane extract and to various doses of blattellaquinone. The pheromone was applied in 5 μl of dichloromethane to a filter paper disk and presented to single males acclimated in the two-choice olfactometer 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 ± 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.

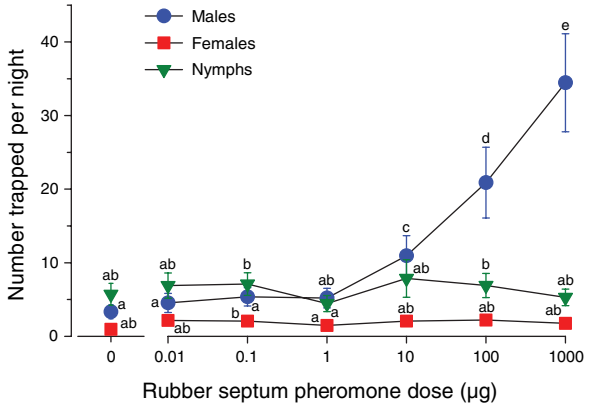

Fig. 4. Trap catches of *B. germanica* using various doses of the synthetic pheromone, blattellaquinone. The pheromone was applied in 50 μl of dichloromethane to a 5-mm sleeve-type rubber septum, which was hung inside a 1-pint (~470 ml) wide-mouth mason jar whose inside wall was treated with a thin layer 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.

References and Notes

1. R. J. Brenner, in *Understanding and Controlling the German Cockroach*, M. K. Rust, J. M. Owens, D. A. Reierson, Eds. (Oxford Univ. Press, New York, 1995), pp. 77–92.
2. L. Zurek, C. Schal, *Vet. Microbiol.* **101**, 263 (2004).
3. D. L. Rosenstreich *et al.*, *N. Engl. J. Med.* **336**, 1356 (1997).
4. S. J. Arbes Jr. *et al.*, *J. Allergy Clin. Immunol.* **112**, 339 (2003).
5. S. J. Arbes Jr. *et al.*, *J. Allergy Clin. Immunol.* **113**, 109 (2004).
6. R. McConnell *et al.*, *Ann. Allergy Asthma Immunol.* **91**, 546 (2003).
7. C. Schal, R. H. Hamilton, *Annu. Rev. Entomol.* **35**, 521 (1990).
8. R. Nishida, H. Fukami, *Mem. Coll. Agric. Kyoto Univ.* **122**, 1 (1983).
9. D. Liang, C. Schal, *Experientia* **49**, 324 (1993).
10. D. Liang, C. Schal, *Tissue Cell* **25**, 763 (1993).
11. D. Liang, C. Schal, *J. Insect Behav.* **6**, 603 (1993).
12. D. Liang, C. Schal, *J. Insect Physiol.* **40**, 251 (1994).
13. S. Nojima, D. J. Kiemle, F. X. Webster, W. L. Roelofs, *J. Chem. Ecol.* **30**, 2153 (2004).
14. Materials and methods are available as supporting material on *Science* Online.
15. T. Eisner, C. Rossini, A. Gonzalez, M. Eisner, *J. Exp. Biol.* **207**, 1313 (2004).
16. R. Brossut, *J. Chem. Ecol.* **9**, 143 (1983).
17. SAS Institute Inc., Cary, North Carolina (2000).

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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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Supporting Online Material

Materials and Methods

***Insects and behavioral assays*.** The *B. germanica* colony was maintained at 27 ± 0.5°C, ~50% relative humidity and 12:12 h light:dark photoperiod, and provided a continuous supply of rat chow 5012 (Purina Mills, St. Louis, MO) and water. Newly emerged adults were separated from the colony on the day of eclosion (day 0), and females and males were maintained in separate rooms.

For behavioral assays in the laboratory, a two-choice olfactometer device was used (*S1*, *S2*, *S3*). Briefly, a straight Plexiglas tube (55 cm long x 3 cm ID) was divided along 15 cm of its upwind end and air was drawn through it at 20 cm/min. A single cockroach was placed in a screen-gated cage at the downwind end 30 min before the start of an assay. A candidate attractant and control (solvent) were loaded in 5 µl dichloromethane onto separate Whatman #1 filter paper discs (5 mm diameter) and after the solvent evaporated (~30 sec) the discs were introduced simultaneously at the upwind end of the olfactometer. The percentage of insects running upwind, the latency of their responses (in sec), and the percentage choosing each arm of the olfactometer were recorded.

***Purification of blattellaquinone*.** We followed a bioassay-guided chromatographic fractionation of the extract and purification of the active fraction. First, the total lipid extract of approximately 2000 pygidia was dried over anhydrous sodium sulfate and then separated by flash

chromatography on silica gel (800 mg) by eluting successively with 8 ml each of mixtures of pentane-ether in the sequence 100% pentane, 5%, 10%, 20% and 40% ether in pentane, and 100% ether. The active fraction (40% ether fraction) was concentrated under a N_2 stream in an ice bath and subjected to preparative high performance liquid chromatography (HPLC).

The HPLC system comprised a Rainin Rabbit-HP (Rainin Instrument Co., Inc., MA, USA) equipped with a Rheodyne injector with a 1.0 ml sample loop (Rheodyne, Inc., CA, USA). The column was an Econosil Silica 5 µm, 250 x 4.6 mm column (Alltech Associates, Inc., Deerfield, IL, USA). The solvent system comprised a hexane-ether gradient, programmed as 5% ether in hexane for 5 min, then increased linearly to 30% at 0.5%/min, to 100% at 5%/min, and kept at 100% ether for 20 min at a flow rate of 1 ml/min. A Dynamax UV-1 (Rainin Instrument Co., Inc., MA, USA) detector was used to monitor the eluate at 210 nm. The effluent was collected every 3 min between 0 and 24 min and from 46 to 73 min, and every 1 min from 24 to 46 min.

Gas chromatographic-electroantennographic detection (GC-EAD). GC-EAD instrumentation used in this study was the same as described previously (*S4*). An HP5890II GC equipped with either a non-polar Equity-1 capillary column (30 m x 0.25 mm ID, 0.25 µm film thickness, Supelco, Bellefonte, PA, USA) or a slightly polar EC-20 capillary column (30 m x 0.25 mm ID, 0.25 µm film thickness, Alltech Associates, Deerfield, IL, USA) was used for analysis in splitless mode. Nitrogen was used as the carrier gas at a head pressure of 79 kPa (flow rate, 1.0 ml/min). The oven temperature was 40°C for 2 min, increased at 15°C/min to 250°C and held for 10 min. Injector and flame ionization detector (FID) temperatures were set at 150 and 250°C, respectively. The column effluent was combined with nitrogen make-up gas (30 ml/min) and then split 1:1 to the FID and EAD. The EAD outlet was secured in a charcoal-filtered and

humidified air stream, refrigerated by a modified condenser flushed with 5°C water, flowing at 500 ml/min over the antennal preparation.

A live male was fixed on a custom-made acrylic holder and used for EAD recordings. The tips of the antennae were brought into contact with a capillary tube on the holder, which was filled with saline. A pure gold recording electrode was connected to the capillary tube, while the indifferent electrode was impaled directly into the mouth of the male. The output signal from the antennae was amplified by a custom-built high-input impedance DC amplifier and filtered by a high-pass filter with a cutoff frequency of ~0.5 Hz (*S4*). The signals were recorded on an HP3390A integrator synchronized with the GC integrator. GC-EAD analyses consistently revealed a single EAD-active compound (fig. S1).

Preparative GC. The behaviorally active HPLC fraction (34–35 min) was further separated on a preparative GC. In preliminary experiments, the pheromone appeared to be thermally unstable, because its FID peak declined by about 50% when the injector temperature was raised from 150°C to 250°C; it disappeared at 300°C. Therefore, we designed a micro-preparative GC system (*S5*) that was integrated with NMR sample preparation for minute amounts of thermally unstable volatile chemicals. The recovery efficiency of volatile chemicals with this technique was >80% with sample sizes of 0.05 to 0.5 µg, and the purity of the acquired NMR samples was sufficient for high sensitivity NMR analyses including two dimensional experiments. An HP5890 GC was equipped with a non-polar Equity-1 mega-bore capillary column (5 m x 0.53 mm ID, 1.5 µm film thickness, Supelco, Bellefonte, PA, USA). Nitrogen was used as the carrier gas at a head pressure of 6.9 kPa and a flow rate of 8.0 ml/min. The time for splitless injection was 1.0 min. The oven temperature was set initially at 40°C for 2 min, increased at 10°C/min to 250°C, and held for 10 min. The injector and collection port temperatures were set at 150°C and 220 °C,

respectively, and the septum purge flow rate was set at 1.5 ml/min with a total flow rate of 100 ml/min.

GC-mass spectrometry (MS). The behaviorally and GC-EAD active fractions from preparative HPLC were subjected to GC-MS analyses on a Shimadzu GC-17A equipped with a 30 m x 0.25 mm ID x 0.25 µm thickness DB-5ms column (J&W Scientific, Folsom, CA, USA) operated in splitless mode and coupled to a Shimadzu QP-5050 quadrupole MS running in the electron impact (EI) (ionization at 70 eV) scan mode. Helium was used as the carrier gas at a head pressure of 54 kPa (flow rate, 1.0 ml/min). Oven temperature was 40°C for 2 min, increased at 5°C/min to 220°C and held for 5 min, and injector and interface temperature were set at 150ºC and 220°C, respectively.

Nuclear magnetic resonance (NMR). A total of ca. 5 µg of pure pheromone was isolated by preparative GC from the extract of ca. 5,000 female pygidia, dissolved in a minimum of "100%" benzene D_6, and used for the NMR analysis on a 600 MHz Burker Avance NMR spectrometer. The ^1H NMR spectrum showed, with a rather high degree of certainty, that it had 14 hydrogen atoms, suggesting either $C_{12}H_{14}O_4$ or $C_{16}H_{14}O$. The first molecular formula has 6 degrees of unsaturation while the second has 10 degrees of unsaturation. Since it is difficult to draw "reasonable" structures with the second formula, $C_{12}H_{14}O_4$ was selected as the most likely formula for the pheromone.

The presence of an isopropyl group is evident in the NMR spectrum. There is a six-proton doublet at δ 0.786 and a one-proton multiplet centered at δ 1.987 (Fig. 1). This methine-derived multiplet is obviously not a first order multiplet; it is clearly coupled to one or more protons in addition to the six-methyl protons. A likely candidate is the two-proton "doublet" at δ 1.920, which is distorted and "leaning" in the direction of the methine group. Assuming that the

methine and methylene groups are coupled, a rough calculation of $\Delta\nu/J$ gives an approximate value of five. This calculation indicates that the patterns should not be first order but that they should retain some resemblance to first order.

The chemical shifts and coupling patterns are reminiscent of esters of isovaleric acid and, in fact, matches NMR spectra of known isovalerate esters. This piece of the structure uses $C_5H_9O_2$ and accounts for one of the degrees of unsaturation. The other piece, the alcohol moiety, must have a formula of $C_7H_5O_2$, noting that the oxygen atom associated with the alcohol portion has already been accounted for in the acid portion.

Thus, a good place to start on the alcohol piece in the NMR spectrum is the two-proton doublet at δ 4.721 (Fig. 1). The coupling constant is small (~2 Hz), which is too small for typical vicinal coupling in a freely rotating system, meaning either long-range coupling or restricted rotation. Since the remaining piece is highly unsaturated, the likely case is that the small coupling is due to long-range coupling. The chemical shift of this methylene group indicates that it is deshielded by an oxygen atom and by some other group, likely some form of unsaturation.

The remaining three protons in the NMR spectrum are likely either olefinic or shielded aromatic protons. The proton at δ 6.356 is an apparent quartet, which is explained by long-range coupling to the methylene group at δ 4.721 and a similar (in magnitude) long range coupling to one of the other olefinic protons (Fig. 1). The other two protons form a multiplet at δ 5.928. This multiplet can be dissected into (1) vicinal coupling to give an AB multiplet, almost a quartet ($\Delta\nu/J$ ~1.7) and (2) long range coupling of one of the AB protons to the proton at δ 6.356. This NMR pattern is consistent with a substituted *para*-benzoquinone. 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.

Synthesis and confirmation of chemical structure. The synthesis is outlined in Fig. 2. Acylation of 2,5-dimethoxybenzyl alcohol in pyridine and CH_2Cl_2 with a catalytic amount of DMAP was carried out with isovaleryl chloride (Aldrich). The crude ester was oxidized with $Ce(NH_4)_2(NO_3)_6$ in acetonitrile and water to give crude blattellaquinone. This crude quinone ester was purified by flash chromatography on silica gel and was recrystallized from hexane-ether. ^{1}H NMR analysis in deuterobenzene (low concentration at 600 MHz) of the synthetic blattellaquinone (fig. S3), which was identical to the natural compound, confirmed the structure.

References and Notes

S1. D. Liang, C. Schal, *J. Insect Behav.* **3,** 211 (1990).

S2 D. Liang, C. Schal, *Experientia* **49,** 324 (1993).

S3. C. Schal, D. Liang, L. K. Hazarika, R. E. Charlton, W. L. Roelofs, *Ann. Entomol. Soc. Am.* **85,** 605 (1992).

S4. S. Nojima, C. Linn, Jr., B. Morris, A. Zhang, W. Roelofs, *J. Chem. Ecol.* **29**, 321 (2003).

S5. S. Nojima, D. J. Kiemle, F. X. Webster, W. L. Roelofs, *J. Chem. Ecol.* **30,** 2153 (2004).

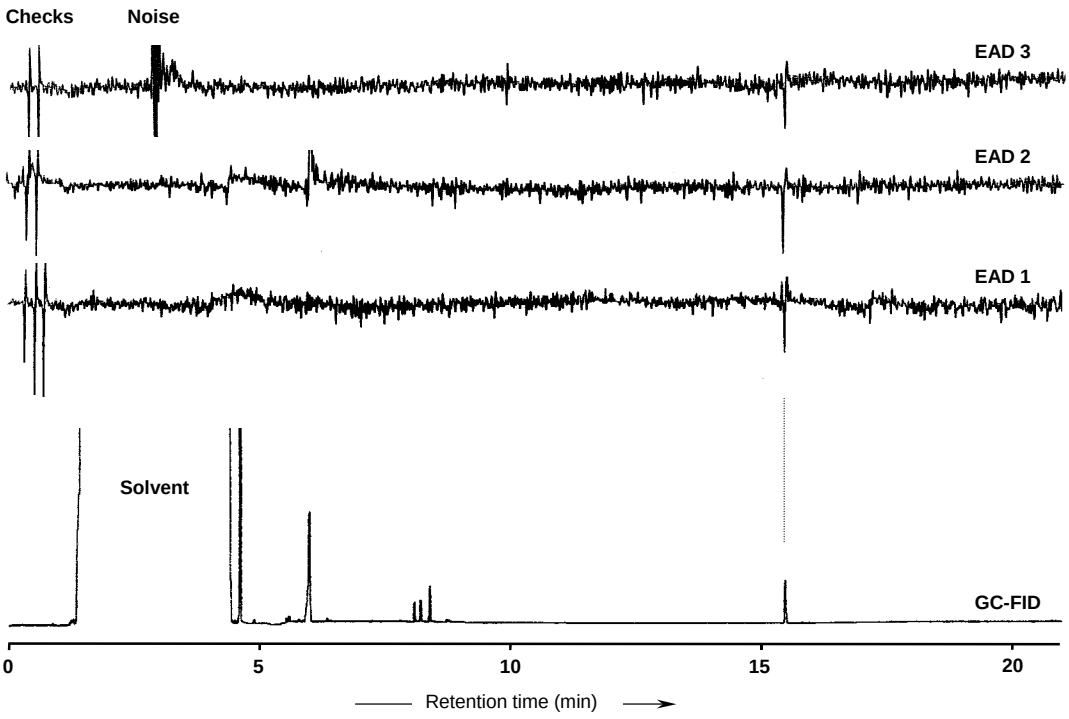
Supplementary Figures

Figure S1. GC-EAD traces of a behaviorally active HPLC-purified fraction. Puffs of the volatiles from a rotten apple are used to check for viability and response of the antenna before each GC-EAD run.

Figure S2. Structural formulas of blattellaquinone and related benzo- and hydroquinones.

Figure S3. NMR analysis of synthetic blattellaquinone.

Figure S1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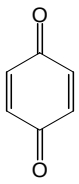
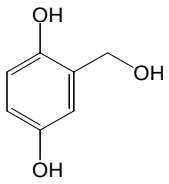
EAD 2
EAD 1
Solvent
GC-FID
0
5
10
15
20
Retention time (min)

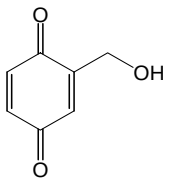
Figure S2



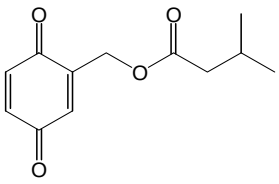
p-benzoquinone



gentisyl alcohol

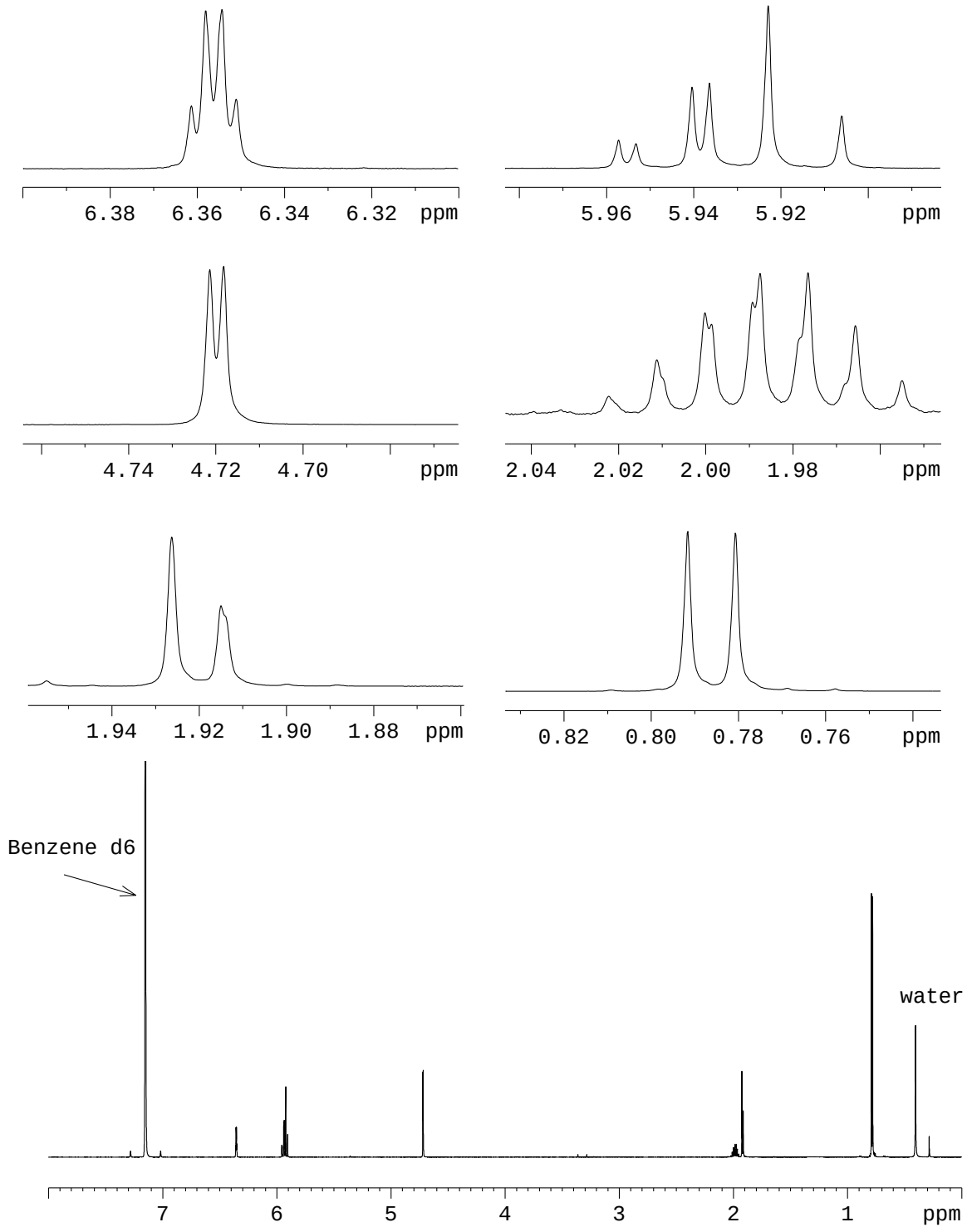


gentisyl quinone



gentisyl quinone isovalerate
"blattellaquinone"

Figure S3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ppm
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1.92
1.90
1.88
ppm
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0.80
0.78
0.76
ppm
Benzene d6
water
7
6
5
4
3
2
1
ppm