large G2 peak after 24 hours (Fig. 3A) when compared with the DMSO control. However, TPA given 6.5 hours after release from synchronizing blockage (that is, in the second half of S) did not cause a larger G2 peak (Fig. 3B). Data obtained 10 hours after release show, in addition, the delayed passage of TPA-treated cells through the S phase (Fig. 3, A and B), thus confirming the interpretation of the incorporation data.

The effects of TPA on the cell cycle of HeLa cells comprise (i) a G1 block prior to entering S, (ii) a delayed passage through S, (iii) a transient blocking in G2, and (iv) a G2 delay of a portion of cells treated with TPA in the early S phase. Since none of these effects were observed with 4-*O*-methyl-TPA (*10*)—a nonpromoting but hyperplasiogenic (*13*) derivative—we conclude that HeLa cell cultures do not offer a target for the mitogenic capacity of phorbol esters. These data and our earlier observations (*6, 7*) seem to be specific for tumor-promoting phorbol derivatives. The effects are reminiscent of results reported from x-ray irradiated cultures. Ionizing radiation can influence the transition from G1 to S in a variety of cells (*14, 15*). Cells in the S phase have been shown to be susceptible to the induction of modification in G2 in their immediate life-span (*9*), and they respond principally with a reduced rate of DNA synthesis and lengthening of the S phase (*9, 15, 16*). A direct blockage of G2 cells by irradiation has also been reported (*9*).

In view of the possibility that TPA may induce sister chromatid exchanges (*17*), although this has been questioned (*18*), the effects of TPA on cells in S as well as in G2 seem to be of particular interest, since sister chromatid exchanges can occur only in these phases of the cell cycle. In that respect also, TPA would mimic irradiation. It remains to be established whether both effects are related.

VOLKER KINZEL
JAMES RICHARDS
MICHAEL STÖHR
Institute of Experimental Pathology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, West Germany

References and Notes

1. I. Berenblum, in *Cancer*, F. F. Becker, Ed. (Plenum, New York, 1975), vol. 1, p. 323.
2. R. K. Boutwell, *CRC Crit. Rev. Toxicol.* **2**, 419 (1974); J. D. Scribner and R. Süss, *Int. Rev. Exp. Pathol.* **18**, 137 (1978).
3. J. E. Trosko, C. Chang, L. P. Yotti, E. H. Y. Chu, *Cancer Res.* **37**, 188 (1977); G. B. Lankas, C. S. Baxter, R. T. Christian, *Mutat. Res.* **45**, 153 (1977).
4. E. Hecker, *Cancer Res.* **28**, 2338 (1968); B. L. Van Duuren, *Prog. Exp. Tumor Res.* **11**, 31 (1969).
5. B. Weinstein, M. Wigler, P. B. Fisher, E. Sisskin, C. Pietropaolo, in *Carcinogenesis*, vol. 2, *Mechanisms of Tumor Promotion and Cocarcinogenesis*, T. J. Slaga, A. Sivak, R. K. Boutwell, Eds. (Raven, New York, 1978), p. 313.
6. V. Kinzel, G. Kreibich, E. Hecker, R. Süss, *Cancer Res.* **39**, 2743 (1979).
7. R. Süss, G. Kreibich, V. Kinzel, *Eur. J. Cancer* **8**, 299 (1972).
8. G. C. Mueller and K. Kajiwara, in *Development and Metabolic Control Mechanisms and Neoplasia. Symposium on Fundamental Cancer Research*, M. D. Anderson Hospital and Tumor Institute, Ed. (William & Wilkins, Baltimore, 1965), p. 452.
9. W. K. Sinclair, *Radiat. Res.* **33**, 620 (1968); M. M. Elkind and G. F. Whitmore, *The Radiation Biology of Cultured Mammalian Cells* (Gordon & Breach, New York, 1967).
10. V. Kinzel, J. Richards, M. Stöhr, in preparation.
11. P. N. Dean and J. H. Jett, *J. Cell Biol.* **60**, 523 (1974); M. Stoehr, U. Gebhardt, K. Goerttler, *Biotechnol. Bioeng.* **18**, 1057 (1976).
12. G. C. Mueller and K. Kajiwara, in *Fundamental Techniques in Virology*, K. Habel and N. P. Salzman, Eds. (Academic Press, New York, 1969), p. 21.
13. G. Fürstenberger, M. de Bravo, S. Bertsch, F. Marks, *Res. Commun. Chem. Pathol. Pharmacol.* **24**, 533 (1979); F. Marks, S. Bertsch, G. Fürstenberger, *Cancer Res.* **39**, 4183 (1979).
14. S. Mak and J. E. Till, *Radiat. Res.* **20**, 600 (1963); S. Lesher, *ibid.* **32**, 510 (1967).
15. J. B. Little, *ibid.* **44**, 674 (1970).
16. T. Terasima and L. J. Tolmach, *Biophys. J.* **3**, 11 (1963).
17. A. R. Kinsella and M. Radman, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 6149 (1978); H. Nagasawa and J. B. Little, *ibid.* **76**, 1943 (1979).
18. K. S. Loveday and S. A. Latt, *Mutat. Res.* **67**, 343 (1979).
19. K. Burton, *Biochem. J.* **62**, 315 (1956).
20. We thank N. König for excellent technical assistance.

22 April 1980; revised 4 June 1980

Chemical Mimicry as an Integrating Mechanism: Cuticular Hydrocarbons of a Termitophile and Its Host

Abstract. *The staphylinid beetle* Trichopsenius frosti *Seevers has the same cuticular hydrocarbons as those of its host termite* Reticulitermes flavipes *(Kollar) and it biosynthesizes them. These cuticular hydrocarbons probably serve as the primary mechanism by which* Trichopsenius frosti *integrates itself into the termite colony.*

The paramount problem of termite-termitophile biology is determining how termitophiles integrate themselves into the social structure of termite colonies. Various authors have suggested that termitophiles use "appeasement chemicals" (*1*), pass as morphological mimics (*2*), or engage in behavioral mimicry (*3*) (or all). Little evidence, however, has been presented to substantiate these hypotheses.

We have reported (*4*) that several species of termites possess caste- and species-specific mixtures of cuticular hydrocarbons, and that these mixtures may be used by the termites as important, perhaps even primary, species recognition cues. We subsequently reasoned that termitophiles associated with these termites may have evolved cuticular hydrocarbon compositions similar enough to those of their host termites to allow them to be perceived as termites. We now report that the staphylinid beetle *Trichopsenius frosti* Seevers, a highly integrated, host-specific termitophile, has cuticular hydrocarbon components identical to those of its termite host *Reticulitermes flavipes* (Kollar). Furthermore, the beetle biosynthesizes its hydrocarbons.

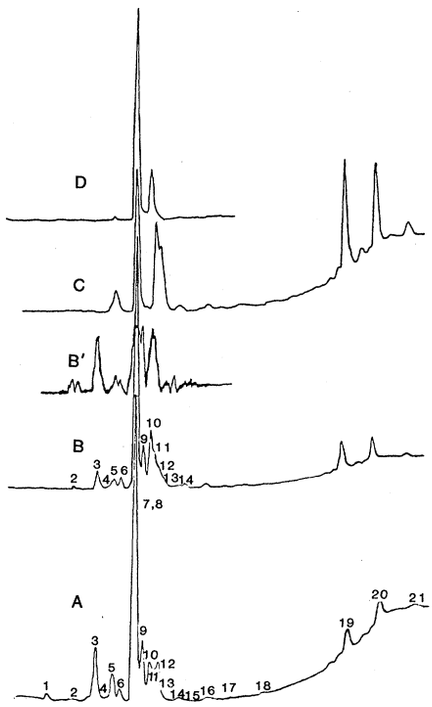

Fig. 1. Gas chromatograph profiles of the total cuticular hydrocarbons of *Reticulitermes flavipes* (A) and *Trichopsenius frosti* (B), the branched hydrocarbons of *T. frosti* (C), and the olefins of *T. frosti* (D); B′ is the radio-GLC trace obtained after incorporation of [1-^{14}C]acetate into hydrocarbons of *T. frosti*.

Portions of several colonies of *R. flavipes* containing *T. frosti* were collected during 1978 from pine logs in Harrison, Jackson, or Stone counties, Mississippi. The beetles were separated from the termites, counted, and stored at −20°C until used. From 715 *T. frosti* (98.69 mg, wet weight), we isolated cuticular hydrocarbons and separated them as described (*4*). Hydrocarbons were characterized by electron impact (EI) and chemical ionization (CI) gas chromatography–mass spectrometry (GC-MS) (*5*). Alkenes

0036-8075/80/1024-0431$00.50/0

Table 1. Cuticular hydrocarbons of *Trichopsenius frosti*: identification and percent composition.

Peak*	Component	ECL†	Carbon number‡	Diagnostic MS ions§	Percent composition‖
1	n-C_{21}	21.0	21	296	4.1
2	2-MeC_{22}	22.7	23	281,309	0.4
3	n-C_{23}	23.0	23	324	3.1
4	5-MeC_{23}	23.5	24	85,253,281	0.3
5	2-Me+3-MeC_{23}	23.7	24	295,323,281,309,323	1.6
6	n-C_{24}	24.0	24	338	1.8
7	9-C_{25}	24.6	25:1	350(157,171,255,269) ¶	37.7
8	2-MeC_{24}	24.7	25	309,337	8.1
9	n-C_{25}	25.0	25	352	7.3
10	7,9-C_{25}	25.4	25:2	348(129,169,255,295) ¶	9.1
11	5-MeC_{25}	25.5	26	85,281,309	1.2
12	3-MeC_{25}	25.7	26	309,337,351	6.5
13	n-C_{26}	26.0	26	366	3.8
14	3-MeC_{26}	26.7	27	323,351,365	1.7
15	n-C_{27}	27.0	27	380	0.4
16	n-C_{28}	28.0	28	394	0.7
17	n-C_{29}	29.0	29	408	0.8
18	n-C_{31}	31.0	31	436	0.2
19	5,17-diMeC_{35}	35.8	37	85,267,281,463,505	5.0
20	5,17-diMeC_{37}	37.8	39	85,267,309,491,533	4.0
21	5,17-diMeC_{39}	39.8	41	85,267,337,519,561	0.7

*See Fig. 1. †Equivalent chain lengths computed from retention times, which were compared to those of n-alkane standards. ‡Determined from CI-MS. §EI-MS, interpreted as described in (*4*). ‖In addition to the components listed, four additional unidentified components of more than 34 ECL units were present and comprised 1.5 percent of the total mixture. ¶Ions in parentheses are for the monomethoxy derivative of the olefin.

were also examined by infrared spectrometry. Double bond positions were located by conversion of the olefins to methoxy derivatives (*6*), which were then analyzed by GC-EI-MS.

In vitro biosynthesis experiments were conducted with beetles from a single colony of *R. flavipes* collected in August 1979. Three groups of 45 live beetles each were chilled to 4°C and cut into slices with a razor blade. This material was added to a 100-μl solution containing phosphate buffer (0.05*M*, *p*H 6.9), sucrose (0.3*M*), and sodium [1-^{14}C]acetate (20 μCi). We incubated the mixture at 37°C in a shaking water bath (60 oscillations per minute) for 2 hours, and then extracted the lipids (*7*) and isolated the hydrocarbons as described (*4*). A portion of the hydrocarbons from each group was assayed for radioactivity (*8*). Another portion was separated by Ag-NO_3-thin layer chromatography into saturated, monounsaturated, and diunsaturated components, which were assayed for radioactivity. We pooled the rest of the samples and subjected them to radio-gas-liquid chromatography (radio-GLC).

Every peak in the chromatographic profile (Fig. 1) of cuticular hydrocarbons from *R. flavipes* workers matches one from *T. frosti* with only minor abundance differences. We previously characterized (*9*) all major components of the *R. flavipes* cuticular hydrocarbons but left uncharacterized several minor components. Components newly identified are either homologs of previously identified components (peaks 4, 14, 15 to 18) or a homologous series of 5,17-dimethylalkanes (peaks 19 to 21).

The cuticular hydrocarbon composition of *T. frosti* exactly mimics that of *R. flavipes* in identity of components, and closely mimics it in relative abundance of components. If termites use cuticular hydrocarbons as species-recognition cues, then *T. frosti* has acquired an infallible integrating mechanism. Support for such an assertion requires knowledge of the source of the beetles' cuticular hydrocarbons. *Trichopsenius frosti* frequently grooms *R. flavipes* and rides on the female termite reproductives (*10*). Both activities could transfer *R. flavipes* cuticular hydrocarbons onto the beetles, either directly or by ingestion. Or, the beetles may biosynthesize their cuticular hydrocarbons. Some combination of these two alternatives is also possible.

To determine whether *T. frosti* can synthesize its hydrocarbons, we conducted the in vitro experiment with [1-^{14}C]acetate (57 mCi/mmole). After 2 hours of incubation with *T. frosti* tissues, 26.7 ± 4.5 pmole (mean ± standard deviation) of [1-^{14}C]acetate was incorporated into hydrocarbon. About 42.6 ± 5.0 percent of the radioactivity was in the alkane fraction, 31.6 ± 8.4 percent was in the alkene fraction, and 25.8 ± 9.7 percent was in the alkadiene fraction. This closely approximates the distribution of the alkanes and olefins in *T. frosti* (Table 1); and radio-GLC showed that each major component was labeled (Fig. 1B′). These results show that *T. frosti* can biosynthesize its cuticular hydrocarbons, and suggest that it has evolved specific cuticular hydrocarbons which function as species-mimicking cues. They further suggest that *T. frosti* is utilizing these chemicals as an important integrating mechanism. The *T. frosti* hydrocarbon proportions do not *exactly* mimic those of any one *R. flavipes* caste. The proportion of branched to normal components most closely approximates that of supplementary reproductives, but the proportion of saturated to unsaturated components more nearly approximates the composition of workers (*9*).

This congruence of hydrocarbon composition between two such phylogenetically remote organisms has not previously been observed. Even among closely related organisms, such a congruence of cuticular hydrocarbons is rare (*11*). That *T. frosti* and *R. flavipes* do have common cuticular hydrocarbon components can only be a result of extensive coevolution over an extended period of time (*12*). We do not know how generally cuticular hydrocarbons have a role as an integrating mechanism for termitophiles, but we note that unpublished data from our laboratory show that two termitophiles associated with *Reticulitermes virginicus* (Banks) (*Trichopsenius depressus* LeConte and *Xenistusa hexagonalis* Seevers) also possess cuticular hydrocarbon compositions that mimic those of their host.

RALPH W. HOWARD*
Southern Forest Experiment Station, Forest Service, U.S. Department of Agriculture, Gulfport, Mississippi 39503
C. A. MCDANIEL
National Monitoring and Residue Analysis Laboratory, U.S Department of Agriculture, Animal and Plant Health Inspection Service, Gulfport 39503
GARY J. BLOMQUIST
Department of Biochemistry, University of Nevada-Reno, Reno 89557

References and Notes

1. E. O. Wilson, *The Insect Societies* (Harvard Univ. Press, Cambridge, Mass., 1971), p. 403.
2. D. H. Kistner, *J. N.Y. Entomol. Soc.* **76**, 213 (1968).
3. _____, in *Social Insects*, H. R. Hermann, Ed. (Academic Press, New York, 1979), vol. 1, pp. 340–413.
4. R. W. Howard, C. A. McDaniel, G. J. Blomquist, *J. Chem. Ecol.* **4**, 233 (1978) (*Reticulitermes flavipes* [Kollar]); G. J. Blomquist, R. W. Howard, C. A. McDaniel, *Insect Biochem.* **9**, 365 (1979) (*Zootermopsis angusticollis* [Hagen]). Also, we have found that *R. virginicus*

(Banks) possesses caste- and species-specific cuticular hydrocarbons (unpublished).
5. R. W. Howard, C. A. McDaniel, D. R. Nelson, G. J. Blomquist, *J. Chem. Ecol.* **6**, 609 (1980); L. L. Jackson and G. J. Blomquist, in *Chemistry and Biochemistry of Natural Waxes*, P. E. Kolattukudy, Ed. (Elsevier, Amsterdam, 1976), pp. 201–233.
6. G. J. Blomquist, R. W. Howard, C. A. McDaniel, S. Remaley, L. A. Dwyer, D. R. Nelson, *J. Chem. Ecol.* **6**, 257 (1980).
7. E. G. Bligh and W. J. Dyer, *Can. J. Biochem. Physiol.* **37**, 911 (1959).
8. Radioactivity was assayed in a Beckman liquid scintillation counter by counting for 10 minutes at about 85 percent counting efficiency. All counting was done with a standard deviation of less than 5 percent. Mention of trade names or companies is solely to identify material used and does not imply endorsement by the U.S. Department of Agriculture.
9. R. W. Howard, C. A. McDaniel, G. J. Blomquist, *J. Chem. Ecol.* **4**, 233 (1978).
10. R. W. Howard, *Sociobiology* **2**, 189 (1976); *Ann. Entomol. Soc. Am.* **72**, 127 (1979).
11. G. J. Blomquist and L. L. Jackson, *Prog. Lipid Res. J.* **17**, 319 (1979).
12. D. H. Kistner, in *Biology of Termites*, K. Krishna and F. M. Weesner, Eds. (Academic Press, New York, 1969), vol. 1, pp. 525–557.
13. Supported in part by NSF grant PCM76-20694 and by the Nevada Agricultural Experiment Station; published as Journal Series No. 450.
* To whom inquiries should be sent.

2 February 1980; revised 5 May 1980

Metabolism of Propachlor by the Germfree Rat

Abstract. *Three major metabolites of propachlor were isolated from the excreta of germfree rats given ^{14}C-labeled propachlor orally. In contrast, 11 urinary metabolites, six of which were 2-methylsulfonylacetanilides not present in excreta of germfree rats, were isolated from control rats given ^{14}C-labeled propachlor orally. Enterohepatic circulation and microbial metabolism in the intestine were necessary for production of the methylsulfonyl-containing and other metabolites of propachlor in the conventional rat.*

The metabolism in vivo of several xenobiotics to methylthio-, methylsulfoxyl-, and methylsulfonyl-containing metabolites has been reported (*1*), and metabolic pathways for their production have been proposed. DeBaun *et al.* (*2*) described one pathway in which methionine supplied the methylthio group in the formation of methylmercapto-2-acetylaminofluorenes. Sumino and Mio (*3*) proposed a similar pathway in which methionine reacted with an arene oxide to form methylsulfide-containing metabolites from 2,5,2′,5′-tetrachlorobiphenyl. Colucci and Buyske (*4*) and Tateishi *et al.* (*1*) showed that rat liver contained an enzyme system, cysteine conjugate β-lyase, that produced thiols from the cysteine conjugates of certain xenobiotics. Tateishi *et al.* showed that the corresponding methylthiols were formed when liver microsomes and *S*-adenosylmethionine were added to their β-lyase system.

Propachlor (**1**, Fig. 1) is also metabolized to methylsulfonyl-containing metabolites by the rat (*5*). Studies on the metabolism of this herbicide in rats have indicated that an intestinal *C-S* lyase participated in the formation of the methylsulfonyl-containing metabolites that were excreted in the urine (Table 1).

In control rats, propachlor was metabolized to 11 urinary metabolites and to fecal residues that could not be solubilized by common solvents or by treatment with dilute acid or base (*5*). The intractable characteristics of these fecal residues indicated that the metabolites were covalently bound. The major urinary metabolite was the mercapturate; six of the metabolites (23 percent of the dose) were 2-methylsulfonylacetanilides. In a separate study, the sulfur from the cysteine and glutathione conjugates of propachlor was shown to be a precursor to the methylsulfonyl sulfur (*6*).

Rats with cannulated bile ducts secreted 67 percent of oral doses of propachlor in the bile as the glutathione conjugate (**2**), the cysteine conjugate (**3**), the mercapturate (**4**), and the mercapturate sulfoxide (**5**); no other ^{14}C-labeled metabolites were detected in the bile (*7*). The discrepancy between the quantities of ^{14}C secreted in the bile of cannulated rats and that excreted in the feces by the conventional rats indicated that the biliary metabolites must have been reabsorbed from the intestine and therefore that enterohepatic circulation of the ^{14}C from the precursors of the mercapturate (*8*) or from mercapturate (or both) participated in the metabolism of propachlor in rats. The identities of the biliary metabolites showed not only that the reabsorbed ^{14}C came from the mercapturate and its precursors, but also that these conjugates were available as substrates for the intestinal flora. Therefore, the metabolism of propachlor was studied in germfree rats and the results were compared with those obtained from control rats to determine what effect the absence of the intestinal flora would have on the metabolic fate of this compound (Table 1).

The germfree rats (*9*) given [^{14}C]-propachlor orally excreted 98.6 percent of the dose in the urine and the feces within 48 hours. Three metabolites were isolated from the excreta, and the fecal radioactive metabolites were water-soluble. The major metabolite was the mercapturate (**4**, Fig. 1), which accounted for 66.8 percent of the dose. The cysteine conjugate (**3**, Fig. 1) was present only in the feces and was the major fecal metabolite. The other metabolite (15.1 percent of the dose) was assigned structure **5** (Fig. 1) from mass spectral data (*10*). This mercapturate sulfoxide is not limited to germfree rats because it has also been isolated from rat bile and from the excreta of chickens given propachlor orally (*7*). The metabolism of propachlor in germfree rats is summarized in Fig. 2.

All of the propachlor was metabolized by the mercapturic acid pathway in the germfree rat. No methylsulfonyl-containing metabolites were formed, and none of the other metabolic transformations of the propachlor molecule found in the control rat had occurred. These transformations included aromatic and aliphatic hydroxylations, *N*-dealkylation, amide hydrolysis, and glucuronide formation. We concluded that enterohepatic circulation and metabolism of the

Table 1. Comparison of the excretion of single oral doses of [^{14}C]propachlor by control rats, control rats with fistulated bile ducts, and germfree rats.

Metabolite	Recovery of ^{14}C (percent of dose)				
	Control		Bile-fistulated	Germfree	
	Urine	Feces	Bile	Urine	Feces
Glutathione conjugate (**2**)*			37		
Cysteine conjugate (**3**)			13		19
Mercapturate (**4**)	17		12	63.1	3.7
Mercapturate sulfoxide (**5**)			4	5.7	9.4
Nonextractable residues		19			
Other metabolites	51†				
Total	68	19	66	68.8	32.1

*Metabolite designations are those used in the figures. †At least ten; see (*5*). Blank spaces indicate that none of the labeled metabolite was detectable.

0036-8075/80/1024-0433$00.50/0