decenyl acetate in mating communication than females of *T. ni*.

Five traps, each of which was baited with an evaporator (*9*) that released *cis*-7-dodecenyl acetate (*10*) at a different rate, were simultaneously placed in a field supporting a mixed population of both species. On each of 30 consecutive mornings, the moths caught in the traps were identified, sexed, and counted, and each trap was randomly moved to a new position. The number of *T. ni* males caught per trap increased with each increase in the rate of evaporation of *cis*-7-dodecenyl acetate (Fig. 1). On the other hand, *A. californica* males were caught only in the traps that released this compound at those low evaporation rates which were largely unattractive to *T. ni* males. These data indicate that the release of the same sex pheromone by females at different rates is a probable mechanism of reproductive isolation between these two species.

Only over a very narrow range of evaporation rates of *cis*-7-dodecenyl acetate will *T. ni* males enter traps baited with this chemical (*11*). Few males are captured in traps releasing the pheromone at rates tenfold above or below the optimum. We believe that the optimum release rate may be that which causes the pheromone concentration near the entrance of the trap to be similar to the concentration found in the immediate vicinity of a free-living, pheromone-releasing female. We further propose that a male encountering this critical concentration stops its long-distance pheromone-orientation behavior and engages in a short-range "searching" behavior, causing it to enter the trap. If these hypotheses are correct, then *T. ni* males responding to the low quantity of *cis*-7-dodecenyl acetate released from *A. californica* females would not reach their critical concentration and thus would neither terminate their long-distance orientation behavior nor enter traps. On the other hand, *A. californica* males approaching a receptive *T. ni* female would presumably reach this concentration, and thus terminate their long-distance orientation behavior, at some point downwind from the female.

It can be seen that reproductive isolation based on prevention of males orienting to the females of the related species is not complete (Table 1). Essentially no *A. californica* males were captured in traps baited with *T. ni* females. On the other hand, a fairly large number of *T. ni* males was captured in the traps baited with females of *A. californica*. Also, at certain intermediate evaporation rates of *cis*-7-dodecenyl acetate, males of both species were captured. However, previous laboratory research (*8*) has shown that an additional mechanism can operate at very close range, causing the female to reject copulation attempts made by males of the wrong species.

Table 1. Capture of males in traps baited with ten virgin noctuid females.

Bait species	No. of males captured per trap*	
	T. ni	*A. californica*
T. ni	1861	1
A. californica	37	79

* Based on 30 trapping nights.

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References and Notes

1. E. Mayr, *Populations, Species, and Evolution: An Abridgment of Animal Species and Evolution* (Harvard Univ. Press, Cambridge, Mass., 1970).
2. S. E. Crumb, *U.S. Dep. Agr. Tech. Bull.* **1135**, 254 (1956).
3. R. S. Kaae, H. H. Shorey, S. U. McFarland, L. K. Gaston, *Ann. Entomol. Soc. Amer.*, in press.
4. V. E. Brady, J. H. Tumlinson, R. G. Brownlee, R. M. Silverstein, *Science* **171**, 802 (1971); E. Priesner, *Z. Vergl. Physiol.* **61**, 263 (1968); W. L. Roelofs and A. Comeau, *Science* **165**, 398 (1969); *J. Econ. Entomol.* **63**, 969 (1970); C. J. Sanders, *Science* **171**, 911 (1971).
5. R. S. Berger, *Ann. Entomol. Soc. Amer.* **59**, 767 (1966).
6. Near identical retention times for the biologically active compound in extracts from female pheromone glands of *T. ni* versus *A. californica* were recorded with a flame ionization detector after gas-liquid chromatographic separation on each of three different columns (Carbowax 20 M, neopentylglycol adipate, and SF 96). Based on the previous identified structure of *T. ni* pheromone, these data limit the structure of *A. californica* phermone to several closely related straight chain 12-carbon monounsaturated acetates. T. L. Payne, H. H. Shorey, L. K. Gaston (*Ann. Entomol. Soc. Amer.*, in press) obtained electroantennograms (EAG) from *A. californica* and *T. ni* in response to *cis*-7-dodecenyl acetate and three closely related compounds (*cis*-Δ*x*-dodecenyl acetate, $x = 6$, 8, and 9). Significant EAG responses were obtained with both *T. ni* and *A. californica* males for all compounds. However, from 100- to 10,000-fold more (compared to *cis*-7-dodecenyl acetate) of each compound was required to obtain the response. In addition, *trans*-7-dodecenyl acetate does not attract males of either species at any of several release rates that we have tested in the field.
7. Double-cone traps [R. K. Sharma, H. H. Shorey, L. K. Gaston, *J. Econ. Entomol.* **64**, 361 (1971)] were baited with ten virgin females of *T. ni* or *A. californica*. Based on earlier experimentation with *T. ni* [R. S. Kaae and H. H. Shorey, *Ann. Entomol. Soc. Amer.* **65**, 436 (1972)], we estimate that no more than two or three of these females were releasing pheromone at any one time.
8. H. H. Shorey, L. K. Gaston, J. S. Roberts, *Ann. Entomol. Soc. Amer.* **58**, 600 (1965). The pheromone extracted from females of each species was bioassayed in a dilution series based on female equivalents.
9. The construction and calibration of the evaporators is described by H. H. Shorey, R. S. Kaae, L. K. Gaston, J. R. McLaughlin, *Environ. Entomol.* **1**, 641 (1972). Five evaporators were designed to release *cis*-7-dodecenyl acetate at 1, 3, 10, 30, and 100 ng/min under constant laboratory conditions of 18°C and 0.4 m/sec airflow. Temperatures and air velocities in the field were lower than these values during the conduct of the experiment, and actual release rates probably varied between one-tenth and one-half of the above-stated rates. Therefore, release rates in Fig. 1 are given as relative, rather than absolute, values.
10. The *cis*-7-dodecenyl acetate used gave only one peak on gas-liquid chromatography (lower limit of detection of other compounds was 0.5 percent), indicating that the material was at least 99.5 percent 7-dodecenyl acetate. An upper limit of 10 percent for the *trans* isomer was estimated by nuclear magnetic resonance (δ and J are slightly different for the *cis* and *trans* olefinic protons).
11. L. K. Gaston, H. H. Shorey, C. A. Saario, *Ann. Entomol. Soc. Amer.* **64**, 381 (1971).
12. This is paper No. 36 of a series entitled "Sex pheromones of Lepidoptera." We thank B. Wilk and S. U. McFarland for their assistance. This research was supported in part by a grant from the Rockefeller Foundation.
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Leaching: Use of a Thermophilic and Chemoautotrophic Microbe

Abstract. *A chemoautotrophic, thermophilic, and acidophilic microorganism capable of oxidizing reduced sulfur and iron compounds and leaching concentrates of molybdenite and chalcopyrite at 60°C has been characterized by transmission and scanning electron microscopy. This constitutes the first direct observations of microorganisms on ore fines.*

Field studies indicate that temperatures within low-grade copper ore dumps may reach 80°C (*1*). These elevated temperatures undoubtedly inhibit the leaching activity of strains of sulfur- and iron-oxidizing bacteria whose optimum temperature range is 25° to 45°C (*2*, *3*). Thermophilic organisms have not previously been considered in leaching activities. We therefore report the characterization of a chemoautotrophic, thermophilic, and acidophilic microorganism, possibly related to *Sulfolobus* (*4*), with the capa-

bility of oxidizing reduced sulfur and iron compounds between 45° and 70°C. The information presented here indicates that this organism may be useful as a biological leaching agent in regions of high temperature within dumps.

The organism was isolated by J. A. Brierley (*5*) from an acid, hot spring in Yellowstone National Park and recently has been described in more detail (*6*). Morphologically, the isolate is pleomorphic, varying in diameter from 1.0 to 1.5 μm. A rigid cell wall structure, typical of most bacteria, is not present. A membrane surrounds the cell; an amorphous layer is evident outside the membrane. The isolate possesses a dense, intracellular body of unknown composition and function (Fig. 1) (*7*).

The chemoautotrophic isolate grows aerobically. The source of energy can be either elemental sulfur or ferrous iron in a basal salt medium (*3*) containing 0.02 percent yeast extract. After 2 weeks of incubation at 60°C, the inoculated medium, containing elemental sulfur, was turbid and the total acidity had increased by 0.54 meq. Incubation of the isolate for 2 weeks in a basal salt medium containing 2.0 mg of ferrous iron per milliliter of solution resulted in the oxidation of 1.9 mg of the ion per milliliter of the solution and microscopically visible growth. Growth also occurs on either sulfur or iron in the absence of yeast extract; however, the growth is much slower.

Leaching studies showed that 98.5 percent molybdenite (MoS_2) with a particle size of 12 to 62 μm is also oxidized by the thermophilic isolate at 60°C. Molybdenum is solubilized at a rate of 6.6 mg liter^{-1} day^{-1} over a 30-day period for a yield of 3.3 percent in 100 ml of the inoculated acid medium, *p*H 2.5 (*3*) when shake flask extractions are used. When 0.02 percent yeast extract is added to the medium, the rate of dissolution is increased to 16.6 mg liter^{-1} day^{-1} (yield after 30 days, 8.3 percent); the addition of both 0.02 percent yeast extract and 1 percent ferrous sulfate increases the rate of dissolution to 26.5 mg liter^{-1} day^{-1} (yield after 30 days, 13.3 percent). In all cases the solubilization of molybdenum in uninoculated controls proceeded at a rate of 0.2 mg liter^{-1} day^{-1} over a 30-day period for a yield of 0.1 percent (*8*). Microscopic observation of inoculated flasks, containing 800 parts per million (ppm) of solubilized molybdenum, after 30 days of incubation, revealed the presence of a dense population of the organism; continuation of the leaching for another 30 days yielded further solubilization of the molybdenum, but at a decreased rate. A manometric measurement of respiration indicated that sulfur oxidation is not inhibited for 1 hour by the addition of 2000 ppm of molybdenum (*9*); however, growth of the isolate on sulfur is slowed by the addition of 1000 ppm of molybdenum (*10*). The tolerance to high concentrations of soluble molybdenum is unique to this organism—other investigators have reported a maximum tolerance of chemoautotrophic bacteria to molybdenum of about 5 to 90 ppm (*11*). Thus, where molybdenum is present, the use of other chemoautotrophic bacteria is necessarily limited.

Although there have been several attempts to investigate the disposition and distribution of bacteria on ore fines by replication electron microscopy (*12*), direct observations have not been made. Figure 2 illustrates several examples of direct observations of the thermophilic isolate on molybdenite fines in which the scanning electron microscope was used in the secondary emission mode (*13*). In Fig. 2A it may be observed that morphologically the isolate consists of clustered (colony) structures, each with an overall shape compatible with that shown in Fig. 1. Several observations of individual isolate clusters similar to that shown in Fig. 2B were made at various imaging angles in order to study the isolate-mineral interface. Apparently the isolate in many cases attaches to the mineral surface. In addition, nondispersive x-ray analysis of isolated organisms showed them to contain trace amounts of copper, detectable above the background in characteristic x-ray maps.

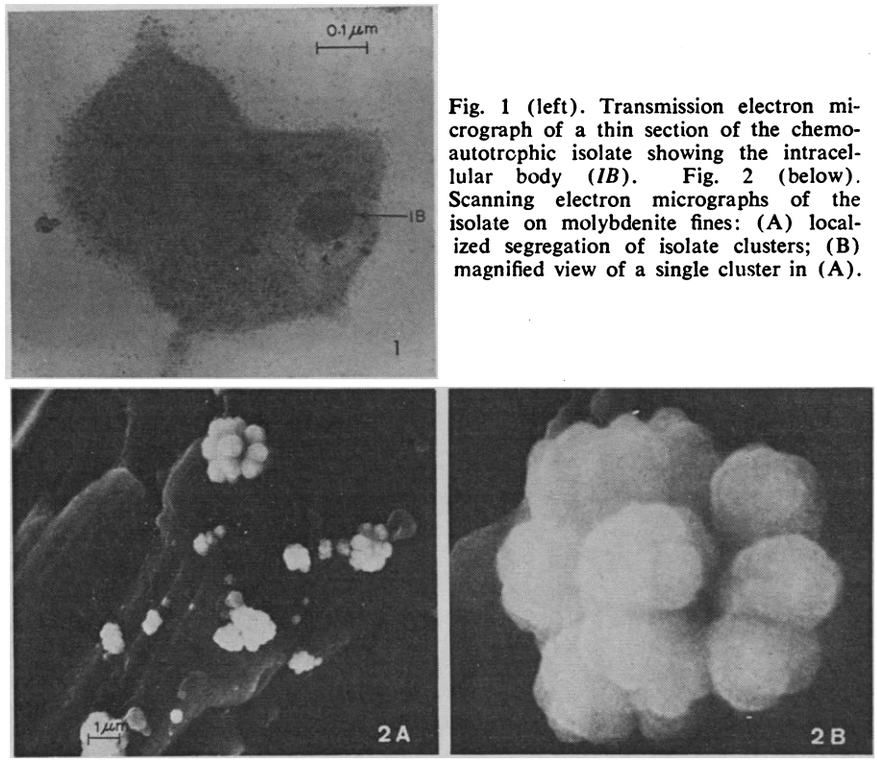

Fig. 1 (left). Transmission electron micrograph of a thin section of the chemoautotrophic isolate showing the intracellular body (*IB*). Fig. 2 (below). Scanning electron micrographs of the isolate on molybdenite fines: (A) localized segregation of isolate clusters; (B) magnified view of a single cluster in (A).

Preliminary evidence indicates that the isolate also oxidizes a chalcopyrite concentrate under the same conditions that it leaches a molybdenite concentrate. The copper is solubilized at a rate of 10 to 16 mg liter^{-1} day^{-1} from a chalcopyrite concentrate (27.6 percent copper; particle size; 74 to 105 μm) over a 30-day period in an inoculated acid medium, *p*H 2.5 (*3*). The solubilization of copper in uninoculated flasks occurs at a rate of 1 to 1.8 mg liter^{-1} day^{-1} over a 30-day period.

These properties, coupled with the isolate's ability to tolerate high concentrations of molybdenum, indicate that it may be a useful agent for leaching in dump regions where temperatures exceed the optimum (25° to 45°C) for most chemoautotrophic microorganisms.

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References and Notes

1. J. V. Beck, *Biotechnol. Bioeng.* **9**, 487 (1967).
2. L. C. Bryner, J. V. Beck, D. B. Davis, D. G. Wilson, *Ind. Eng. Chem.* **46**, 1587 (1954).
3. L. C. Bryner and R. Anderson, *ibid.* **49**, 1721 (1957).
4. T. D. Brock, K. M. Brock, R. T. Belly, R. L. Weiss, *Arch. Mikrobiol.* **84**, 54 (1972).
5. J. A. Brierley, thesis, Montana State University (1966).
6. C. L. Brierley and J. A. Brierley, *Can. J. Microbiol.*, in press.
7. Thin sections were prepared as follows: the cells were cultured in basal salt medium with 0.02 percent yeast extract and colloidal sulfur; thin sections fixed with glutaraldehyde and osmium tetroxide were examined in an electron microscope (Hitachi HU-11B).
8. The intial concentration of molybdenum in all cases was 6000 ppm.
9. C. L. Brierley, *N.M. State Bur. Mines Miner. Resour. Target Explor. Rep. E-2* (1972).
10. In tolerance studies, molybdenum was supplied as $Na_2MoO_4 \cdot 2H_2O$. Manometric tolerance studies were performed on a differential respirometer (Gilson).
11. O. H. Tuovinen, S. I. Niemela, H. G. Gyllenberg, *Antonie van Leeuwenhoek J. Microbiol. Serol.* **37**, 489 (1971).
12. W. I. Schaeffer, P. E. Holbert, W. W. Umbreit, *J. Bacteriol.* **85**, 137 (1963); D. G. Zvyaginstev, A. F. Pertsovskaya, V. I. Duda, D. I. Nikitin, *Mikrobiologiya* **38**, 937 (1969).
13. The samples were prepared for scanning electron microscopy as follows: molybdenite fines from inoculated acid media placed on aluminum specimen stubs were air-dried and metallized with a mixture of gold and palladium (60 : 40) in vacuum to a thickness of roughly 150 Å. Observations were made in a scanning electron microscope (Hitachi HHS-2R) operated at 20 kv and in a scanning electron microscope (Cambridge Stereoscan Mark II) fitted with a nondispersive x-ray analyzer (Princeton Gamma-Tech) operated at an accelerating potential of 20 kv. L. E. Murr, *Electron Optical Applications in Materials Science* (McGraw-Hill, New York, 1970).

14. Research at the New Mexico Bureau of Mines and Mineral Resources was supported in part by U.S. Bureau of Mines grant GO122123. We thank Dr. J. V. Scaletti, University of New Mexico Medical School, for preparing the transmission electron micrograph.

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Lack of Tolerance to Δ^9-Tetrahydrocannabinol in Chimpanzees

Abstract. *Five chimpanzees were given Δ^9-tetrahydrocannabinol (Δ^9THC): 1.0 milligram per kilogram of body weight for 21 days and 4.0 milligrams per kilogram of body weight for 42 days. Although accuracy and speed of performance on a delayed matching-to-sample task were significantly affected by both doses, tolerance to Δ^9THC did not develop. No long-term behavioral effects of Δ^9THC were observed after termination of the drug regimens.*

Although the short-term (acute) effects of marihuana have been determined, the long-term effects of marihuana have not been well defined (*1*). For example, the issue of whether marihuana tolerance develops in man has not been settled experimentally, and some discrepancy still exists regarding the effects of repeated marihuana administrations in nonhuman species. In those animal experiments where the effects of marihuana on unlearned behavior or on learned shock-avoidance behavior have been studied, no consensus as to the occurrence of marihuana tolerance has been reached (*2, 3*).

In contrast, the development of a marked tolerance to the effects of marihuana on simple behavioral tasks maintained by appetitive rewards has been consistently demonstrated in a wide range of animal species (*2, 4, 5*). Most of these behavioral tasks have involved situations of the "go, no-go" type in which delivery of reward is made dependent on the repeated occurrence, as compared to the nonoccurrence, of a simple learned response. We now report a lack of tolerance in the chimpanzee to the behavioral effects of Δ^9-*trans*-tetrahydrocannabinol (Δ^9THC), the major active constituent of marihuana, on a conditional discrimination task which makes reward dependent on correct choice responses.

The effects of repeated Δ^9THC administrations on short-term memory have not been studied in animals or man. We chose the delayed matching to sample task for our chimpanzee experiments since this task has proved useful for the study of conceptual processes and short-term memory in nonhuman primates, as well as for the study of short-term effects of psychotropic drugs (*6*). In addition, both the delayed matching to sample performance of nonhuman primates and human short-term memory have been shown to be impaired by Δ^9THC (*7, 8*).

Each of the three male and two female adult chimpanzees had extensive behavioral and Δ^9THC histories (*5, 8–10*) but had not been given drugs for approximately 3 weeks prior to the experiments reported below. The indoor area of the chimpanzee's living cage was modified to hold a stimulus-response panel containing three horizontally aligned choice-response keys and a sample response key located above the middle choice key. A delayed matching-to-sample trial was initiated by illuminating the sample response key with one of three white geometric forms (Δ, $\times$, or $-$) or one of three colors (green, red, or blue). The chimpanzee was required to make ten consecutive responses to the sample key after which the sample stimulus was turned off and a 20-second delay period was initiated. After the delay period, each of the three choice keys was simultaneously illuminated with a different choice stimulus. Although the positions of the choice stimuli were determined randomly, the choice stimuli for a given trial were from the same stimulus dimension as the sample stimulus for that trial. A response to the choice stimulus, which was the same as the previously presented sample stimulus, produced a reward consisting of a 1-g banana pellet (Ciba) and terminated the trial. A response to either of the two incorrect choice stimuli simply terminated the trial. Successive trials were separated by 15 seconds. Each daily experimental session consisted of 100 trials or 85 minutes, whichever came first.

After the behavior of individual chimpanzees appeared stable over seven consecutive sessions, the chimpanzees were given six control sessions in which the drug was not given, 21 consecutive Δ^9THC sessions at 1.0 mg/kg, and 21 recovery sessions in which no drug was given. Finally, 42 consecutive Δ^9THC sessions at 4.0 mg/kg were followed by 33 recovery sessions during which no drug was given. The Δ^9THC (*11*) was orally administered 2.5 hours before each drug session in a vehicle consisting of water, corn syrup, and orange extract. The drug vehicle alone was administered 2.5 hours before each session in which no drug was given.

Since no systematic differences were obtained between color and form stimuli, the percentage of correct matching responses was plotted as an average for color and form trials (Fig. 1). Separate *t*-tests indicated that the initial Δ^9THC doses of 1.0 mg/kg and 4.0 mg/kg produced significant ($P < .01$) decreases in matching accuracy as compared to the immediately preceding sessions without the drug. The magnitudes of these performance decrements were apparently not dose related. More importantly, no significant decrease in the drug effect on matching accuracy was observed during either long-term drug regimen. Recovery after the drug was more rapid after termination of the 1.0 mg/kg dose. In fact, a significant ($P < .05$) decrease in matching