taxa identified with the phylogeny of the Attini[7]. On the other hand, *Mycocepurus* is one of a series of small genera whose members cultivate specialized fungi that differ considerably from those produced by polymorphic attines such as *Acromyrmex* and *Atta*[7]. If the mandibular gland chemistry of *M. goeldii* is typical of other attine genera that have diverged from the main stem of attine evolution in not emphasizing simple aliphatic ketones and alcohols, then a large potential treasure-trove of natural products remains to be characterized. This could be particularly true for species in the genera *Mycetophylax* and *Mycetarotes*, two divergent taxa that are considered to be closely related to *Mycocepurus*[7].

Although *o*-aminoacetophenone exhibits demonstrable pheromonal activity for workers of *M. goeldii*, it possesses no pronounced activity for workers of attine species in more specialized genera. Species of the latter, such as *Atta texana*, utilize 4-methyl-3-heptanone as an alarm pheromone and in view of the great olfactory acuity they manifest for their own alarm pheromone[1], it is not surprising that they can readily distinguish this minty ethyl ketone from the unrelated grape-like aromatic ketone produced by *M. goeldii*. It is worth noting that another ant pheromone, methyl anthranilate[12], also is characterized by a powerful grape-like odor, and it may not be insignificant that both *o*-aminoacetophenone and methyl anthranilate possess similar shapes. Since insect pheromones have been utilized as paradigms for studying the relationships of molecular shape to odor quality[13], it would appear that these natural products, because they can be evaluated behaviorally, may be particularly useful for studying olfactory theory. Finally, as the degradation of tryptophan could possibly lead to the synthesis of *o*-aminoacetophenone, this species may provide a particularly useful model for a worthwhile biosynthetic study.

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The effect of diphosphonates on periosteal and bone cells in culture

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Summary. Calvaria cells were separated into periosteal and bone cells and cultured in the presence of ethane-1-hydroxy-1,1-diphosphonate (EHDP) or dichloromethanediphosphonate (Cl_2MDP). Both cell types were affected to the same degree with respect to the effect on cell number and lactate production. The action of the diphosphonates seems therefore not to be specific for one of the cell types.

Diphosphonates are compounds which contain a P-C-P bond and are thus related to pyrophosphate, but they are resistant to metabolic destruction. They have a strong affinity for calcium phosphate crystals; they inhibit both formation and dissolution of this mineral in vitro and in vivo, and they prevent ectopic calcification and bone resorption[2,3]. Recently these effects have been used clinically. Thus, EHDP has been found to decrease the development of ectopic ossification after total hip replacement[4] and in paraplegia[5]. Furthermore various diphosphonates have proved useful in the management of Paget's disease, a disease in which bone turnover is increased[6–8], and in tumoral bone disease[9,10].

These effects of diphosphonates in vivo have been mainly attributed to their physicochemical interactions with calcium phosphate crystals. Recently, however, it was found that the diphosphonates also influence cellular metabolism. Thus, in cultured calvaria and rabbit ear cartilage cells both Cl_2MDP and EHDP decrease the production of lactate[11,12]. Furthermore Cl_2MDP increases glycogen content[13], palmitate oxidation[14] and alkaline phosphatase activity in cultured calvaria cells[15], and increases the synthesis of collagen and glycosaminoglycans in cultured cartilage cells[16,17].

Bone cells have been separated into osteoclast- and osteoblast-like cells by Luben et al.[18], and into periosteal and bone cells by Peck et al.[19]. According to the latter authors, the periosteal cells seemed to be the osteoclast-like and the bone cells the osteoblast-like cells.

In previous studies on the effect of diphosphonates on calvaria cells a mixture of these cells has always been used. The question arises whether the action of the diphosphonates varies according to the type of cells; it is also possible that only certain types of cells survive when cultured in the presence of diphosphonates. To answer these questions, the cells were separated according to Peck[19] and the effect of EHDP and Cl_2MDP on cell number and lactate production was studied in both types of cells.

Material and methods. Bovine parathyroid hormone (PTH) (248 units/mg) was obtained from Inolex Corp., Chicago, Ill., USA. It was dissolved in 0.01 M HCl, 10 mM ascorbic acid, 2 mg/ml bovine serum albumin at a concentration of 40 units/ml, divided into aliquots and frozen at −80 °C. Salmon calcitonin was obtained in solution from Sandoz AG, Basel, Switzerland. 100 units were dissolved in 1.0 ml water containing 2 mg acetic acid, 2 mg sodium acetate and 7.5 mg NaCl. The solution was divided into aliquots and frozen at −80 °C until used. EHDP and Cl_2MDP were obtained as the disodium salt from Procter & Gamble Co., Cincinnati, OH, USA.

The cells were cultured as described earlier[11]. Briefly, calvaria of 1-day-old Wistar rats were digested with collagenase and the liberated cells cultured in an atmosphere

of 5% CO_2 in minimum essential medium with 10% (v/v) foetal calf serum. The cells were plated at a density of 200,000 cells/ml. Either 0.5 ml of cell suspension was added to 24-well tissue culture cluster-dishes 3542 (diameter 1.6 cm, Costar, Cambridge, MA, USA) or 1.5 ml to 3.5-cm petri dishes (Corning Glass Works, Corning, NY, USA). The day of plating was called day zero. The medium was changed on days 1, 4 and 7. The diphosphonates were given from day 1 until the end of the experiment.

To separate calvaria cells into periosteal and bone cells, we used a procedure similar to that described by Peck et al.[19]. To obtain periosteal cells, the periosteum was scraped from the calvaria of 1-day-old Wistar rats with a blunt scalpel and transferred with minimum essential medium, containing 2.7 mM $NaHCO_3$ and 140 mM NaCl, to a centrifuge tube. After centrifugation ($350 \times g_{av}$ for 7 min) it was digested with collagenase[11] and the cell suspension filtered through a 35 µm nylon mesh. The cells were plated at a density of 200,000 cells per ml medium. To obtain the bone cells, the scraped calvaria were digested with collagenase[11] and the cell suspension treated as described above.

If the cells had to be counted at the end of the culture period, a Coulter counter (model Industrial D, Coulter Counter Electronics, Dunstable, Beds, U.K.) was used[11]. The distribution curves (number of cells as function of size) of the 2 kinds of cells, namely bone and periosteal cells, were the same, both having a mean diameter of 14 µm.

The lactate produced during an incubation time of 16 h was measured in the medium after precipitation of the proteins with $HClO_4$, using lactate dehydrogenase[20].

To measure c-AMP, the old medium was sucked off the petri dish and replaced with 1.5 ml of minimum essential medium containing 2.7 mM $NaHCO_3$ and 140 mM NaCl, 0.5% (w/v) bovine serum albumin and 10 mM theophylline. After incubation for 10 min at 37 °C in air, PTH, calcitonin or the vehicle solution were added. After 2.5 min the medium was sucked off and discarded, and the cells were washed once with 1.5 ml of ice-cold Hanks solution. Then 0.5 ml of ice-cold ethanol −0.2 N-HCl[21] was added to the dishes, the cells were scraped off with a rubber policeman and transferred to a tube. The dishes were washed 3 times with 0.5 ml or 0.2 ml respectively of ethanol −0.2 N-HCl and these solutions were also added to the tubes. After leaving the tubes for 24 h at −20 °C, the suspensions were centrifuged at $6500 \times g_{av}$ for 10 min, the supernatants transferred to a conical tube and the solvent evaporated under vacuum. The solid residues were kept dry at −20 °C until the assay was done. The c-AMP was estimated by competitive protein binding assay[22] using the kit supplied by Amersham, Bucks., U.K.

Results. To prove that 2 types of cells were really obtained by the separation technique applied, the stimulation of c-AMP production by PTH and by calcitonin in periosteal and bone cells was measured. As seen in table 1, periosteal cells were more sensitive than bone cells to calcitonin, and bone cells were more sensitive than periosteal cells to PTH, indicating that the separation was successful.

As seen in table 2, the diphosphonates acted on both cell types to the same degree. EHDP had no effect on the cell number of either population, while 0.25 mM Cl_2MDP diminished it considerably. Lactate production was inhibited in a dose-dependent fashion in a similar way in the 2 populations, Cl_2MDP being more active than EHDP.

Discussion. The effect of the 2 diphosphonates was the same on both types of cells and was similar to that observed earlier for a mixed population of cultured calvaria cells[11], i.e. EHDP and Cl_2MDP treatment selected neither periosteal nor bone cells. A similar conclusion was obtained earlier when the effect of Cl_2MDP on the alkaline phosphatase of cultured calvaria cells was studied[15]. A rise of this enzyme under treatment with Cl_2MDP was observed whether cell multiplication was stopped or not. These data therefore also suggested that the effect was not due to cell selection.

Diphosphonates given in vivo affected both cell types, osteoblasts and osteoclasts[23]. Since periosteal cells are osteoclast-like and bone cells are osteoblast-like[19], the data presented here agree with that finding. The situation in vivo, however, is more difficult to interpret, since the concentration in the environment of the bone cells is not known.

Table 1. The effect of PTH and calcitonin on c-AMP production in cultured bone and periosteal cells

	c-AMP production (pmole/dish)	
	Periostal cells	Bone cells
Control	9.1 ±0.6 (6)	8.2 ± 0.4 (4)
Calcitonin 0.5 units/ml	15.6 ±1.6 (5)	10.1 ± 0.2* (3)
PTH 0.4 units/ml	65.2 ±6.6 (5)	188.8 ±21.5** (4)
Calciton/control	1.71±0.21 (5)	1.23± 0.06 (3)
PTH/control	7.2 ±0.9 (5)	23.0 ± 2.9** (4)
PTH/calcitonin	4.2 ±0.6 (5)	18.7 ± 2.2** (3)

After 6 days of culture in dishes with a diameter of 3.5 cm the cells were incubated with hormone, and c-AMP was measured in the cells as described under 'Methods'. The mean of n dishes ±SEM is given.

* Significantly different from periosteal cells $p < 0.05$; ** significantly different from periosteal cells $p < 0.001$.

Table 2. The effect of diphosphonates on bone and periosteal cells

	Control	EHDP		Cl_2MDP		
		0.025 mM	0.25 mM	0.0025 mM	0.025 mM	0.25 mM
a) Effect on cell number		Cell number (% of control)				
Bone cells	100.0± 1.8 (8)	99.9± 5.4 (7)	100.4± 1.9 (8)	102.7± 1.7 (8)	90.3± 3.7* (8)	62.9± 1.7*** (8)
Periosteal cells	100.0± 0.6 (8)	96.3± 1.7 (8)	97.8± 1.4 (8)	91.7± 4.2* (6)	85.7± 4.2** (8)	55.8± 1.4*** (8)
b) Effect on lactate production		Lactate production (% of control)				
Bone cells	100.0± 2.3 (8)	89.2± 5.3 (7)	62.5± 4.7*** (8)	87.0± 2.0*** (8)	68.0± 4.6*** (8)	16.3± 1.3*** (8)
Periosteal cells	100.0± 1.0 (8)	85.9± 4.9* (8)	72.5± 3.3*** (8)	91.3± 4.2* (6)	72.4± 7.4** (7)	7.8± 2.5*** (7)

Cells were cultured in dishes with a diameter of 1.6 cm from days 1–7 in the presence or absence of diphosphonates. Lactate production was measured during an incubation of 16 h from the evening of day 7 until the next morning. The results are the mean ± SEM (n) and are expressed as percent of the control. The absolute values for the control were the following: bone cells: the mean value for the cell number was 588,925± 13,821 (8) cells per dish and for the lactate production: 5.57± 0.13 (8) µmoles lactate/10^6 cells; periosteum cells: the mean for the cell number was 799,325± 28,171 (8) cells per dish and for the lactate production: 3.48± 0.07 (8) µmoles lactate/10^6 cells.

* Significantly different from control $p < 0.05$; ** significantly different from control $p < 0.005$; *** significantly different from control $p < 0.001$.

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Multiple sex-linked reciprocal translocations in a termite from Jamaica[1]

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Summary. Males of the termite *Incisitermes schwarzi* from Jamaica have a diploid chromosome number of 32. In male meiosis there are 7 bivalents, and a ring of 18 chromosomes equivalent to sex chromosomes, 9 X's and 9 Y's. This is the largest reciprocal translocation complex so far described for any plant or animal species.

The males of several species in the primitive termite family Kalotermitidae are multiple translocation heterozygotes, having rings or chains of chromosomes in meiosis; females are structural homozygotes, showing only bivalents in meiosis[2,3]. Disjunction of chromosomes from the rings or chains in the first meiotic division in males takes place in regular alternate fashion, with the translocated set of chromosomes segregating to male-determining sperm, the standard set with which it is associated segregating to female-determining sperm. The geographic variation seen in the chromosome rearrangements in some species[3] suggests that these multiple sex chromosome systems have recently been built up by successive reciprocal translocations or Robertsonian fusions between autosomes and the original sex chromosomes. Among these species is *Incisitermes schwarzi* Banks, which occurs in southern Florida, throughout the West Indies, in Mexico, and probably also in Central America and northern South America. The male meiotic chromosome arrangements found in *I. schwarzi* prior to this report are shown in the first 3 lines of the table[2,3].

Several colonies were collected in northwestern Jamaica. Good chromosome preparations were obtained from the males of one colony about 2 miles east of Discovery Bay. Reproductives were injected with Colcemid; the gonads were dissected out several hours later in a 0.45% sodium citrate solution, fixed and spread by the method of Imai, Crozier and Taylor[4], and stained with Giemsa.

The diploid number from spermatogonial mitoses is 32 (fig. 1). There are 2 acrocentric pairs; most of the other chromosomes are metacentric or submetacentric. Among the several chromosomes that do not occur as precise homologous pairs are 3 distinctive and easily recognizable ones (arrows, fig. 1): a large submetacentric (the largest chromosome of the diploid set), and 2 very small chromosomes (the 2 smallest of the diploid set, one slightly larger than the other). In male meiosis these 32 chromosomes are arranged as 7 bivalents and a multivalent ring of 18 (fig. 2). The bivalents comprise 4 pairs of metacentrics, 2 pairs of acrocentrics, and one small submetacentric pair. The elements of the ring are joined by terminal and subterminal chiasmata; in about 30% of the meiotic cells, the multivalent takes the form of a chain (fig. 3) rather than a ring, probably because of the failure of one chiasma. The multivalent has proved difficult to analyze in detail, but fairly consistently the large submetacentric, the second smallest, and the smallest chromosomes can be recognized at positions numbered 1, 7 and 15, respectively (arbitrarily starting with the large submetacentric as No. 1, and counting in the direction of its long arm) (see fig. 3). With alternate segregation, all even-numbered chromosomes (in

Geographic variation in male meiotic chromosome arrangements in *Incisitermes schwarzi*

Meiotic chromosomes	Location	References
$10^{II} + C^{XI}$	Tulum (Yucatan, Mexico)	Luykx and Syren[3]
$9^{II} + O^{XIV}$	Dania, South Miami, Florida Keys, Everglades City (Florida)	Syren and Luykx[2]; Luykx and Syren[3]
$8^{II} + O^{XVI}$	Miami (Florida) and New Providence Island (Bahamas)	Syren and Luykx[2]; Luykx and Syren[3]
$7^{II} + O^{XVIII}$	Discovery Bay (Jamaica)	This report

II = bivalents; C = chain; O = ring.