tion has been reported for the influence of chloroquine upon the melting of DNA (*17*).

Intercalation into helical duplex structures is not the only type of binding of berberine to polynucleotides. Changes in the absorption spectrum of berberine are also induced by RNA (*8*), and enhancement of the fluorescence of the alkaloid is produced not only by double-helical DNA but also by denatured DNA and ribosomal RNA (*9*). These effects are not merely results of the binding of berberine to polyanions; interaction with chondroitin sulfate, a high-molecular (5×10^3 to 5×10^4 daltons) polyanionic polysaccharide, produces only weak enhancement of berberine's fluorescence (*9*). Monoribonucleotides do not enhance this fluorescence (*9*).

A biological effect of berberine is the conversion of yeast to respiratory-deficient cells which grow in "petite" colonies (*13*). This phenomenon has recently been studied in detail for ethidium bromide with the conclusion that this converting agent is intercalated into supercoiled mitochondrial DNA and produces configurational changes in this DNA which are responsible for the "mitochondrial mutation" (*12*). It is worthy of consideration that berberine acts in an analogous manner.

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References and Notes

1. L. Michaelis, *Cold Spring Harbor Symp. Quant. Biol.* **12**, 131 (1947).
2. D. Gottlieb and P. D. Shaw, Eds., *Antibiotics*, vol. 1, *Mechanism of Action* (Springer, New York, 1967).
3. W. H. Elliot, *Biochem. J.* **86**, 562 (1963); I. B. Weinstein, R. Chernoff, I. Finkelstein, E. Hirschberg, *Mol. Pharmacol.* **1**, 297 (1965); R. L. O'Brien, J. G. Olenick, F. E. Hahn, *Proc. Nat. Acad. Sci. U.S.* **55**, 1511 (1966).
4. R. D. Estensen, A. K. Krey, F. E. Hahn, *Fed. Proc.* **27**, 713 (1968).
5. R. H. F. Manske and W. R. Ashford, in *The Alkaloids*, R. H. F. Manske and H. L. Holmes, Eds. (Academic Press, New York, 1954), vol. 4, p. 78.
6. O. Stickl, *Z. Hyg. Infektionskr.* **108**, 567 (1928); G. F. Foley, R. E. McCarty, V. M. Binns, E. E. Snell, B. M. Guirard, G. W. Kidder, V. C. Dewey, P. S. Thayer, *Ann. N.Y. Acad. Sci.* **76**, 413 (1959).
7. R. L. Varma, *Indian Med. Gaz.* **62**, 84 (1927); R. A. Neal, *Ann. Trop. Med. Parasitol.* **58**, 420 (1964); T. V. Subbaiah and A. H. Amin, *Nature* **215**, 527 (1967).
8. F. W. Morthland, P. P. H. DeBruyn, N. S. Smith, *Exp. Cell Res.* **7**, 201 (1954).
9. H. Yamagishi, *J. Cell. Biol.* **15**, 589 (1962).
10. M. Klimek and L. Hnilica, *Arch. Biochem. Biophys.* **81**, 105 (1959).
11. B. Ephrussi, H. Hottinguer, A. M. Chimenes, *Ann. Inst. Pasteur Paris* **76**, 351 (1949).
12. P. P. Slonimski, G. Perrodin, J. H. Croft, *Biochem. Biophys. Res. Commun.* **30**, 232 (1968).
13. M. N. Meisel and T. S. Sokolova, *Dokl. Akad. Nauk S.S.S.R.* **131**, 436 (1959).
14. L. S. Lerman, *J. Cell. Comp. Physiol.* **64**, Suppl. 1, 1 (1964).
15. ———, *Proc. Nat. Acad. Sci. U.S.* **49**, 94 (1963).
16. R. L. O'Brien, J. L. Allison, F. E. Hahn, *Biochim. Biophys. Acta* **129**, 622 (1966); C. Nagata, M. Kodama, Y. Tagashira, K. Imamura, *Biopolymers* **4**, 409 (1966).
17. J. L. Allison, R. L. O'Brien, F. E. Hahn, *Science* **149**, 1111 (1965).
18. We thank the Medical Audio-Visual Branch of our institute for producing the figures.

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Host Finding by Odor in the Myrmecophilic Beetle Atemeles pubicollis Bris. (Staphylinidae)

Abstract. *The beetle* Atemeles publicollis *orients to the odor of its host ants by positive anemotaxis and osmoclinotaxis. The chemical response changes with age of the beetles. After hatching they are attracted by* Myrmica *odors, and, after hibernating, by* Formica *odors.*

Atemeles pubicollis, a staphylinid beetle, lives as a guest in colonies of ants. Soon after hatching in a nest of its first host *Formica polyctena* Foerst. the beetle migrates to and hibernates in a nest of its second host *Myrmica* sp. In the spring, *Atemeles* returns to the nest of *Formica*, where it reproduces. Although this life cycle was discovered almost 60 years ago (*1*), it remained unknown how the guests find their hosts' nests.

Laboratory experiments combined with field studies on the life cycle of *Atemeles* yielded the following results. Six to ten days after hatching, *Atemeles* leave the nest of *Formica*. Positive phototaxis and a high locomotor and flight activity lead them out of the ants' dark nest mounts and out of the wood biotope. Its second host *Myrmica* has its colonies in open grassland. However, in this biotope many other species of ants occur also. The myrmecophilic beetles cannot recognize the nests of *Myrmica* by optical cues because the nests are all situated either below the surface or under stones. The entrances of *Myrmica* nests look very similar to those of many other ant species.

Therefore, the involvement of olfactory orientation was investigated. Many ant species of the subfamily Myrmicinae mark their trails by pheromones (*2*). Some myrmecophiles living among army ants (*3*), and also the beetle *Amphotis marginata* Fabr. (*4*), a guest of *Lasius fuliginosus* Latr., can follow the trail pheromones of their hosts. *Atemeles*, in contrast, does not respond to trails of *Myrmica* when tested in the laboratory. Colonies of *Myrmica laevinodis* Nyl., *Lasius niger* L., *Camponotus ligniperda* Latr., and *Formica fusca* L. were placed around an arena, with the exits of these colonies covered by nylon netting which made it possible for the beetles to establish odor contact with the ants. In none of these experiments were beetles attracted to the exits of any of the colonies. If, however, weak air currents (0.5 m/sec) were blown through a nest of *Myrmica laevinodis* Nyl. and through *Formica fusca* L. and *Lasius niger* L. nests into the arena,

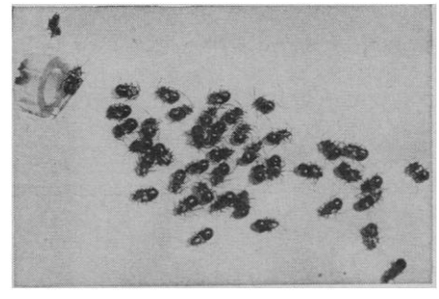

Fig. 1 (top right). *Atemeles pubicollis* follow an air current carrying the odor of their host ants' nest. They thus reach the nest entrance.

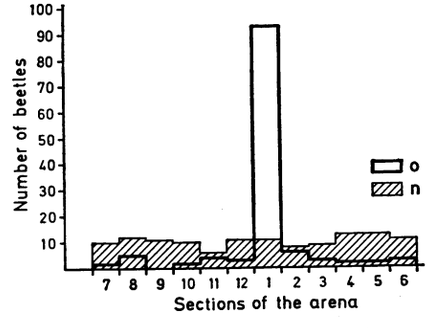

Fig. 2 (bottom right). Distribution of *Atemeles* in an arena when three different ant odors, carried by air currents, are offered simultaneously. The arena is divided into 12 sections. Ants' nest entrances are situated in sections 1 (*Myrmica laevinodis*), 4 (*Formica fusca*), and 10 (*Lasius niger*). Distribution of beetles, when no air is blown into the arena (*n*); distribution when odors are offered (*o*).

the beetles assembled before the entrance of the *Myrmica* nest (Fig. 1). When odors of various ant species were offered simultaneously, the scent of *Myrmica* was always preferred (Fig. 2). The beetles were even attracted if the *Myrmica* scent was added to an air current carrying the odor of different ant species. Only when *Myrmica* odors were absent did other species of the subfamily Myrmicinae, for example, *Tetramorium caespitum* L. and *Solenopsis fugax* Latr., weakly attract the beetles. Thus, a host-specific odor is the releasing signal which attracts *Atemeles pubicollis*.

Olfactory and anemotactic orientation are intimately connected. An air current without host scent releases only a short turning reaction but no clear upwind crawling. Also, the beetles orient osmoclinotactically, that is, in a zigzag pattern toward the source of the scent. Thus they are able to detect and follow the increasing concentration of the scent in the air current. Together these mechanisms enable the guests to find their hosts with high accuracy. *Atemeles* react to the chemical stimuli coming from *Myrmica* only from 1 to 14 days after leaving the *Formica* colony. After this time, no accumulation of beetles at a *Myrmica* scent source is observed. In laboratory colonies, all beetles are adopted by *Myrmica* soon after they reach the peripheral nest area. Adoption is released by substances which are secreted from integumental glandular cells situated dorsolaterally in the beetles' abdominal segments. These substances are highly attractive for the host ants (*5*).

After hibernating and reaching maturity, *Atemeles* leave *Myrmica* and return to *Formica*. Again the beetles orient to specific odors of their host, which is now *Formica*; the positive anemotactic and osmoclinotactic behavior is identical in beetles migrating to *Myrmica* or to *Formica*.

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References and Notes

1. E. Wasmann, *Deut. Entomol. Nationalbibliothek* **1**, 1 (1910).
2. E. O. Wilson, *Science* **129**, 643 (195 → *ibid.* **149**, 1064 (1965); M. S. Blum, I. C. Moser, A. D. Cordero, *Psyche J. Entomol. (Cambridge, Massachusetts)* **71**, 1 (1964); M. S. Blum and G. N. Ross, *J. Insect Physiol.* **11**, 857 (1965).
3. R. D. Akre and C. W. Rettenmeyer, *J. Kans. Entomol. Soc.* **41**, 165 (1968).
4. B. Hölldobler, *Naturwissenschaften* **55**, 397 (1968).
5. ———, *Zool. Anz. Suppl.*, in press.
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Acid Polysaccharides from Invertebrate Connective Tissue: Phylogenetic Aspects

Abstract. *Polyfucose sulfate and a chondroitin sulfate were isolated from echinoderm connective tissue. Coelenterate and poriferan connective tissues were devoid of these acid polysaccharides.*

Mathews *et al.* (*1*) have isolated chondroitin sulfates from the cartilages of a mollusk (squid) and of an arthropod (horseshoe crab). They speculated on the possible existence of a related acid polysaccharide precursor in the connective tissue of some common ancestor. In an investigation on the structure of the carbohydrate moiety of invertebrate collagens (*2*), we have isolated and studied the acid polysaccharides present in the connective tissue of various phyla. We report the isolation of a polyfucose sulfate and a chondroitin sulfate from the connective tissue of an echinoderm (*Thyone briareus*) and the absence of these acid polysaccharides in the connective tissue of a coelenterate (*Metridium dianthus*) and a poriferan (*Hippospongia gossypina*).

Connective tissue from the body wall of *Thyone briareus* was rendered completely soluble with a proteolytic enzyme (Pronase at 70°C, *p*H 8.5 for 2 hours). The acid polysaccharides were precipitated with cetyltrimethylammonium bromide and the precipitate was converted into the soluble potassium salt by potassium thiocyanate and treated with mild alkali (0.1*M* NaOH, 65°C for 2 hours) to remove traces of mannose, xylose, and glucose, as well as residual peptide. The acid polysaccharides, as potassium salts, were separated into two components by the addition of ethanol (Table 1). The optical rotation and composition of the fraction precipitated with 15 to 19 percent ethanol are close to those calculated for chondroitin sulfate, except for excess sulfate. The hexuronic acid moiety was shown to be glucuronic by reduction of the methyl ester to glucose. Hexosamine was identified as galactosamine on an amino acid analyzer. The infrared spectrum in the 8- to 10-μm range indicated that there was a sulfate group in the 6-position of the D-galactosamine residue. The location of the other sulfate groups was not determined. The preparation was completely hydrolyzed (*3*) by testicular hyaluronidase (E.C. 3.2.1.35) (turbidity test and ratio of Elson-Morgan reagent to carbazole). The fraction precipitated by 38 to 52 percent ethanol (Table 1) is a poly-L-fucose sulfate. Preliminary analysis indicated that the L-fucose residues are joined primarily by $\alpha(1\rightarrow2)$ linkages and that the sulfate groups are located at the 3- or 4-position (*4*). Neither of these acid polysaccharides was detected in intact connective tissue, in derived gelatins, or in the carbohydrate fractions of *Metridium dianthus* and *Hippospongia gossypina* obtained by gel filtration after proteolysis. Indeed, no acid polysaccharides (containing hexuronic acid, sulfate, or sialic acid) were found in *M. dianthus* (*5*). Polyfucose sulfate has now been found in the connective tissue of at least three species of echinoderms and in the egg jelly-coat of three additional echinoderm species (*6*). To the best of our knowledge, it has not been reported to occur in any other phylum. It does occur, curiously enough, in one division of plants also consisting of L-fucose residues joined primarily by α-L-$(1\rightarrow2)$ linkages (*7*).

Polyglucose sulfate (*8*) is another example of a connective tissue acid polysaccharide which, thus far, has been encountered in only one animal phylum. The polysaccharide, hyaluronic acid, has been found only in protozoa and in

Table 1. Analysis of acid polysaccharides from *Thyone briareus*.

Residues and elements	Molar ratio of ethanol fractions: 15 to 19 percent (to hexosamine)	38 to 52 percent (to fucose)
Fucose	0.04	1.00
Galactose	0	0.06
Hexosamine	1.00	< .03
Hexuronic acid	1.07	< .02
Sulfate	1.6	.97
Nitrogen	1.02	.04
$[\alpha]_D^{21}$ (water, *c* 1.0)	−42°	−150°