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Absence of Antimicrobial Substances in the Egg Capsules of Millipedes

MILLIPEDES lay their eggs in hollow capsules of their own construction, within which the emerged young spend the early part of their existence. The capsules are moulded primarily of soil and vegetable debris, and are built with the apparent addition of maternal enteric constituents, both oral and aboral in origin[1,2], which suggests that they might be impregnated chemically for defence against microorganisms. Here we present evidence indicating that for at least two species of millipede this hypothesis does not apply, because neither antibacterial nor antifungal substances could be detected in the walls of their capsules.

The millipedes studied were *Glomeris marginata* (order Glomerida) and *Narceus annularis* (order Spirobolida), from Europe and Ithaca, New York, respectively. They produced their capsules in laboratory cages, which were set up with soil and leaf litter, simulating their natural habitat. In the case of *Narceus*, the contents in the cage came from the actual collection sites. Several hundred *Narceus* capsules were obtained and several dozen of *Glomeris*. Extracts were prepared by separating the eggs from the capsules, homogenizing the capsules in water (ratio of capsule to water = 1 : 2 for antibacterial tests; 3 : 10 for antifungal tests), and sterilizing the homogenates by membrane filtration.

The bacteria tested included *Pseudomonas fluorescens*, *Escherichia coli*, *Aerobacter aerogenes*, *Sarcina lutea*, *Bacillus megaterium* and *B. subtilis*, as well as several Gram-negative cocci and bacilli and Gram-positive spore-forming rods isolated from the soil in which the capsules had been deposited. The tests were carried out by addition of capsule extract (droplets or soaked filter paper disks) to tryptose blood agar base (Difco) plates on the surface of which were spread standardized bacterial suspensions. The plates were incubated at 30° C for 18 h and examined for antibiotic effects at a magnification of 12. Even at maximal concentrations, the extracts had no inhibitory effect.

The fungi tested were *Candida albicans*, *Histoplasma capsulatum*, *Cryptococcus neoformans*, *Coccidioides immitis* and—with the *Glomeris* extract only—*Paecilomyces varioti*. Growing cultures of the fungi, in suitable defined media, were uninhibited by the addition of extract to medium in the ratio of 1 : 5. Moreover, capsules of *Narceus* placed directly on peptone glucose agar were quickly overgrown with saprophytic moulds.

It seems probable that the principal function of the capsules is to offer concealment and mechanical protection from predators. A single negative finding of this sort should not be taken to indicate that antimicrobial agents are likely to be generally absent from soil-inhabiting arthropods and other animals. Clues to the occurrence of such agents are not numerous (probably because biologists are not generally alert to their possible significance), but they do occur. In some centipedes, for example, the eggs are tended by the mother, and when unattended become overgrown with mould[3,4]. Comparable brooding behaviour has been reported for crickets[5], earwigs[6] and ants[7]. In our opinion, a systematic pursuit of such leads is likely to pay off, particularly with regard to the eventual discovery of new antifungal agents.

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THOMAS EISNER
STANLEY A. ZAHLER
JAMES E. CARREL
DOROTHY J. BROWN

Division of Biological Sciences,
Cornell University,
Ithaca, New York.

GEORGE W. LONES

Laboratory of Microbiology,
National Institute of Allergy and Infectious Diseases,
National Institutes of Health,
Bethesda, Maryland.

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Sterol Metabolism as a Basis for a Mutualistic Symbiosis

MUTUALISM makes it possible for certain organisms with unusual nutritional requirements to survive and reproduce[1]. Species of this kind may be invaluable in studies of metabolism if the aposymbiotic (symbiote-free) organisms can ultimately be raised easily on defined substrates in sterile conditions[2]. During efforts to develop such a laboratory culture of the beetle *Xyleborus ferruguineus*, fungus-free females feeding on media containing cholesterol as the sterol source produced second-generation larvae that did not pupate[3]. Here we report evidence that the mutualistic fungus *Fusarium solani* associated with the beetle produced ergosterol as its only sterol in pure culture on a chemically defined substrate in sterile conditions. Ergosterol proved to be adequate as the sole sterol source for continued growth, development and reproduction of the fungus-free beetle. Subsequent tests of other sterols showed that 7-dehydrocholesterol was similarly adequate.

The fungal sterol fraction was isolated from *F. solani* grown on Neutral-Dox-Yeast medium[4]. Batches of fungus harvested after five, ten or fifteen days' growth were washed, freeze-dried, weighed and analysed for sterols. For each analysis, 15 g of freeze-dried fungus of a given age was homogenized in and extracted with a 2 : 1 mixture of chloroform–methanol. The total lipid extract was chromatographed on a silicic acid column and the sterol-containing fraction was eluted with chloroform. Thin-layer chromatography of this fraction using several solvent systems showed that significant amounts of sterol esters were not present. Free sterols were precipitated by digitonin treatment[5].

Preliminary identification of the sole sterol, ergosterol, in the fungus was based on its retention time as the free form and as the trimethylsilyl derivative on 1 per cent 'QF–1' on 'Gas-Chrom Q' at two temperature programmes using dual-hydrogen gas–liquid chromatography. Retention times of the isolated ergosterol were compared with recrystallized commercial ergosterol. This identification