now been shown to be functionally involved in the photoperiodic reaction of both an insect and a mite might indicate that the photoperiodic mechanism in different classes of arthropods may comprise certain common functional elements.

It is remarkable that the effect of carotenoid deficiency on the photoperiodic response is fully expressed already in the first generation reared on the diet; in all cases investigated so far the effect was delayed for one or more generations, because of the transmission of small but sufficient amounts of carotenoids from the mother via the eggs[11–15]. However, the eggs of *Apanteles*, like those of other parasitoid wasps, contain very little yolk[26], which may explain the absence of a maternal effect in our work with carotenoid-free diets. The rather variable incidence of diapause in wasps reared under short-day conditions on the diet without vitamin A (table) may be explained by slight variations in the carotenoids present in the natural products (wheat germ, casein, agar) used in the diet. In some studies, carotenoid deprivation did not show an effect on the photoperiodic response or on the entrainment of circadian rhythms; these negative results, however, may well have been due to the presence of maternally derived carotenoids. Rearing the insects for one or more further generations on the carotenoid-free diet might have shown a different effect, but this was not always possible[21,27,28].

When its host is reared on cabbage, the *Apanteles* larvae spin yellow cocoons. However, *A. glomeratus* larvae emerging from caterpillars reared on the artificial diet, both with and without vitamin A, spin white cocoons. Preliminary analyses of the yellow cocoons indicate that lutein is the main carotenoid pigment present. All carotenoids present in *A. glomeratus* are necessarily sequestered from its host; a rather close conformity has been found between the carotenoids present in *A. glomeratus* and in *P. brassicae*[29].

The larvae of *Apanteles* have no external ocelli. It is likely, therefore, that photoperiodic photoreception occurs extraretinally, possibly directly in the brain. The photoreceptors must be very sensitive, considering the fact that light has to pass through the rather heavily pigmented cuticle of the host before it reaches the parasitoid larvae inside. Nevertheless, photoperiodic time measurement is executed by the wasp larvae with remarkable accuracy, quite independent of the host's own response to the photoperiod[23,24]. One of the main goals of further research will be the localization of the photoperiodic photoreceptor in *Apanteles*.

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Prokaryote-eukaryote interactions in trace element metabolism. *Desulfovibrio* sp. in *Helix aspersa*

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Summary. Snails *(Helix aspersa)* contain sulphate-reducing bacteria in their crops. Feeding such animals on food containing sulphate and molybdate ions does not induce a copper deficiency and in fact the bacteria appear to facilitate metal absorption. This is in contrast to the effects of these bacteria in ruminant cattle.
Key words. Copper metabolism; sulphate-reducing bacteria; molybdate; snails.

There are at least 16 metals that are essential for all prokaryotic and eukaryotic forms of life and about 12 of these are only required in trace amounts. Deficiencies in these trace elements are, however, among the most common of naturally occurring diseases probably because most minerals cannot be absorbed in their natural state by cells. Trace elements are to a large extent absorbed in a complexed form and what attaches to the metal ion frequently determines its degree of absorption by the organism[1].

Microorganisms have evolved a wide range of systems for accumulating the trace elements that they require and low mol.wt molecules in the form of water soluble chelates (siderochromes) are secreted by many microorganisms in order to assimilate metals from the environment[2]. The importance of these microbial systems in facilitating metal ion uptake by other organisms is well known and is frequently a crucial influence in the rhizosphere region around plant roots. In fact the microbe-plant interaction is so important in its effect on the growth, devel-

opment and chemical composition of the plant that it is currently being exploited as a method of crop management. Microorganisms may be used to increase production[3] or to inhibit infection[4] by manipulating the availability of metals to plants within the agricultural system.

The interactions between the metal metabolism of microorganisms and animals are poorly understood although competition for these trace elements appears to be one of the basic defence mechanisms for limiting microbial infections[5]. Part of the difficulty in understanding prokaryote–eukaryote interactions is, of course, the wide variety of possible effects that can be involved. In some preliminary experiments we observed, however, that a number of terrestrial molluscs contained sulphate-reducing bacteria in their crops. Sulphate reducing bacteria convert sulphate ions (SO_4^{2-}) to sulphide (S^{2-}) and they are of particular interest in relation to trace element metabolism for the following reasons:

1) Sulphide ions are very strong ligands for many of the essential soft acid metals (e.g. Cu, Zn, Fe, Ni) upon which all animals depend. Sulphate reducing bacteria may, therefore, deprive other organisms of a number of trace elements by rendering them insoluble. 2) Alternatively, since these bacteria are likely to live in a metal deficient environment of their own making they probably have very strong siderophores to chelate metals and make them available for absorption[6]. 3) In cattle there is a strong interaction between sulphate-reducing bacteria and molybdenum in plants. This produces a dramatic and often lethal disease due to copper deficiency apparently caused by thiomolybdates (e.g. MoS_4^{2-}, $MoOS_3^{2-}$ etc.) synthesized by bacteria in the rumen[7].

Molluscs are very copper dependent, mainly because of their use of the copper containing respiratory pigment hemocyanin and they contain special pore cells that are responsible for recycling copper within the body[8]. The occurrence of sulphate reducing bacteria in the crop of these copper dependent organisms provided, therefore, a unique opportunity for investigating the interactions between the metal metabolism of these two groups of organisms.

The common garden snail, *Helix aspersa,* was collected from a single flint walled site near Bradfield, England (Grid reference SU 623713) in April 1984. Specimens were weighed, divided into 3 size ranges, small (< 3.4 g), medium (3.5–7.0 g), and large (7.1–10 g), dissected and analyzed for copper. Similar animals from the same population were maintained in the laboratory from April to July and either fed 3 times per week on a diet of lettuce and carrot that were grown on and sprayed with water containing 0.2 g $NaMoO_4 \cdot l^{-1}$ and 0.1 g $Na_2SO_4 \cdot l^{-1}$ or maintained on untreated lettuce and carrots. After 4 and 10 weeks on this treatment animals were weighed, killed, dissected, digested in acid and analyzed for total copper content by atomic absorption spectroscopy (Varian 175). The crops of the animals that were killed after 10 weeks were removed and their contents incubated in a medium for sulphate reducing bacteria.

This culture medium was based on Postgate's medium D and consisted of 0.5 g $KH_2PO_4 \cdot l^{-1}$; 1.0 g $NH_4Cl \cdot l^{-1}$; 1.0 g $Na_2SO_4 \cdot l^{-1}$; 1.0 g $CaCl_26H_2O \cdot l^{-1}$; 2.0 g $MgSO_4 \cdot 7H_2O \cdot l^{-1}$; 0.5 g $FeSO_4 \cdot 7H_2O \cdot l^{-1}$; 3.5 g sodium lactate $\cdot l^{-1}$; 1.0 g yeast extract $\cdot l^{-1}$; 0.1 g ascorbic acid $\cdot l^{-1}$; 0.1 g thioglycolic acid $\cdot l^{-1}$; 2.5 g agar $\cdot l^{-1}$ autoclaved, adjusted to pH 7.6 and gassed with nitrogen[6].

The culture conditions imposed by this medium are largely specific for sulphate reducing bacteria. The presence of these bacteria is also shown by the reduction of SO_4^{2-} in the medium to S^{2-}. This is identified after about 7–10-day incubation by the formation of black deposits of FeS at the sites of bacterial colonies. Typically a crop that contained sulphate reducing bacteria had about 10^6 of the bacteria per cm^3 and the organisms were approximately 1.9 × 0.7 μm (McHale, unpublished). On the basis of these characteristics the bacteria were provisionally identified as *Desulphovibrio* sp. which is the commonest and most widespread genus of sulphate reducing bacteria.

The copper content of freshly collected snails in April is shown in table 1. There was no significant difference in μmoles Cu · g wet weight^{-1} for the different size groups but in all subsequent work medium or large snails were used (i.e. > 3.5 g). Animals maintained in the laboratory for 4 and 10 weeks showed a progressive increase in copper content whether they were exposed to molybdate/sulphate suppliments or not (fig.). At the end of 10 weeks all the snails that had been exposed to molybdate/sulphate diets contained sulphate reducing bacteria in their crops while only 50% of the control animals contained these bacteria. When this population of control animals was divided into those containing sulphate reducers and those without these bacteria there was a statistically significant difference ($p < 0.01$) in soft tissue copper content (table 2).

Individual growth of the snails was not monitored during these

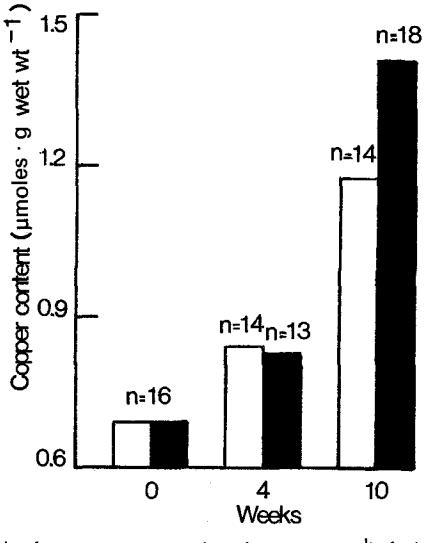
Table 1. Copper content of various size ranges of *H. aspersa* collected in April

Body weight (g)	Copper content soft tissues (μmoles · g wet wt^{-1})
< 3.4	0.62 ± 0.32
3.5–7.0	0.71 ± 0.53
7.1–10	0.72 ± 0.48

Table 2. Copper content of snails in relation to the presence or absence of sulphate reducing bacteria

Treatment	Mean wt of snails (g)	Presence of sulphate reducers	Copper content (μmoles · g wet wt^{-1})
Mo/SO_4	7.8 ± 2.23 ns (n = 17)	+	1.42 ± 1.14
Controls	7.7 ± 1.8 ns (n = 9)	+	1.42 ± 0.50*
Controls	6.6 ± 2.8 ns (n = 8)	–	0.88 ± 0.19*

n, number of specimens; ns, no statistical difference between weights of animals in various treatments. * Specimens statistically different at $p < 0.01$ level.



Increase in the copper content (μmoles · g wet wt^{-1}) during laboratory feeding of diets for up to 10 weeks. White columns, control animals; black columns, animals fed food sprayed with molybdate and sulphate salts (n = number of specimens).

experiments but it is clear from the figure that the animals had accumulated copper and that the concentration of this metal virtually doubled during the 10 weeks of these experiments. The presence of molybdate/sulphate supplements did not influence the ability of snails to take up copper even though sulphate-reducing bacteria were present in their crops. There is, therefore, no evidence of the complex copper-molybdenum-sulphate interactions that occur in ruminants and which have been interpreted as copper deficiency diseases resulting from the bacterial synthesis of thiomolybdates or the precipitation of copper sulphides[7]. In fact the copper content of the snails fed supplemented diets appeared to be greater than those of controls (fig.). One explanation for this seems to be that all the snails on these supplemented diets were found, at the end of the experiment, to contain sulphate reducing bacteria whereas these bacteria occurred in only 50% of the controls. This suggests that the presence of sulphate in the diet encourages the growth of these bacteria in the animals crop. In addition if the copper analyses of the control snails are considered in relation to the presence or absence of bacteria (table 2) it becomes clear that not only is there a significant difference between these groups but the snails with the bacteria have a higher copper content that is equivalent to those fed molybdate/sulphate diets. This indicates that far from being inhibitory the sulphate reducing bacteria are actually beneficial and increase the availability of copper to the snail presumably by the effects of their own metal chelating secretions. This raises a number of interesting questions. Firstly, although the digestive tracts of many molluscs are known to contain bacteria it is only the sulphate reducers that have been considered in these experiments. Does this imply either that these bacteria are good indicators of total bacterial populations or, as seems more likely, that sulphate reducers are particularly good at making copper available to their hosts? Second, is the effect specific for copper or are other metals such as zinc also made more available to the snail? Finally, is this part of a general phenomenon i.e. that those invertebrates that lack an acidic stomach may facilitate their uptake of trace metals by absorbing bacterial metal-chelate complexes in a way analogous to plant root systems. Certainly it appears that ecologists who use invertebrates as environmental monitoring systems should recognize that particular metals may be made more available to these animals by bacterial faunas which may themselves vary with the occurrence of certain pollutants.

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Nature of the sound produced by courtship-inhibiting behavior of the male *Drosophila mercatorum*[1]

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Summary. The male of *Drosophila mercatorum* was found to produce a courtship-inhibiting sound when he was courted by another male. The nature of the sound was significantly different from the courtship sounds emitted by a courting male.
Key words. Drosophila mercatorum; courtship sound; courtship-inhibiting sound; courtship-inhibiting behavior; male-male interaction.

The role of courtship sounds (songs) released by wing vibration of a courting male is known to act positively as a species recognition signal and/or as a sexually stimulating signal that lowers the threshold level of female receptivity[2-5]. It has also been shown in some drosophilid species that the female produces sounds in response to male courtship[2, 6, 7]. Some of these are thought to act as a signal of rejection by unreceptive females, resulting in inhibition of further male courtship. The author reports here that when one male courts another, the latter produces a unique sound that results in inhibition of the courtship of the first male. Such a sound, produced during male-male interaction, has not been described previously in *Drosophila*.

Materials and methods. The wild type laboratory strain of *Drosophila mercatorum* used was R (Rochester, New York, collected in 1957). The male of *D. mercatorum* displays two kinds of courtship behavior accompanying wing vibration, which consequently produces two kinds of courtship sound. The first (A sound) is produced by wing vibration and wing flicking by the male when positioned behind or by the side of the female. After producing the A sound, the male approaches much more closely behind the female, extends one wing at about 40°, and vibrates both wings. The sound thus produced is referred to as the B sound, after which the male finally attempts to mount. The results obtained so far suggest that the A sound may be essential for male recognition by the female and that the B sound is more closely associated with copulation itself[3].

The flies used were raised and aged on the standard *Drosophila* medium seeded with live yeast. They were kept at 25 ± 1 °C on a 12-h light-dark cycle. Mating tests and recording of the sounds were carried out during the beginning of the dark period, at 6 days of age. This species is known to be sexually most active in the evening, and at this age[8].

Observations of mating behavior were made at 10 × magnification. Procedures both for recording sounds and for obtaining oscillograms have been described elsewhere[3]. Interpulse intervals of the sounds were determined from the oscillograms. Sonograms were produced using a Kay Electronic Co. 7030A sound spectrograph with the reproducing circuit of the sonograph set at 20–2000 Hz. The relative quantification of sound levels was made by a sectioner analysis which permits the individual intensity of each frequency component to be displayed at any preselected point in time; this permits one to estimate both a funda-