between the stromatolite-bearing carbonates and evidence of extensive volcanicity is also of note. In all the other early Precambrian occurrences, stromatolites are associated with lavas, and we speculate that the exhalative activities of lava extrusions provided optimum conditions for stromatolite growth. The Earth's atmosphere at the time was probably anaerobic[18], and some Recent algal species can thrive in such an environment. If ancient stromatolites produced oxygen by photosynthesis, this could have been immediately removed from the environment as free oxygen, and transported to one of the oxygen sinks then operative. The thin banded ironstones in the overlying Mozaan might owe their presence to the local production of biogenically derived oxygen during those times.

Cloud[19] adapted many of his former ideas concerning the major features of crustal evolution to account for the anomalous presence of banded ironstones and stromatolites in the Pongola. He suggested that the events taking place in southern Africa in the early Precambrian were out of phase with those of the rest of the planet in that they anticipated many later developments in other areas.

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T. R. Mason

Department of Geology,
University of Natal,
Durban 4001,
South Africa

V. Von Brunn

Department of Geology,
University of Natal,
Pietermaritzburg 3201,
South Africa

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Pigmentation of the ladybird beetle *Coccinella septempunctata* by carotenoids not of plant origin

Many insects contain carotenoid pigments[1], and some notable examples are found in the Coleoptera, where carotenoids are responsible for the yellow colour of the yellow and black striped wing cases of the Colorado beetle, *Leptinotarsa decemlineata*, and the orange-red colour of the ladybird beetles, *Coccinella* spp. There has, however, been no report of any insect species being able to synthesise carotenoids *de novo*, and it is generally accepted that in insects, as in other animals, any carotenoids present are of dietary origin[2]. In July 1976 the Liverpool area, like many regions of the UK, experienced an invasion by enormous numbers of ladybirds, almost entirely of the seven-spot variety, *Coccinella septempunctata*. With such large numbers of insects available we were able to use modern analytical techniques to identify the carotenoids of these beetles. We report here that the carotenoids identified were mostly hydrocarbons, and the carotenoid pattern indicated that these pigments in the ladybird are likely to be of microbial rather than plant origin, thus suggesting the involvement of symbiotic microorganisms.

Preliminary thin-layer chromatographic analysis showed that the carotenoid composition of isolated red wing cases (elytra) was the same as that of the entire insect, so whole insects were used in the large-scale analyses. Carotenoid hydrocarbons formed the bulk of the pigment, but there were also small amounts of many xanthophylls, of which only lutein (β, ε-carotene-3,3′-diol) was identified with certainty. The carotenes were characterised by chromatographic properties, light absorption and mass spectra, and whenever possible by comparison with authentic samples. Eighteen compounds were identified, including β-carotene (β,β-carotene), previously reported by Lederer[3] and by Valadon and Mummery[4]. One of the main carotenes present was torulene (3′,4′-didehydro β,ψ-carotene), and smaller amounts of γ-carotene (β,ψ-carotene), β-zeacarotene (7′8′-dihydro-β,ψ-carotene), 'cyclic ζ-carotene' (7′,8′,11′,12′-tetrahydro-β,ψ-carotene), lycopene (ψ,ψ-carotene) and 3,4-didehydrolycopene (3,4-didehydro-ψ,ψ-carotene) were present. A second series of compounds present possessed a methylenecyclohexane ring system (γ-ring) (ref. 5), a very unusual structural feature. β,γ-Carotene, γ,γ-carotene, γ,ψ-carotene, 3′,4′-didehydro-γ,ψ-carotene, 7′,8′-dihydro-γ,ψ-carotene and 7′,8′,11′,12′-tetrahydro-γ,ψ-carotene were all identified. The natural occurrence of the last three of these carotenoids has not been reported previously. A series of more saturated acyclic compounds that are intermediates in carotenoid biosynthesis[6] was identified—phytoene (7,8,11,12,7′,8′,11′,12′-octahydro-ψ,ψ-carotene), phytofluene (7,8,11,12,7′,8′-hexahydro-ψ,ψ-carotene), neurosporene (7,8-dihydro-ψ,ψ-carotene) and 'ζ-carotene' (a mixture of symmetrical 7,8,7′,8′,-tetrahydro-ψ,ψ-carotene and unsymmetrical 7,8,11,12-tetrahydro-ψ,ψ-carotene). The carotenoids present in the largest amounts were torulene and β,β-, β,γ-, β,ψ- and γ,ψ-carotenes.

Phytoene, phytofluene, 'ζ-carotene', neurosporene, 'cyclic ζ-carotene' and β-zeacarotene are possible intermediates (as are lycopene and γ-carotene) in the biosynthesis of β-carotene, and have not been detected before in any insect or other animal. The presence of lycopene in the ladybird has finally been proved, long after evidence for its occurrence was first reported[3]. The γ-ring carotenoids and the carotenes of the torulene series are also unusual and their presence in an animal is surprising.

The question of the origin of these pigments cannot be answered in terms of the accepted view that insect carotenoids are of dietary origin[2]. The food chain involving the ladybird is short and simple. The beetles feed on aphids which in turn feed on the juices of green plant tissues. Within this food chain only the green plants are known to be capable of synthesising carotenoids, and there is no known metabolic process whereby the bicyclic compounds with β- and ε-rings, produced by the plants, could be transformed into acyclic, monocyclic or γ-ring carotenes. The ladybird carotenes must therefore originate elsewhere.

The γ-ring carotenes, β,γ-carotene and γ,γ-carotenes, together with β-carotene have, however, been isolated from a green variant of the aphid, *Macrosiphum liriodendri*, whereas pink variants of the same species contained β-carotene, γ-carotene, torulene, lycopene and 3,4-didehydrolycopene[7]. We stress that the ladybirds used in our investigation were members of a huge, migratory, starving population, and nothing was known of their previous food intake. Although it is unlikely that the ladybirds or their larvae would have had access to the aphid species *M. liriodendri*, which seems to be a specific parasite of the tulip tree (*Liriodendron tulipifera*), it is not unreasonable to expect that the γ-ring and torulene series carotenoids may be present in other species of aphid which may have formed part of their diet. Even so, the aphids themselves could not obtain these carotenes from the green plants on which they feed.

We therefore conclude that these carotenes are synthesised

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within the aphid and/or the ladybird. The possibility of synthesis *de novo* by the insect cannot be ruled out, although this would be the first instance to be discovered of carotenoid synthesis by any animal. The involvement of symbiotic microorganisms seems more probable, and the nature of the carotenoids present gives indirect support for this theory. Apart from these reports of their occurrence in the insect species, γ-ring carotenoids have been found only in a discomycete fungus *Caloscypha fulgens*[8]; torulene and related compounds are the characteristic pigments of red yeasts (*Rhodotorula* spp.)[9]. Further, the 'ζ-carotene' isolated from ladybirds was a mixture of the symmetrical and unsymmetrical isomers, a situation encountered commonly in bacteria and fungi but not normally found in higher plants[10]. Weisgraber *et al.*[11] have summarised evidence which they believe suggests strongly that the γ-ring and torulene series carotenes present in the aphid *M. liriodendri* are of microbial origin, and aphids in general are rich in symbionts (yeasts, bacteria, etc.) some of which are red[12].

For the ladybird, many of the carotenes present could presumably have been derived from aphids (or their symbiotic microbes) devoured by the beetles, but it seems unlikely that phytoene and the other desaturation and cyclisation intermediates would be absorbed and accumulated by the ladybird if they were obtained only in minute amounts in its food. Their presence suggests a site of synthesis within the ladybird itself. Although no information is available about the microflora of the ladybird, it is reasonable to believe that carotenogenic microorganisms present in its aphid prey could be acquired and cultivated by the beetle. Thus it is likely that the carotenoids responsible for the characteristic red colour of *C. septempunctata* are synthesised within the insect, probably through an association with symbiotic microorganisms. Preliminary analysis indicates that the situation may be similar in other species of ladybird.

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GEORGE BRITTON
WILLIAM J. S. LOCKLEY
GARRY A. HARRIMAN
TREVOR W. GOODWIN

Department of Biochemistry,
The University of Liverpool,
P.O. Box 147,
Liverpool, UK

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Naturally occurring viral R plasmid with a circular supercoiled genome in the extracellular state

PHOTOSYNTHETIC bacteria have been used extensively in studies on photosynthesis, photochemical nitrogen fixation and photoevolution of hydrogen. A major obstacle to combined biochemical and genetic studies of these processes is a lack of knowledge of gene transfer in such bacteria. The only transfer mechanism known is that reported by Marrs for the facultative phototroph *Rhodopseudomonas capsulata*[1]. In this bacterium gene transfer is mediated by a filterable agent of small size (Gene-Transfer-Agent) estimated to carry between 10^5 and 10^6 daltons of DNA; the nature of these GTAs is unknown. Although GTAs have been used to construct a preliminary map of the bacteriochlorophyll and carotenoid biosynthetic genes in *R. capsulata* (ref. 2), the small amount of DNA transferred limits their usefulness in genetic analysis. To obtain alternative means of gene transfer we examined isolates of the closely related species *R. sphaeroides* for temperate transducing bacteriophage and conjugative R plasmids.

Several temperate, but evidently non-transducing bacteriophage have been isolated from *R. sphaeroides* (ref. 3) and *R. capsulata* (ref. 4). Many of our wild type isolates carry between one and three prophages and more than 90% are resistant to penicillin because of a diffusible penicillinase (unpublished data). Since the production of diffusible penicillinases by *Staphylococcus aureus*[5], *Pseudomonas aeruginosa*[6] and various enteric bacteria[7] is encoded by plasmid genes, extrachromosomal DNA isolated from *R. sphaeroides* (refs 8, 9) could constitute extrachromosomal prophages and/or penicillinase plasmids.

Initial experiments with a penicillinase-producing strain of *R. sphaeroides* RS601 (prototrophic, penicillin resistant) showed that a sensitive mutant RS602 (prototrophic, penicillin sensitive), isolated after treatment with the mutagen nitrosoguanidine, was 'cured' of a prophage. Bacteriophage released spontaneously by the wild type RS601 (10^3–10^4 plaque-forming units per ml of supernatant) produced small (1 mm diameter) turbid plaques on the 'cured' derivative RS602.

Although attempts to perform generalised transduction with this bacteriophage (designated Rφ6P) were unsuccessful, when recipient cells (RS602) were grown in aerobic conditions at 32 °C and at a multiplicity of infection of 1.0, 50% of 'survivors' of bacteriophage infection were penicillin resistant. An analysis of 100 penicillin resistant transductants derived from this experiment showed that each produced a diffusible penicillinase.

The question now arises as to the exact nature of this high frequency transduction; at least two explanations seem possible. Either the transduction is mediated by a defective specialised transducing bacteriophage analogous to Plide in *Staphylococcus aureus*[10], or the wild type bacteriophage

Table 1 The relationship between penicillin resistance and lysogenisation by Rφ6P in *R. sphaeroides*

Possible phenotypes	Number of colonies
$Pen^s \varphi^-$	1127
$Pen^s \varphi^+$	0
$Pen^r \varphi^-$	0
$Pen^r \varphi^+$	123

An exponential culture (about 5×10^8 colony forming units per ml) of RS602 (penicillin sensitive, Rφ6P sensitive) grown with aeration at 32 °C, was infected with Rφ6P at a multiplicity of 0.1. The bacteriophage were allowed to adsorb for 30 min at 32 °C without aeration and then excess free bacteriophage were removed with anti-bacteriophage antiserum. The bacteriophage infected cells were diluted and plated on PYEA (0.3% peptone, 0.3% yeast extract, 1.5% agar) to give approximately 50 colonies per plate. After 2 d of incubation at 32 °C the colonies were patched on to PYEA plates and later replicated onto PYEA + 1 μg ml^{-1} penicillin to determine their resistance/sensitivity phenotype. Lysogeny, as determined by release of free bacteriophage, was assayed by overlaying the patch plates with soft PYEA (0.8% agar) seeded with RS602.

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