Transgenic plants

Insecticidal toxin in root exudates from *Bt* corn

Bt corn is corn (*Zea mays*) that has been genetically modified to express insecticidal toxins derived from the bacterium *Bacillus thuringiensis* to kill lepidopteran pests feeding on these plants. Here we show that *Bt* toxin is released into the rhizosphere soil in root exudates from *Bt* corn.

The insecticidal toxin produced by *B. thuringiensis* subsp. *kurstaki* remains active in the soil, where it binds rapidly and tightly to clays[1] and humic acids[2]. The bound toxin retains its insecticidal properties[3] and is protected against microbial degradation by being bound to soil particles[4], persisting in various soils for at least 234 days (the longest time studied), as determined by larvicidal bioassay[5]. Unlike the bacterium, which produces the toxin in a precursor form, *Bt* corn contains an inserted truncated *cry1Ab* gene that encodes the active toxin.

In laboratory studies, caterpillars of the monarch butterfly (*Danaus plexippus*) were killed as a result of feeding on milkweed (*Asclepias curassavica*) that had been artificially contaminated with pollen from transgenic corn that expressed the *cry1Ab* gene from *B. thuringiensis* subsp. *kurstaki*[6], and green lacewings (*Chrysoperla carnea*), which are insect predators of insect pests, were killed by ingesting European corn borers (*Ostrinia nubilalis*) reared on *Bt* corn[7].

We germinated surface-sterilized seeds[8] of *Bt* corn (NK4640Bt) and of the isogenic strain without the *cry1Ab* gene on agar. The seedlings were aseptically placed on plastic screening (6-mm mesh), which, to minimize contamination by products of endosperm hydrolysis, was suspended over 200 ml of Hoagland's solution in 4-litre beakers covered with aluminium foil, which was removed after 18 to 20 days when the tops of the plants had reached it. After 7, 15 and 25 days of growth in a plant-growth room (26 ± 2 °C, 12 h light–dark cycle), the soil-free medium was replaced with fresh solution and analysed.

Total protein in the medium (average of 105 μg protein per plant) was determined by the Lowry procedure[9]. A major band migrating on SDS–PAGE (sodium dodecyl sulphate–polyacrylamide gel electrophoresis) to a position corresponding to a relative molecular mass of 66,000 (66K), the same as that of the Cry1Ab protein, was evident after 7 and 15 days only in the exudates from *Bt* corn, although several protein bands of smaller relative molecular mass were seen in exudates from both *Bt* and non-*Bt* corn. We confirmed the presence of the toxin in the exudates from *Bt* corn by immunological assay with Lateral Flow Quickstix (from EnviroLogix, Maine; detection limit < 10 parts per billion) and verified that it was active in an insecticidal bioassay using larvae of the tobacco hornworm (*Manduca sexta*), a model for testing antilepidopteran activity[3,5].

Larvae placed on medium containing exudates from *Bt* corn stopped feeding and began to die after 2 to 3 days and had a mortality of 90 to 95% after 5 days (dose lethal to 50% of larvae, LC_{50}, was 5.2 μg protein). There was no immunological reaction or larval mortality obtained with the exudates from non-*Bt* corn. After 25 days of growth, when the medium was no longer sterile (as demonstrated by streaking it on various microbiological media), the 66K band had disappeared, although there were several new protein bands of smaller relative molecular mass, and the immunological and larvicidal assays were negative, indicating that microbial, and probably also corn, proteases had hydro-lysed the toxin.

Samples of soil from the rhizosphere of seedlings that had been transplanted into either sterile or non-sterile soil in test-tubes were taken from randomly selected tubes, vortexed with extraction buffer (EnviroLogix) and centrifuged. We analysed the supernatants using the immunological and larvicidal assays and found that these were positive, even after 25 days of growth, for samples from *Bt* corn (100% mortality; $LC_{50} = 1.6$ μg protein per soil tube) but were negative for non-*Bt* corn. Moreover, particles of rhizosphere soil in suspension placed directly on the bioassay medium caused mortality comparable to the supernatants.

Although the concentration of protein in the rhizosphere soil was approximately 95 μg per g soil, the concentration of the actual toxin in the extraction buffer was apparently too low to be detected by SDS–PAGE. These results agree with earlier findings showing that the toxin binds rapidly to surface-active soil particles and that the bound toxin retains its larvicidal activity and is protected by this binding against biodegradation[1–5].

About 15 million acres of *Bt* corn were planted in the United States in 1998, which was just under 20% of the total acreage of corn[10]. The *Bt* toxin that is released into soil from roots during the growth of a *Bt* corn crop would add to the amount of toxin introduced into soil from pollen during tasselling and as a result of the incorporation of plant residues after harvesting the crop.

We have no indication of how soil communities might be affected by *Bt* toxin in root exudates in the field. *Bt* toxin in the rhizosphere might improve the control of insect pests, or it might promote the selection of toxin-resistant target insects. Receptors for the toxin are present in non-target as well as target insects, so there may be a risk that non-target insects and organisms in higher trophic levels could be affected by the toxin. Further investigations will be necessary to shed light on what might happen underground.

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Palaeobiology

Herbivorous diet in an ornithomimid dinosaur

In 1997, twelve well-articulated skeletons of an ornithomimid dinosaur[1] from the Upper Cretaceous Ulansuhai Formation[2] in China were discovered. Each skeleton contained a preserved gastrolith mass inside the ribcage that was attached on the medial surface of the articulated dorsal ribs and gastralia. The occurrence and characteristics of gastrolith masses in this ornithomimid indicate that these non-avian toothless theropods may have had gizzards and been herbivores, like modern herbivorous birds that use grit to grind up plant matter.

The gastroliths found in these dinosaurs (Fig. 1) are mainly composed of grains of silicate, with no bony elements (energy dispersive X-ray analysis detected hardly any phosphorus pentoxide in the matrix) or insect remains. The gastroliths are found in the same region of each individual, generally close to the middle dorsal vertebrae. One of the isolated gastrolith masses belongs to a large individual (15 × 11 × 3 cm), as judged by the size of its rib impressions, and occupies a greater total volume than those belonging to juveniles (10 × 7.5 × 2.5 cm; Fig. 1c), which is indicative of a body-size correlation to total gastrolith volume like that observed in crocodilians[3].

Between species, grit size in modern birds is correlated with body weight, as demonstrated by a regression of mean grain size against the logarithm of mean body weight[4]. We sonically disaggregated part of one gastrolith mass, found in a juvenile, which contained roughly 170 grains per cm^3 (grains were counted if they were larger than 0.5 mm along the intermediate axis). The estimated body weight of the juvenile (femur circumference, 57 mm) was about 10 kg (ref. 5), and the mean intermediate diameter of the grain was 1.27 mm, which is small with respect to the estimated body weight and lies outside the range of modern birds (Fig. 1e).

Most gastrolith grains less than 1 mm in diameter vary from angular to very angular, and those between 1 and 2 mm are mainly subangular (Fig. 1f). Small gastrolith grains may have spent only a short time in the theropod body, whereas larger grains were probably subjected to more abrasion from other grains. Excluding grains ranging from very angular to subangular, the mean grain size is 2.41 mm, which fits the predicted size based on values in modern birds (Fig. 1e). There are over a thousand grains in total, with a concentration of 37 grains per cm^3.

The fully terrestrial habitat of this ornithomimid[1] rules out the possibility that the gastroliths were used for hydrostatic adjustment, as has previously been recorded for plesiosaurs[6], crocodilians[3] and a tangasaurid[7]. The small calcium content of the grains indicates that they were unlikely to have been used for the uptake of calcium nutrient, as in birds[8]. The limited food-processing ability of the oral cavity of ornithomimids, indicated by a lack of teeth and the weakly developed jaw adductor musculature[1], indicates that the gastroliths may have been used for the mechanical breakdown of food, as in herbivorous dinosaurs[9] and omnivorous and herbivorous birds[4].

In modern birds, there is a clear relation between diet and the characteristics of the grit they use[4]: carnivorous birds have no muscular gizzard or grit; frugivorous birds use only a little grit; and herbivorous birds retain more grit than insectivorous or omnivorous birds[4]. The occurrence of gastroliths in this ornithomimid and the large number of grains they contain are consistent with a herbivorous diet and the possession of a muscular ventricular stomach, or gizzard, like that found in modern herbivorous birds.

Gastroliths in non-avian theropods are found in this ornithomimid and in *Caudipteryx*[10]. Phylogenetic analysis of Theropoda does not support a close relationship of Ornithomimidae with *Caudipteryx* or the bird clade[10,11], indicating that the use of gastroliths in this ornithomimid is the result of convergent evolution, and that herbivory in theropods may have evolved on several occasions.

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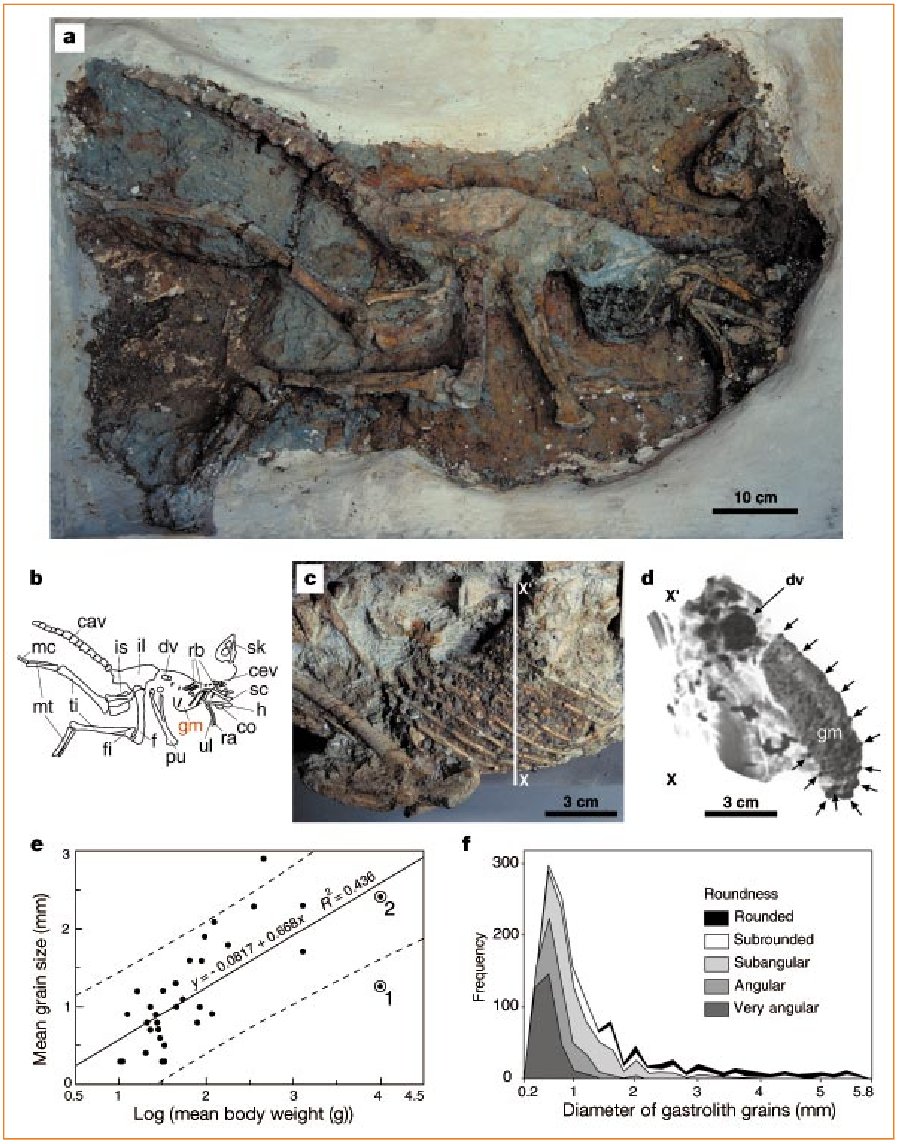

Figure 1 Gastroliths found in the ribcage of skeletons of ornithomimid theropod dinosaurs. **a,** Articulated ornithomimid specimen IVPP-V11797-1. **b,** Schematic representation of the skeleton shown in **a**. **c,** Gastrolith mass in stomach region of IVPP-V11797-4. **d,** Computed tomography scan at the white line (X–X′) in **c**. Unlabelled arrows point to ribs. **e,** Scatter plot based on modern birds (single dots)[4, 12] and the ornithomimid (1). Broken lines indicate the 95% confidence interval. The ornithomimid value, excluding very angular to subangular gastrolith grains (2), is shown for comparison. **f,** Grain size frequency subdivided by grain roundness. Abbreviations in **b** and **d**: cav, caudal vertebra; cer, cervical vertebra; co, coracoid; dv, dorsal vertebra; f, femur; fi, fibula; gm, gastrolith mass; h, humerus; il, ilium; is, ischium; mt, metatarsus; pu, pubis; ra, radius; rb, rib; sc, scapula;

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