

Entomology

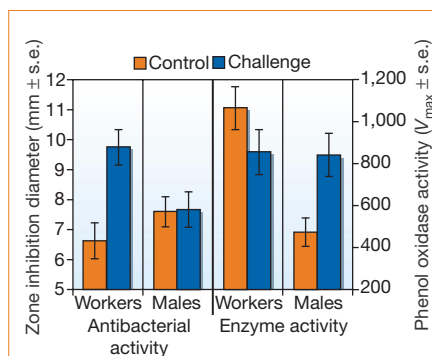
# Immune defence in bumble-bee offspring

Immune-challenged vertebrate females transfer specific antibodies to their offspring<sup>1–3</sup>, but this gratuitous immunity cannot operate in invertebrates<sup>4</sup>. Here we show that constitutive immune defence is enhanced in sexual offspring of the bumble-bee *Bombus terrestris* L. when the parental colony is immune-challenged. Our findings indicate that invertebrates may use a different component of the immune system to generate a facultative trans-generational increase in the immune response.

Insect immunity is characterized by the inducible expression of a large array of antimicrobial peptides and by the constitutive melanization–encapsulation response, which is based on a cascade involving an inactive precursor of the enzyme phenol oxidase<sup>4,5</sup>. Antibacterial activity can be induced, for example, by lipopolysaccharide (LPS) extracted from bacterial surfaces. The operation of the cascade is indicated by the phenol oxidase activity in the insect haemolymph<sup>6,7</sup> and can be monitored by measuring the rate of conversion of a phenol substrate into quinone, which then polymerizes to form melanin. Because both quinone and melanin are toxic to microorganisms<sup>5</sup>, hosts with high phenol oxidase activity are less susceptible to microbial infection<sup>8</sup>.

Social insects cooperate in brood care and make a considerable investment in their offspring. In annual species such as bumble-bees, reproduction occurs at the end of the colony cycle, when sexuals (daughter queens and males) emerge — here the term ‘trans-generational’ distinguishes the queen and workers from sexual offspring. Unlike daughter queens, males do not hibernate, so their reproductive success depends on post-emergence survival after they leave the parental colony and are exposed to parasites in the same habitat<sup>9</sup>. Assuming facultative adjustment of offspring immunity, we investigated whether parasite-challenged parental colonies could enhance their males’ immunocompetence.

We used a split-colony design<sup>10</sup> with 11 colonies, each equally split into treatment and control groups. In the immune-challenged group, 70–80% of workers were injected weekly with LPS (Sigma L-2755, 0.5 mg ml<sup>-1</sup> in Ringer’s solution (5 µl)), which activates the immune system for long periods<sup>11</sup>. Control workers were treated in the same way, but with the omission of LPS. Colonies completed their life cycle in the laboratory under standard conditions (24 °C, 60% relative humidity). We counted the number of sexuals and haemocytes (Neubauer haemocytometer, 1/6 dilution) and used standard protocols to measure



**Figure 1** Antibacterial and phenol oxidase activities in the haemolymph of workers and males from control (orange bars) and challenged (blue bars) groups from 11 bumble-bee colonies. In workers, antibacterial activity was higher in challenged groups than in controls (Wilcoxon’s paired signed-rank test:  $T_+ = 6$ ,  $n = 11$ ,  $P < 0.02$ ), but phenol oxidase activity was lower ( $T_- = 3$ ,  $n = 11$ ,  $P = 0.005$ ). In males, antibacterial activity was the same ( $T_+ = 31$ ,  $n = 11$ , NS) but phenol oxidase activity was higher ( $T_+ = 6$ ,  $n = 11$ ,  $P < 0.02$ ) in the challenged side than in controls. Further experiments showed that increased activity in males correlates with an increased encapsulation response against an invader ( $F_{1,23} = 5.25$ ,  $P = 0.031$ ).  $V_{max}$  is measured as the maximum change in optical density  $\times 10^{-3}$  per minute.

antibacterial<sup>12</sup> and phenol oxidase<sup>13</sup> activity (1/20 dilution).

As expected, workers in the challenged groups showed more antibacterial activity than controls (Fig. 1). Their phenol oxidase activity, however, was lower (Fig. 1), indicating that there could be a possible trade-off between these two immune responses in challenged workers. Haemocyte counts were similar between the two groups (Wilcoxon’s paired signed-rank test,  $T_- = 29$ ,  $n = 11$ , NS). Immune-challenged groups had lower reproductive output (repeated measures-MANOVA for log-transformed number of males and queens: Hotelling’s  $T = 1.297$ ,  $F_{2,9} = 5.839$ ,  $P = 0.024$ ), notably producing fewer queens ( $F_{1,10} = 12.082$ ,  $P = 0.006$ ), indicating a possible trade-off between reproductive output and immune response. Male offspring from challenged groups showed higher phenol oxidase activity than controls, but antibacterial activity (Fig. 1) and haemocyte counts were comparable between the two groups ( $T_- = 29$ ,  $n = 11$ , NS).

As insects do not produce antibodies, they cannot transfer specific immunity as mammals do<sup>1</sup>. Male bumble-bees from immune-challenged groups have increased constitutive immunity relative to controls, which both enhances encapsulation (Fig. 1) and protects against microorganisms<sup>5,8</sup>. As the phenol oxidase enzyme cascade provides a broader immunity than the costly antibacterial immune response<sup>12</sup>, males may benefit by enhancing their most general means of prophylaxis. Although the physiological mechanism by which this trans-generational transfer is achieved is unknown, the enhanced immunity could be

the result of monitoring cues from worker bees, as in the density-dependent prophylaxis observed in other insects<sup>13</sup>.

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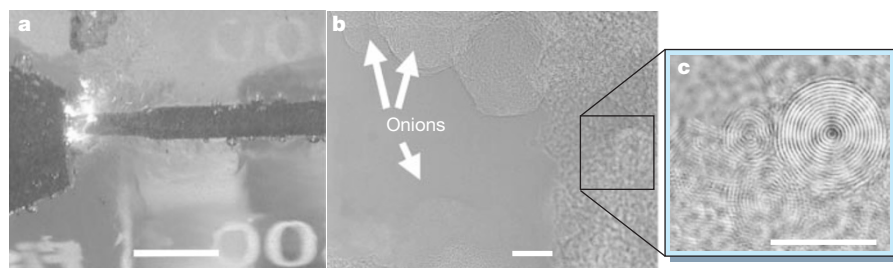
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Nanotechnology

# Synthesis of carbon ‘onions’ in water

The fabrication of carbon nanomaterials usually calls for expensive vacuum systems to generate plasmas<sup>1,2</sup> and yields are disappointingly low. Here we describe a simple method for producing high-quality spherical carbon nano-‘onions’ in large quantities without the use of vacuum equipment. The nanoparticles, which have C<sub>60</sub> cores surrounded by onion-like nested particles, are generated by an arc discharge between two graphite electrodes submerged in water. This technique is economical and environmentally benign, and produces uncontaminated nanoparticles which may be useful in many applications.

Nanoparticles have previously been prepared using a range of vacuum and non-vacuum methods<sup>3–5</sup>. Ours is a non-vacuum method in which the carbon arc is sustained in deionized water. The apparatus consists of two submerged graphite electrodes, and the arc discharge is initiated by contacting a pure grounded graphite anode (tip diameter, 5 mm) with the carbon cathode (tip diameter, 12 mm) of similar purity; the discharge voltage and current were 16–17 V and 30 A, respectively. The nano-onions are mostly found floating on the water surface, with the rest falling to the bottom of the beaker through natural segregation, giving material of high purity.



**Figure 1** Carbon nano-'onions' created by arc discharge in water. **a**, Image of a carbon arc discharge in water. Scale bar, 12 mm. **b, c**, Low- and high-magnification electron micrographs of carbon nano-onions floating on the water surface after their production. Scale bars, 10 nm.

A digital image of the arc discharge in water is shown in Fig. 1a.

We investigated the unpurified material from the water surface using a JEOL 4000EX transmission electron microscope. A typical high-resolution micrograph of the material is shown in Fig. 1b — several spherical carbon nano-onions are evident, as well as polyhedral, nested onion-like particles. At higher magnification (Fig. 1c), spherical nano-onions with 7, 10 or 15 walls are seen. The core of the larger 'onion' in Fig. 1c has a diameter of 7–8 Å, which is consistent with that of the C<sub>60</sub> molecule. A series of images obtained at intervals through the features verified that they were spherical nanoparticles and not nanotubes<sup>6</sup>.

The average diameter of the nano-onions is 25–30 nm (range 5–40 nm), a useful size range for many lubrication applications. Nanoparticles composed of halogens, tungsten and sulphur (IF–WS<sub>2</sub>), which are similar to the carbon onions reported here, are more effective as solid-state lubricants when dispersed in oil than are the conventionally used 2H–MoS<sub>2</sub> crystals<sup>7</sup>.

Although the production rate (3 mg min<sup>-1</sup>) of these carbon onions is faster than in conventional processes, it is not yet adequate for industrial application. The apparatus used in the initial experiments was unsophisticated, however, and minor adjustments (such as increasing the arc current to 50 A) could improve yields significantly. The loss of water through evaporation is slight, but chilling and circulating it may enhance production; auto-feeding the carbon anode would allow the process to run continuously for several hours. With these and other minor modifications, our process should be adaptable for mass production of nanoparticles.

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COMMUNICATIONS ARISING

Palaeoecology

Fossils and avian evolution

Discoveries of archaic Mesozoic fossil birds ('opposite' birds, or enantiornithines) during the past decade have revolutionized our understanding of early avian evolution, but the rarity of Early Cretaceous ornithurines — birds that are closely related to the modern avian radiation — has meant that information about these species has lagged behind.

Norell and Clarke<sup>1</sup> describe the newly discovered and well preserved Late Cretaceous Mongolian *Apsaravis* as an ornithurine, cladistically slotting it between the well known and abundant marine hesperornithiforms and ichthyornithiforms. They claim that this specimen provides evidence against a so-called near-shore or marine 'ecological bottleneck' of ornithurines at the Cretaceous/Tertiary (K/T) boundary<sup>2</sup>, as well as insight into the assembly of the modern flight apparatus. However, I question their conclusions on the grounds of their inconsistency with literature on earlier ornithurine birds and of comparison of *Apsaravis* with other abundant ornithurines of that age.

Contrary to the claims of Norell and Clarke<sup>1</sup>, morphologically well defined ornithurines are known from highly preserved fossil material from the Early to the latest Cretaceous period, including *Ambiortus* from the Early Cretaceous of Mongolia (which shares no particular adaptations for near-shore habitats and has a highly developed flight apparatus with a deeply keeled ornithurine sternum) and the closely allied *Otogornis* (Early Cretaceous of China). Other notable examples include *Chaoyangia*, a toothed ornithurine from the Early Cretaceous of continental China<sup>3</sup>, the Early

Cretaceous *Gansus* (possibly a shore-dwelling bird), from Gansu Province in the continental interior of China, and *Liaoningornis*, perhaps the oldest known ornithurine bird from the famous Early Cretaceous *Confuciusornis* locality<sup>2</sup>.

As hesperornithiform birds (highly derived ornithurines) were already well adapted for foot-propelled diving by the Aptian epoch (the latter part of the Early Cretaceous), their divergence must have been from even earlier ornithurine birds. By the Late Cretaceous, numerous taxa of hesperornithiform birds, together with the volant ornithurines *Ichthyornis* and *Apatornis*, are known from many marine localities in both the Northern and Southern Hemispheres.

What correlation is there between the distributions of shore birds (waders; Charadriiformes) and the marine, shoreline habitats of the continental margins? These birds are quite cosmopolitan, occurring in shallow waters of continental margins and interiors, and in freshwater and marine habitats.

As *Apsaravis* is not related to any modern order of birds, it has no bearing on what types of ornithurine bird breached the K/T boundary. It has been noted previously<sup>2,4</sup> that no modern avian order (except Charadriiformes) can be confidently dated to before the K/T boundary<sup>5</sup>. The enantiornithines were the predominant land birds of the Mesozoic era, identified from numerous localities from the Lower to the Upper Cretaceous, in contrast to the scant finds of ornithurines; no enantiornithine is known to have lived after the K/T boundary. Hesperornithiforms from the Lower to Upper Cretaceous and ichthyornithiforms from the Upper Cretaceous have been found from many marine localities, but not after the K/T boundary; early Palaeocene fossil birds are known predominantly from morphotypes representative of shore birds.

The K/T extinction event was therefore likely to have been devastating for birds. 'Shore birds' may represent one of the major bottlenecks of avian morphotypes, transcending the K/T boundary, and could represent the wellsprings of an explosive Tertiary radiation of the modern bird orders that closely paralleled a similar event in Tertiary mammal history (this explosive model is supported by 'gap' analyses<sup>6,7</sup>).

Because shoreline habitats were as common in continental interiors as on continental margins during the Late Cretaceous<sup>2</sup>, and shore birds are equally at home in freshwater and/or marine environments, *Apsaravis* has no bearing on the habitat of Late Cretaceous birds. If, for example, an individual of *Larus pipixcan*, the common gull of summer prairies of the American great plains, were to be fossilized, the logic of Norell and Clarke would imply that it was a member of a land-bird order.