Immune defence in bumble-bee offspring

Immune-challenged vertebrate females transfer specific antibodies to their offspring1–3, but this gratuitous immunity cannot operate in invertebrates4. 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 oxidase5,6. 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 haemolymph7,8 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 microorganisms9, hosts with high phenol oxidase activity are less susceptible to microbial infection10.

Social insects cooperate in brood care and make a considerable investment in their offspring. In annual species such as bumblebees, 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. Assuming facultative adjustment of offspring immunity, we investigated whether parasite-challenged parental colonies could enhance their males’ immunocompetence.

We used a split-colony design10 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\(^{-1}\) in Ringer’s solution (5 \(\mu\)l)), which activates the immune system for long periods11. 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 antibacterial12 and phenol oxidase13 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, \(T = 9, n = 11, P = 0.94\)). Immune-challenged groups had lower reproductive output (repeated measures MANOVA for log-transformed number of males and queens; Hotelling’s T\(^2\) = 1.297, \(F_{1,10} = 5.839, P = 0.024\), 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 (\(T = 9, n = 11, NS\)).

As insects do not produce antibodies, they cannot transfer specific immunity as mammals do14. Male bumble-bees from immune-challenged groups have increased constitutive immunity relative to controls, which both enhances encapsulation (Fig. 1) and protects against microorganisms15,16. As the phenol oxidase enzyme cascade provides a broader immunity than the costly antibacterial immune response17, 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 insects18.

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Synthesis of carbon ‘onions’ in water

The fabrication of carbon nanomaterials is usually called for expensive vacuum systems to generate plasmas and yields are disappointingly low. Here we describe a simple method for producing high-quality spherical carbon nanomaterials in large quantities without the use of vacuum equipment. The nanoparticles, which have C\(_{60}\) 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.

 Nanotechnology

The fabrication of carbon nanomaterials is usually called for expensive vacuum systems to generate plasmas and yields are disappointingly low. Here we describe a simple method for producing high-quality spherical carbon nanomaterials in large quantities without the use of vacuum equipment. The nanoparticles, which have C\(_{60}\) 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.

We used 1-watt graphite electrodes to generate carbon nanoparticles using an arc discharge in water. The electrodes were heated to a temperature of 1,800 °C, which is sufficient to produce carbon nanoparticles. The arc discharge was generated using an electrical circuit consisting of a high-voltage power supply and a circuit for generating the arc. The circuit was designed to produce a maximum voltage of 10 kV and a current of 20 A. The arc discharge was generated by short-circuiting the power supply and the graphite electrodes. The arc discharge was sustained by the feedback of the plasma generated during the discharge. The carbon nanoparticles were produced by extracting the plasma generated during the arc discharge.

The arc discharge generated plasma by discharging a high-voltage power supply at the graphite electrodes. The plasma was generated by the high temperature of the graphite electrodes, which is sufficient to generate carbon nanoparticles. The plasma was extracted from the arc discharge by passing it through a water-cooled condenser. The water-cooled condenser was designed to cool the plasma to a temperature of 0 °C, which is sufficient to produce carbon nanoparticles.

The carbon nanoparticles were characterized using a number of techniques, including electron microscopy, X-ray diffraction, and Raman spectroscopy. The electron microscopy images showed that the carbon nanoparticles were spherical in shape and had a diameter of 10 nm. The X-ray diffraction patterns showed that the carbon nanoparticles had a graphitic structure. The Raman spectroscopy spectra showed that the carbon nanoparticles had a graphitic structure.

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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 C₆₀ molecule. A series of images obtained at intervals through the features verified that they were spherical nanoparticles and not nanotubes.

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 (1F–WS₅), 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–MoS₂ crystals. The average diameter of the nano-onions is 25–30 nm (range 5–40 nm), which is consistent with the C₆₀ molecule. A series of images obtained at intervals through the features verified that they were spherical nanoparticles and not nanotubes.

Although the production rate (3 mg min⁻¹) 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-feed the carbon anode would allow the process to run continuously for several hours. With these and other minor modifcations, our process should be adaptable for mass production of nanoparticles.

**Fossils and avian evolution**

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

Norell and Clarke describe the newly discovered and well-preserved Late Cretaceous Mongolian Apsaravis as an ornithure, cladistically slotting it between the well known and abundant marine hesperornithiforms and ichthyornithiforms. They claim that this specimen provides evidence against a previously proposed hypothesis that no modern avian order existed before the K/T boundary. 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 ornithures; 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 predominately 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 wellspring 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).

Because shoreline habitats were as common in continental interiors as on continental margins during the Late Cretaceous, 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.