Entomology

Immune defence in bumble-bee offspring

Immune-challenged vertebrate females transfer specific antibodies to their offspring[1–3], but this gratuitous immunity cannot operate in invertebrates[4]. 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[4,5]. 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[6,7] 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[5], hosts with high phenol oxidase activity are less susceptible to microbial infection[8].

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[9]. Assuming facultative adjustment of offspring immunity, we investigated whether parasite-challenged parental colonies could enhance their males' immunocompetence.

We used a split-colony design[10] 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 μl)), which activates the immune system for long periods[11]. 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

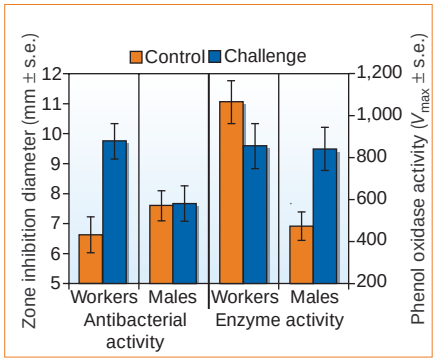

Figure 1 Antibacterial and phenol oxidase activities in the haemolymph of workers and males from control (orange bars) and challenged (blue bars) groups from 11 bumble-bee colonies. In workers, antibacterial activity was higher in challenged groups than in controls (Wilcoxon's paired signed-rank test: $T_+ = 6$, $n = 11$, $P < 0.02$), 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 × 10^{-3} per minute.

antibacterial[12] and phenol oxidase[13] 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[1]. Male bumble-bees from immune-challenged groups have increased constitutive immunity relative to controls, which both enhances encapsulation (Fig. 1) and protects against microorganisms[5,8]. As the phenol oxidase enzyme cascade provides a broader immunity than the costly antibacterial immune response[12], 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[13].

Yannick Moret, Paul Schmid-Hempel
Eidgenössische Technische Hochschule Zürich, Ecology and Evolution, ETH-Zentrum NW, 8092 Zürich, Switzerland
e-mail: moret@eco.umnw.ethz.ch

1. Hanson, L. A. *Ann. Allergy Asthma Immunol.* **81,** 523–537 (1998).
2. Coste, A., Sirard, J. C., Johansen, K., Cohen, J. & Kraehenbuhl, J. P. *J. Virol.* **74,** 8966–8971 (2000).
3. Rollier, C., Charollois, C., Jamard, C., Trepo, C. & Cova, L. *Vaccine* **18,** 3091–3096 (2000).
4. Hoffmann, J. A., Reichhart, J. M. & Hetru, C. *Curr. Opin. Immunol.* **8,** 8–13 (1996).
5. Söderhäll, K. & Cerenius, L. *Curr. Opin. Immunol.* **10,** 23–28 (1998).
6. Nappi, A. J. & Vass, E. *Pigment Cell Res.* **6,** 117–126 (1993).
7. Thörnqvist, P.-O. & Söderhäll, K. in *Diseases in Asian Aquaculture* vol. 3 (eds Flegel, T. W. & MacRae, I. H.) 203–218 (Fish Health Section, Asian Fisheries Soc., Manila, Philippines, 1996).
8. Nigam, Y., Maudlin, I., Welburn, S. & Rattcliffe, N. A. *J. Invertebr. Pathol.* **69,** 279–281 (1997).
9. Schmid-Hempel, P. *Parasites in Social Insects* (Princeton Univ. Press, Princeton, New Jersey, 1998).
10. Schmid-Hempel, R. & Schmid-Hempel, P. *Funct. Ecol.* **12,** 22–30 (1998).
11. Kato, Y. *et al. Insect Biochem. Mol. Biol.* **24,** 539–545 (1994).
12. Moret, Y. & Schmid-Hempel, P. *Science* **290,** 1166–1168 (2000).
13. Barnes, A. I. & Siva-Jothy, M. T. *Proc. R. Soc. Lond. B* **267,** 177–182 (2000).

Nanotechnology

Synthesis of carbon 'onions' in water

The fabrication of carbon nanomaterials usually calls for expensive vacuum systems to generate plasmas[1,2] 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_{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.

Nanoparticles have previously been prepared using a range of vacuum and non-vacuum methods[3–5]. 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.