Kin recognition in honeybees

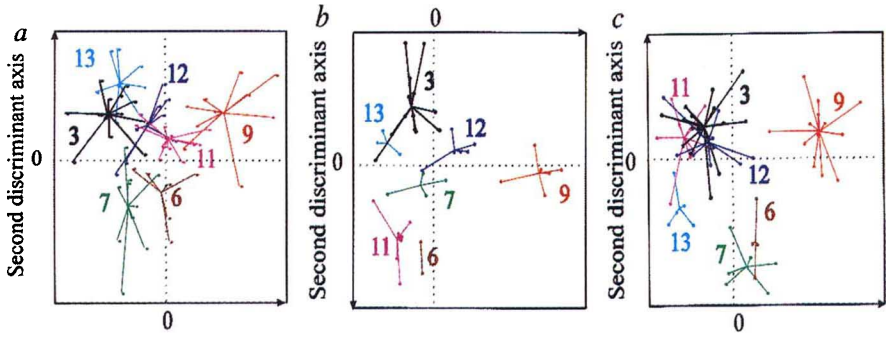
SIR — In honeybee colonies, polyandry leads to the presence of 7–20 subfamilies[1]. Workers can discriminate members of the same subfamily (super-sisters) from workers of other subfamilies (half-sisters)[2,3], and this may allow them to act nepotistically. How they discriminate is unknown, but the process is likely to involve subfamily-specific chemical labels which bees learn[2]. Page *et al.*[4] showed that laboratory-reared workers from artificial colonies with just two subfamilies had cuticle hydrocarbon profiles that were more similar between super-sisters than between half-sisters. This may not reflect the natural situation, however, where nest-mates can exchange hydrocarbons either by direct contact or through comb wax.

We have examined the cuticle hydrocarbon composition of honeybee workers from a colony headed by a naturally inseminated queen. We assigned each bee to one of 16 subfamilies using two highly variable microsatellite loci (*A76*, *A107*; ref. 1), genetic markers unrelated to cuticle hydrocarbons. To determine the relative importance of genetic and environmental factors to cuticle hydrocarbon profiles, adult bees were matured under three conditions: isolated, in groups of 10, and in their parental hive. After 5 days, workers of each set (117, 77 and 117 bees, respectively) were individually analysed (see figure).

Hydrocarbons were extracted for 5 minutes in 1 ml of pentane, analysed by gas chromatography (Girdel 300) on a 30-m nonpolar capillary column and constituents identified by mass spectrometry (Nermag R 10-10-C GC-MS). Twenty-six compounds were identified, belonging to four major classes of long-chain hydrocarbons: alkenes, alkadienes, methyl-branched alkanes, but mostly *n*-alkanes. All *n*-alkanes in the C_{21}–C_{33} series were present. Compounds with an even number of carbons predominated. For the statistical analyses, 12 compounds were excluded because their concentrations were too low to be measured reliably. The 14 remaining compounds were C_{23}, $C_{23:1}$, C_{25}, C_{27}, $C_{27:1}$, MeC_{27}, C_{29}, $C_{29:1}$, MeC_{29}, C_{31}, $C_{31:1}$, MeC_{31}, $C_{33:1}$, $C_{33:2}$.

A generalized linear model[5] (Splus 3.0) was applied to the data matrix. Each element of this matrix is the mean percentage of a given hydrocarbon for a family in a given rearing condition. Each matrix line thus represents a mean hydrocarbon profile. Our analysis shows that hydrocarbon profiles differ significantly between subfamilies. This demonstrates that these profiles are conserved even within the hive, and that cuticle hydrocarbons possess the necessary prerequisites of sufficient variability and genetic determinism for use as labels for subfamily recognition.

A potential consequence of subfamily recognition is that workers could improve the reproductive success of their own subfamily in several circumstances, such as rearing full-sister queen larvae[6] or preferentially feeding their full-sister laying workers. Such nepotistic behaviour, claimed by some[6,7] but denied by others[8,9], implies subfamily recognition. Breed *et al.*[10] argued that few areas in sociobiology have received as much experimental attention, yet yielded so little in the way of supportable conclusions, as the question of subfamily nepotism in honeybees. Our demonstration of genetically determined chemical markers shows that honeybees possess a system with sufficient discriminating power for subfamily recognition.



The distinctiveness of subfamily profiles is illustrated by a two-dimensional projection on the first plane of a factorial discriminant analysis (Statgraphics 7.0). First and second axes are linear combinations of the hydrocarbon concentrations. In the multi-dimensional space of the hydrocarbon profiles they form the plane where the projected distances between subfamilies are largest. In this analysis, performed on each set separately (*a*, isolated bees; *b*, grouped bees; *c*, hive bees), only the seven best represented subfamilies were kept. Not only were bees of the same subfamily well discriminated, but the relative positions of the subfamilies in the plane remained roughly the same in the three analyses. Each subfamily is given a different colour, and bee dots of the same subfamily are linked together through their common barycentre.

Gérard Arnold, Brigitte Quenet
Jean-Marie Cornuet, Claudine Masson
Laboratoire de Neurobiologie Comparée des Invertébrés, INRA-CNRS (URA 1190), BP 23, 91440 Bures-sur Yvette, France
Benoît De Schepper
Laboratoire de Chimie Agro-industrielle, ENSCT, 31077 Toulouse, France
Arnaud Estoup
Laboratoire Populations, Génétique et Evolution, CNRS, 91198 Gif-sur-Yvette, France
Patrick Gasqui
Laboratoire de Biométrie, INRA, 78352 Jouy-en-Josas, France

1. Estoup, A., Solignac, M. & Cornuet, J. M. *Proc. R. Soc.* B**258,** 1–7 (1994).
2. Getz, W. M. & Smith, K. B. *Nature* **302,** 147–148 (1983).
3. Moritz, R. F. A. & Hillesheim, E. *Anim. Behav.* **40,** 641–647 (1990).
4. Page, R. E., Metcalf, R. A., Metcalf, R. L., Erickson, E. H. & Lampman, R. L. *J. chem. Ecol.* **17,** 745–756 (1991).
5. McCullagh, P. & Nelder, J. A. *Generalized Linear Models* (Chapman & Hall, London, 1989).
6. Page, R. E., Robinson, G. E. & Fondrk, M. K. *Nature* **338,** 576–579 (1989).
7. Visscher, P. K. *Behavl Ecol. Sociobiol.* **18,** 453–460 (1986).
8. Oldroyd, B. P., Rinderer, T. E., Schwenke, J. R. & Buco, S. M. *Behavl Ecol. Sociobiol.* **34,** 169–173 (1994).
9. Oldroyd, B. P., Rinderer, T. E. & Buco, S. M. *Nature* **346,** 707–708 (1990).
10. Breed, M. D. *et al. Behavl Processes* **33,** 25–40 (1994).

Fossil mesothele spiders

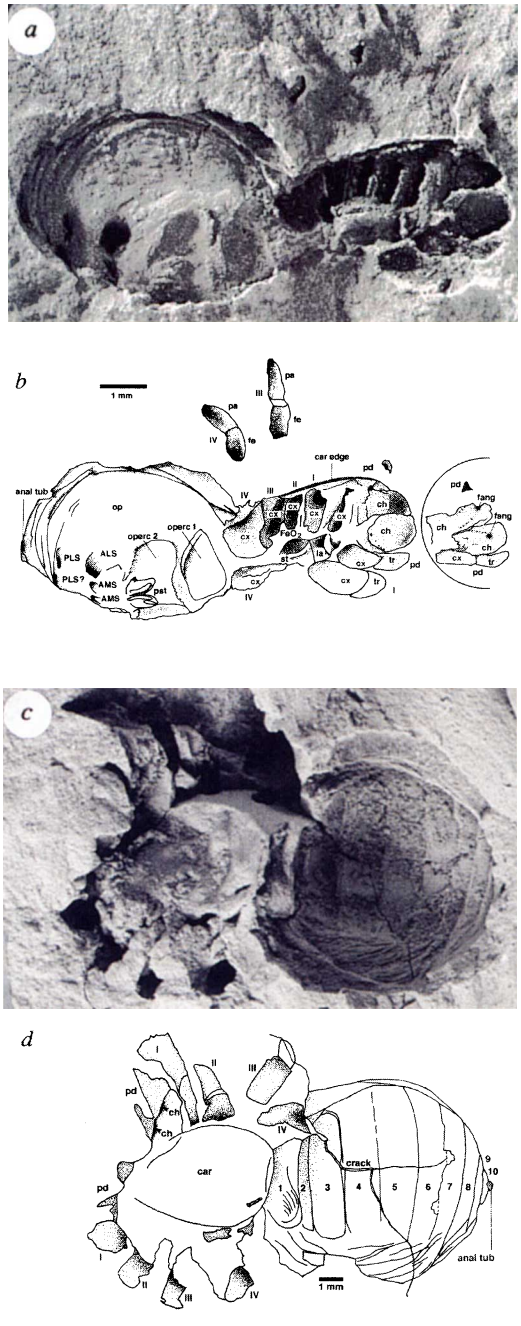
SIR—The living spiders *Liphistius* and *Heptathela* constitute the suborder Mesothelae, which is a sister group to Opisthothelae to which all other spiders belong[1]. Mesotheles exhibit the most primitive characteristics of all living spiders and would be expected to appear earlier in the fossil record than the oldest opisthothele, *Rosamygale* from Triassic (240 Myr) strata[2]. Indeed, many Carboniferous (355–290 Myr) spiders were once referred to this suborder[3]. Re-examination of all available types of Carboniferous spiders reveals that some are not spiders and that none shows autapomorphies of Mesothelae. New fossils from Montceau-les-Mines, near Autun, France, however, confirm the presence of mesotheles in the late Carboniferous (around 295 Myr).

The two fossils came to light in the collections of the Natural History Museums of Autun and London (details of the new species will be published elsewhere[4]). The Autun specimen (*a*, *b* in the figure) reveals a deep, narrow sternum as wide as the labium, pro- and retromarginal cheliceral tooth rows, and two opisthosomal book-lung opercula. Paired internal structures lying above the second operculum and opening to its posterior border are similar to the supposed tracheal organs of *Heptathela*[5]. Posterior to the second

operculum, three holes represent external moulds of the left anterior lateral spinneret and the anterior median spinneret pair. Posterior to these, two more holes of approximately the same size and straddling a mid-ventral line, suggest a single pair of posterior spinnerets. Four posterior spinnerets (smaller posterior median and larger posterior lateral) are present in all spiders, except when the posterior median spinnerets are missing (some specialized opisthotheles), or a single posterior median spinneret is present (*Heptathela*)[6]. The posterior median spinnerets are usually small or lost, and so the relatively large posterior spinnerets present in the fossil are most probably posterior lateral spinnerets. The London specimen (*c*, *d* in the figure) shows the dorsal surface of the opisthosoma, with a series of at least ten tergites, including microtergites at the posterior end of the opisthosoma, adjacent to the anal tubercle.

The specimens are assumed to be conspecific. The animal is a spider because of the presence of spinnerets (and therefore, presumably, silk glands), a pedicel, a flexible opisthosoma with discrete tergites, carapace and sternum morphology, and lack of autapomorphies of other arachnid groups. One autapomorphy of mesotheles, a deep and narrow sternum[7], can be seen; in addition, dorsal opisthosomal tergites, two ventral opercula (covering the two pairs of book-lungs), orthognath chelicerae, and fully developed anterior median spinnerets, are indicative of Mesothelae. Supposed tracheal organs are a synapomorphy for *Heptathela* and the Montceau spider (or a symplesiomorphy if homologous with amblypygid eversible sacs[8]). The position of the spinnerets in the fossils, neither bunched together just behind the second operculum as in modern mesotheles, nor close to the anal tubercle as in Opisthothelae, but widely spaced between the second operculum and the anal tubercle, could reflect either the start of their rearward movement towards the opisthothelate condition, or a more ancestral arrangement prior to their bunching close to the second operculum. If the posterior spinnerets in the fossils are posterior lateral spinnerets, it would represent a new configuration for Araneae, an advance over the *Heptathela* condition, and an autapomorphy for the fossil species. Another autapomorphy of the Montceau spider is biserial cheliceral dentition (*b* in the figure; inset); only a promarginal row is present in living mesotheles[9,10].

The Montceau spider is plesiomorphic with respect to Opisthothelae; the deep and narrow sternum confirms that it belongs in Mesothelae. It is thus the first record of a fossil mesothele. The fossil species cannot be ancestral to modern mesotheles because of autapomorphies. As the sister clade to Mesothelae, Opisthothelae must have originated some time before the age of these fossils.



Upper Carboniferous mesothele spider from Montceau-les-Mines, France. *a*, Photograph of Autun specimen 51961, whitened with NH_4Cl (×7.4); *b*, explanatory drawing of *a* (inset: dorsal view of external moulds of chelicerae, to same scale); *c*, photograph of London specimen In 50260, whitened with NH_4Cl (×3.3); *d*, explanatory drawing of *c*. Abbreviations: 1–10, opisthosomal tergite numbers; I–IV, walking legs I–IV; ALS, anterior lateral spinneret; AMS, anterior median spinneret; anal tub, anal tubercle; car, carapace; ch, chelicera; cx, coxa; pst, paired structures; fe, femur; la, labium; operc, book-lung operculum; op, opisthosoma; pa, patella; pd, pedipalp; PLS, posterior lateral spinneret; st, sternum; te, tergite; tr, trochanter. Photos: J. Almond (*a*) and L. Anderson (*c*).

Paul A. Selden
Department of Earth Sciences,
University of Manchester,
Manchester M13 9PL, UK

1. Platnick, N. I. & Gertsch, W. J. *Am. Mus. Novit.* **2607,** 1–15 (1976).
2. Selden, P. A. & Gall, J.-C. *Palaeontology* **35,** 211–235 (1992).
3. Petrunkevitch, A. in *Treatise on Invertebrate Paleontology. Pt P. Arthropoda* Vol. 2 (ed. Moore, R. C.) 42–162 (Geol. Soc. Am. & Univ. Kansas Press, Lawrence, KA, 1955).
4. Selden, P. A. *Rev. Suisse Zool.* (in the press).
5. Yoshikura, M. *Kumamoto J. Sci.* **B1,** 37–40 (1954).
6. Marples, B. J. *J. Linn. Soc. (Zool.)* **46,** 209–222, pl. 1 (1967).
7. Raven, R. J. *Bull. Am. Mus. nat. Hist.* **182,** 1–180 (1985).
8. Kaestner, A. *Invertebrate Zoology* Vol. 2 (transl. Levi, H. W. & Levi, L. R.) 123 (Wiley, New York, 1968).
9. Abraham, H. C. *Proc. zool. Soc. Lond.* **1929,** 671–677, pls 1–3 (1929).
10. Bristowe, W. S. *Proc. zool. Soc. Lond.* **1932,** 1015–1057, pls 1–6 (1932).

A plant oncogene as a phosphatase

SIR — The plant oncogene *rolB,* from *Agrobacterium rhizogenes,* induces differentiation and growth of neoplastic roots (hairy-roots[1]) in dicotyledonous plants. *rolB*-transformed plant cells show an increased membrane sensitivity to[2,3], and binding capacity of[4], auxin, the most extensively studied plant hormone. The oncogene *rolB* may thus provide a tool for elucidating the still elusive mechanism of auxin signal perception/transduction and for shedding light on the role of this plant hormone in the control of plant growth and differentiation. So far, all attempts to clarify the biochemical activity and subcellular localization of the *rolB* gene product have been inconclusive. Here we show that the RolB protein overproduced in *Escherichia coli* has tyrosine phosphatase activity, and that in transformed plant cells it is localized in the plasma membrane.

The full-length *rolB* gene was cloned in vector ptrc97B (provided by E. Amman) and the resulting construct (pMTB4) transferred in *E. coli* W3110*lac* I^qL8. On induction by isopropyl-β-D-thiogalactoside, the production of the RolB protein was confirmed by immunoblotting. The lysates of bacteria producing RolB (MTB4) have a fivefold higher phos-

Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. Priority will be given to letters of fewer than 500 words.