

*This copy is for your personal, non-commercial use only.*

If you wish to distribute this article to others, you can order high-quality copies for your colleagues, clients, or customers by [clicking here](#).

Permission to republish or repurpose articles or portions of articles can be obtained by following the guidelines [here](#).

**The following resources related to this article are available online at [www.sciencemag.org](http://www.sciencemag.org) (this information is current as of February 18, 2010):**

**Updated information and services**, including high-resolution figures, can be found in the online version of this article at:

<http://www.sciencemag.org/cgi/content/full/296/5568/741>

A list of selected additional articles on the Science Web sites **related to this article** can be found at:

<http://www.sciencemag.org/cgi/content/full/296/5568/741#related-content>

This article **cites 16 articles**, 10 of which can be accessed for free:

<http://www.sciencemag.org/cgi/content/full/296/5568/741#otherarticles>

This article has been **cited by** 138 article(s) on the ISI Web of Science.

This article has been **cited by** 46 articles hosted by HighWire Press; see:

<http://www.sciencemag.org/cgi/content/full/296/5568/741#otherarticles>

This article appears in the following **subject collections**:

Psychology

<http://www.sciencemag.org/cgi/collection/psychology>

## REPORTS

In this large multicenter sample, we were unable to detect a schizophrenia susceptibility locus of major effect on chromosome 1q. It remains possible that the genes identified as disrupted in the Scottish translocation finding (10, 11), or genes in the regions supported by the Finnish (9) and/or Canadian (6) samples, will be shown to have small effects on schizophrenia susceptibility in other populations, or that the pathways in which these genes participate will have more major effects. Identifying such genes to elucidate the pathogenesis of this devastating disorder remains a major goal of schizophrenia research.

### References and Notes

- M. T. Tsuang, W. S. Stone, S. V. Faraone, *Br. J. Psychiatry* **178** (suppl. 40), s18 (2001).
- N. J. Bray, M. J. Owen, *Trends Mol. Med.* **7**, 169 (2001).
- H. H. Goring, J. D. Terwilliger, J. Blangero, *Am. J. Hum. Genet.* **69**, 1357 (2001).
- Schizophrenia Linkage Collaborative Group, *Am. J. Med. Genet.* **67**, 580 (1996).
- D. F. Levinson *et al.*, *Am. J. Hum. Genet.* **67**, 652 (2000).
- L. M. Brzustowicz, K. A. Hodgkinson, E. W. C. Chow, W. G. Honer, A. S. Bassett, *Science* **288**, 678 (2000).
- Markers and locations are from the Applied Biosystems (Foster City, CA) high-density map ([www.appliedbiosystems.com/products/linkmapping.cfm?prod\\_id=681&linkmap\\_id=41](http://www.appliedbiosystems.com/products/linkmapping.cfm?prod_id=681&linkmap_id=41)). Locations on the Marshfield map (Center for Medical Genetics, Marshfield, WI; [http://research.marshfieldclinic.org/genetics/Map\\_Markers/maps/IndexMapFrames.html](http://research.marshfieldclinic.org/genetics/Map_Markers/maps/IndexMapFrames.html)) are typically 5 to 6 cM farther from the p terminus.
- H. M. Gurling *et al.*, *Am. J. Hum. Genet.* **68**, 661 (2001).
- J. Ekelund *et al.*, *Hum. Mol. Genet.* **10**, 1611 (2001).
- J. K. Millar *et al.*, *Hum. Mol. Genet.* **9**, 1415 (2000).
- D. H. Blackwood, *Am. J. Hum. Genet.* **69**, 428 (2001).
- Sixteen fluorescein-labeled CA-repeat markers covered 107.5 cM of chromosome 1q (sex-averaged genetic distance). Heterozygosity averaged 0.768; intermarker spacing averaged 7.14 cM [see Web table 2 (14) for details]. Primers (Applied Biosystems) were distributed to each laboratory, and optimal conditions were suggested after testing in Cardiff. The AU/US, JHU, and NIMH data sets were genotyped at the Australian Genome Research Facility (Melbourne, Australia).
- The eight samples and references to their methods are as follows: AU/US (24, 25) (molecular methods apply also to JHU and NIMH), University of Bonn (26), Cardiff (27), University of Chicago (28), CNRS (29, 30), JHU (31), NIMH (32) [for a publicly available data set, see (33)], and VCU/Ireland (34, 35). Research diagnostic interviews were completed by research clinicians and best-estimate diagnoses were made based on interviews, records, and informant reports. Affected cases had DSM-III-R/DSM-IV diagnoses of schizophrenia or schizoaffective disorder. Predominant ethnic origins were as follows: Bonn, German, Israeli/Sephardic; Chicago, AU/US, JHU, and NIMH, European, African American; CNRS, French, French/African/Indian mixtures (Reunion Island); VCU/Ireland, Irish; Cardiff, English, Welsh. The NIMH sample was ethnically diverse. For details of sample sizes, see Web table 1 (14).
- Supplementary material is available on Science Online at [www.sciencemag.org/cgi/content/full/296/5568/739/DC1](http://www.sciencemag.org/cgi/content/full/296/5568/739/DC1).
- P. Holmans, *Am. J. Hum. Genet.* **52**, 362 (1993).
- L. Kruglyak *et al.*, *Am. J. Hum. Genet.* **58**, 1347 (1996).
- D. A. Dorr, J. P. Rice, C. Armstrong, T. Reich, M. Blehar, *Genet. Epidemiol.* **14**, 617 (1997).
- Multipoint ASP and NPL analyses were performed on each data set and on all pedigrees combined (using separate allele frequencies for each data set). Web tables 7 to 9 (14) show linkage scores for all analyses. ASP analyses considered all possible pairs [ $S \times (S - 1) / 2$  for  $S$  affected sibs]. Region-wide  $P$  values were computed empirically by simulating 5000 replicates (assuming no linkage). Logistic regression analyses [Web table 3 (14)] tested intersite heterogeneity in ASP sharing proportions and overall significance of linkage allowing for intersite heterogeneity, with  $P$  values based on simulation. See (14) for details. For NPL scores, the  $Z_{\text{all}}$  scoring function was used (16), which considers allele sharing among all genotyped affected cases in the pedigree including ill siblings, parents, offspring, and other relatives, whereas the MLS statistic considers only sharing within affected sibling pairs.
- J. D. Terwilliger, K. M. Weiss, *Curr. Opin. Biotechnol.* **9**, 578 (1998).
- See Web table 4 (14) for detailed results for 679 European-origin and 58 African-origin pedigree subgroups. "Other" ancestries ( $n = 42$ ; Asian, Micronesian, Indian, Sephardic, or uncertain) were not analyzed separately.
- L. L. Cavalli-Sforza, P. Menozzi, A. Piazza, *The History and Geography of Human Genes* (Princeton Univ. Press, Princeton, NJ, 1994), pp. 268–272.
- Recessive analyses [Web table 6 (14)] used the model associated with the largest  $Z_{\text{max}}$  for the Canadian sample (6): disease allele frequency = 0.065,  $f(AA) = 0.50$ ,  $f(Aa) = f(aa) = 0.0015$ . GENE-HUNTER 2.0 was used to compute two-point heterogeneity lod scores for D1S484, D1S2878, and D1S196, and multipoint lod scores using 16 markers; multipoint analyses were repeated with disease allele frequency = 0.13 and with unaffected cases coded as diagnosis unknown.
- The recessive model described above (22), assuming 75% of families linked (6), predicts a population-wide  $\lambda_{\text{sibs}}$  of 3.55 (36). With 800 ASPs there is 100% power to detect MLS = 3 at  $\lambda_{\text{sibs}} = 1.8$  (10-cM map), with expected MLS > 20 for  $\lambda_{\text{sibs}} = 3$  (no parents typed) (37). For comparable families containing 800 ASPs, simulation studies determined power (to detect genome-wide significant linkage) ranging from 66 to 94% (dominant model) as  $\lambda_{\text{sibs}}$  varied from 1.27 to 1.36, and from 48 to 68% (recessive) for  $\lambda_{\text{sibs}}$  from 1.24 to 1.31 (38).
- D. F. Levinson *et al.*, *Am. J. Psychiatry* **155**, 741 (1998).
- K. R. Ewen *et al.*, *Am. J. Hum. Genet.* **67**, 727 (2000).
- S. G. Schwab *et al.*, *Mol. Psychiatry* **5**, 638 (2000).
- N. M. Williams *et al.*, *Hum. Mol. Genet.* **8**, 1729 (1999).
- Q. Cao *et al.*, *Genomics* **43**, 1 (1997).
- F. Bonnet-Brilhault *et al.*, *Eur. J. Hum. Genet.* **7**, 247 (1999).
- D. Campion *et al.*, *Psychiatry Res.* **51**, 215 (1994).
- J. L. Blouin *et al.*, *Nature Genet.* **20**, 70 (1998).
- C. R. Cloninger *et al.*, *Am. J. Med. Genet.* **81**, 275 (1998).
- Schizophrenia Genetics Initiative Data Archive (<http://zork.wustl.edu/nimh/sz.html>).
- R. E. Straub *et al.*, *Nature Genet.* **11**, 287 (1995).
- R. E. Straub *et al.*, *Am. J. Med. Genet.* **81**, 296 (1998).
- J. W. James, *Ann. Hum. Genet.* **35**, 47 (1971).
- E. R. Hauser *et al.*, *Genet. Epidemiol.* **13**, 117 (1996).
- P. A. Holmans, D. F. Levinson, unpublished data.
- The authors gratefully acknowledge participation of family members as well as the assistance of D. Nancarrow, N. Hayward, D. P. Lennon, M. Gladis, J. Endicott, M. S. O'Brien, C. E. Thornley, and H. L. Jones. Supported by NIMH grant MH61602 (D.F.L., C.L., B.R., A.E.P., P.V.G., D.B.W., M.J.O.). Additional support provided by NIMH grants MH 41953, 52537, and 45390 (B.R. and K.S.K.); the U.K. Medical Research Council (M.J.O.); Deutsche Forschungsgemeinschaft grant SFB 400 (D.B.W., W.M.); the German-Israeli Foundation for Scientific Research (B.L., D.B.W.); NIMH grants KO2-01207 and K24-MH64197 (D.F.L.); National Health and Medical Research Council of Australia (NHMRC) grants 33505 and 35016, Rebecca L. Cooper Medical Research Foundation, Queensland Department of Health, and NHMRC Network for Brain Research into Mental Disorders (B.J.M.); the NIMH Intramural Program and the Brain Research Foundation, University of Chicago (P.V.G.); NIMH grant RO1-MH57314 (A.E.P.); and CNRS and Aventis Pharma SA (J.M., C.L.). Specimens from the NIMH Schizophrenia Genetics Initiative (NIMH SGI) were used in this study. Data and biomaterials were collected in three projects that participated in the NIMH SGI. From 1991 to 1997, the principal investigators and co-investigators were Harvard University (grant U01 MH46318) (M. T. Tsuang, S. Faraone, and J. Pepple); Washington University, St. Louis (grant U01 MH46276) (C. R. Cloninger, T. Reich, and D. Svrakic); and Columbia University (grant U01 MH46289) (C. Kaufmann, D. Malaspina, and J. Harkavy Friedman).

16 January 2002; accepted 25 March 2002

## Influence of Gene Action Across Different Time Scales on Behavior

Y. Ben-Shahar,<sup>1</sup> A. Robichon,<sup>3</sup> M. B. Sokolowski,<sup>4</sup> G. E. Robinson<sup>1,2\*</sup>

Genes can affect natural behavioral variation in different ways. Allelic variation causes alternative behavioral phenotypes, whereas changes in gene expression can influence the initiation of behavior at different ages. We show that the age-related transition by honey bees from hive work to foraging is associated with an increase in the expression of the *foraging* (*for*) gene, which encodes a guanosine 3',5'-monophosphate (cGMP)-dependent protein kinase (PKG). cGMP treatment elevated PKG activity and caused foraging behavior. Previous research showed that allelic differences in PKG expression result in two *Drosophila* foraging variants. The same gene can thus exert different types of influence on a behavior.

Some genes influence behavior via genetic polymorphisms, whereas other genes influence behavior via developmental polymorphisms. But little is known about whether the same gene, or orthologs of a gene, can influence behavior in both ways. This knowledge

is necessary to develop a comprehensive understanding of how genes and the environment influence behavior, because both involve genomic responsiveness, albeit over vastly different scales of time.

The *foraging* gene (*for*) affects naturally

REPORTS

occurring variation in insect behavior (1). Two *for* alleles are commonly present in populations of *Drosophila melanogaster*: *for<sup>R</sup>* (rover) flies have higher levels of *for* mRNA and PKG activity and collect food over a larger area than do *for<sup>S</sup>* (sitter) flies. Patchy food and high population densities provide a selective advantage for rovers; more uniformly distributed food and low population densities favor sitters (2). These results suggest that behavioral evolution in flies has involved selection for alternative *for* alleles under different ecological conditions.

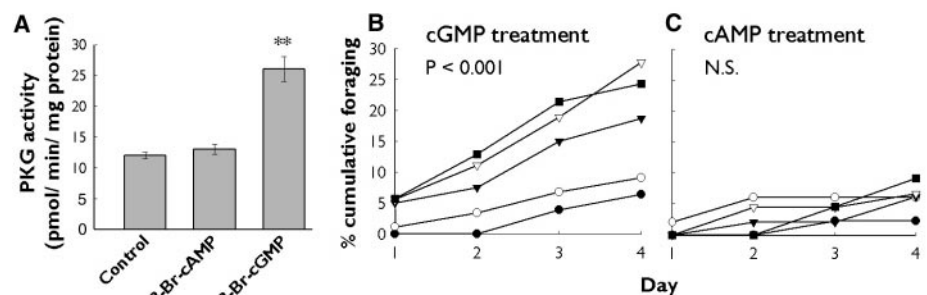
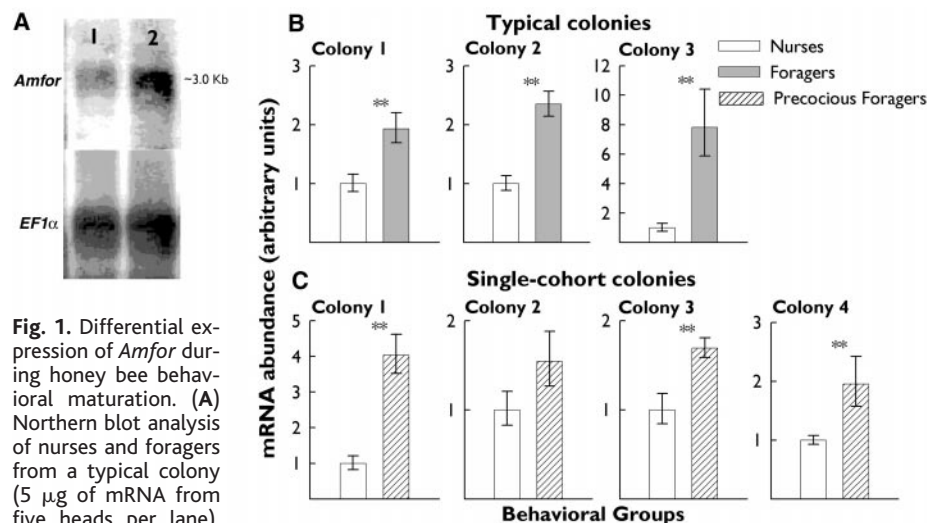
We used the honey bee (*Apis mellifera*) to study the possibility that *for* also is involved in developmentally regulated behavioral variation. Unlike in flies, foraging in honey bees unfolds as part of a complex process of behavioral maturation, and in a social context. Honey bee colonies exhibit an age-related division of labor; adult worker bees perform tasks in the hive such as brood care (“nursing”) when they are young, and then shift to foraging for nectar and pollen outside the hive. The transition to foraging typically occurs at about 2 to 3 weeks of age, is preceded by a series of orientation flights, and involves changes in brain chemistry, brain structure, endocrine activity, and gene expression (3). The age at onset of foraging is not rigid; it depends on the needs of the colony, mediated in part by inhibitory social interactions with older individuals and pheromones from the brood and queen (3). Foraging in honey bees is also different from flies because foragers collect food for their colony, and not necessarily when they themselves are hungry (4).

We hypothesized that foraging in honey bees is associated with an up-regulation of the *for* transcript in the brain, with foragers having higher levels than nurses. This hypothesis was based on the notion that nurse bees loosely resemble sitter flies because they obtain food only in the more restricted confines of the beehive, whereas forager bees display rover-like behavior by ranging widely throughout the environment. Specifically, we investigated whether the same gene that results in alternative allelic-based phenotypes (sitters and rovers in *Drosophila*) is also involved in developmentally regulated alternative phenotypes, nursing, and foraging in honey bees.

To test this hypothesis, we cloned a honey bee *for* ortholog (*Amfor*) (5). The predicted pro-

tein sequence of *Amfor* contains all regulatory, cGMP binding, and kinase domains typical of a PKG and is >80% similar to PKGs from other

organisms (5). Northern blot analysis (6) indicated the presence of a single transcript in the head and suggested higher expression in forager



**Fig. 2. Effects of treatment with cGMP or cAMP on honey bee foraging behavior.** (A) Treatment with 8-Br-cGMP (500 μM), but not 8-Br-cAMP (1000 μM), significantly increased PKG activity [ANOVA, N = 8 heads per group; PKG activity measured as in (7)]. (B) Treatment with 8-Br-cGMP induced precocious foraging. Two trials were performed with no significant differences between them (P = 0.62), allowing the data to be pooled. P value on the graph is based on a survival analysis for dose dependence [Cox proportional hazards test, see (28)]; N = 35 to 45 bees per trial for each treatment. One-day-old bees were treated for 4 days in the laboratory and then introduced to single-cohort colonies. Observations at the hive entrance were made (9) to ensure that the onset of foraging was identified; the graph indicates the cumulative percentage of bees initiating foraging on each of the first 4 days of observation, when they were 4 to 10 days old. (C) Treatment with 8-Br-cAMP did not induce precocious foraging. Design and analysis were as in (B). One trial was performed: N = 44 to 49 bees per group. No significant (P = 0.22) effect on precocious foraging was found in a second trial with 3000 mM 8-Br-cAMP (11). ●, 0 μM; ○, 100 μM; ▼, 250 μM; ▽, 500 μM; ■, 1000 μM.

<sup>1</sup>Department of Entomology, <sup>2</sup>Neuroscience Program, University of Illinois at Urbana-Champaign, 320 Morrill Hall, 505 South Goodwin Avenue, Urbana, IL 61801, USA. <sup>3</sup>CESG/CNRS, Université de Bourgogne, 15 rue Hugues Picardet, Dijon 21000, France. <sup>4</sup>Department of Zoology, University of Toronto, 3359 Mississauga Road, Mississauga, Ontario L5L1C6, Canada.

\*To whom correspondence should be addressed. E-mail: generobi@life.uiuc.edu

## REPORTS

heads relative to nurse heads (Fig. 1A). Real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR) (7) demonstrated that foragers had significantly higher brain levels of *Amfor* mRNA (by a factor of 2 to 8) than did nurses in all three colonies studied (Fig. 1, A and B). Foragers also exhibited about four times as much PKG activity as did nurses [ $34.9 \pm 3.1$  versus  $12.5 \pm 1.2$  pmol min<sup>-1</sup> mg<sup>-1</sup> protein ( $\pm$ SE), foragers and nurses, respectively;  $N = 8$  heads per group, analysis of variance (ANOVA),  $P < 0.001$ ; assayed as in (1)].

These results are consistent with our hypothesis; however, foragers typically are also older than nurses. To resolve whether *for* up-regulation is associated primarily with foraging behavior or with the foragers' advanced age, we manipulated colony social structure to obtain precocious foragers. We established "single-cohort colonies" initially composed only of 1-day-old bees (8); the absence of foragers results in some colony members initiating foraging as much as 2 weeks earlier than usual (9). In support of our hypothesis, 7- to 9-day-old precocious foragers

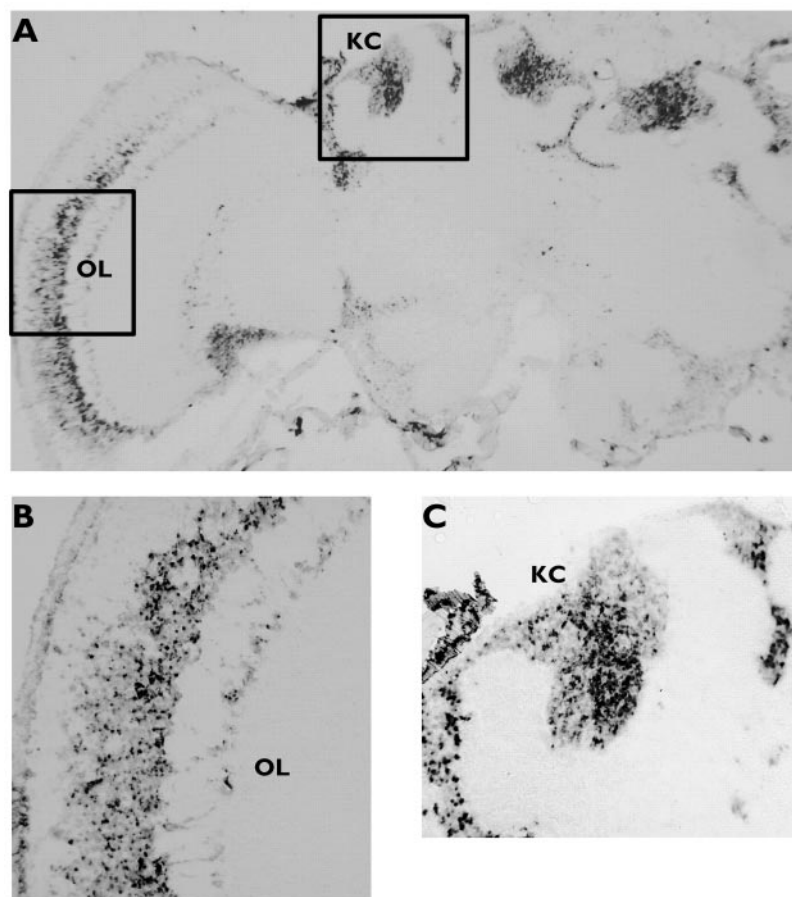
had significantly higher levels of *Amfor* mRNA (by a factor of 2 to 4) than did same-age nurses in three of four colonies (Fig. 1C).

We used a pharmacological approach to test the hypothesis that increased PKG activation causes an increase in the likelihood of precocious foraging. Bees were chronically treated with 8-Br-cGMP (10), a membrane-permeable analog that is relatively resistant to degradative phosphodiesterases. As expected, the treatment significantly elevated PKG activity (Fig. 2A); treated bees had forager-like levels of PKG activity, whereas control bees had levels similar to nurse bees. This treatment significantly increased the likelihood of precocious foraging in a dose-dependent manner (Fig. 2B). In contrast, 8-Br-cAMP treatment, which elevated cAMP (adenosine 3',5'-monophosphate)-dependent protein kinase activity (11), did not elevate PKG activity and did not affect the likelihood of precocious foraging (Fig. 2C). These results demonstrate a specific treatment effect and suggest that PKG activation can influence the initiation of foraging behavior.

In situ hybridization analysis was performed (12) to explore where *Amfor* might exert its effects in the brain (Fig. 3). *Amfor* is highly expressed in the lamina of the optic lobes and in the mushroom bodies. The mushroom bodies constitute the main center for multimodal sensory processing in the insect brain (13). In the mushroom bodies, *Amfor* is preferentially expressed in a central column of intrinsic (Kenyon) cells that receive mainly visual input (14). On the basis of these results, we speculate that *Amfor* is involved in higher order integration of visual information associated with orientation and foraging behavior; involvement in other neural functions related to division of labor is also possible.

Division of labor in honey bees involves intricate processes that integrate the effects of age, social interactions, colony needs, and resource availability on the likelihood of engaging in foraging behavior. Other genes show changes in brain expression in association with the transition from hive work to foraging (3), and quantitative trait loci for pollen versus nectar foraging also have been identified (15). Our results suggest that the up-regulation of *Amfor* in the brain and the resultant increase in PKG activity is causally related to the transition from hive work to foraging outside. Hence, *Amfor* apparently influences the division of labor in honey bees and is one of only a few genes implicated in the organization of an animal society (16, 17).

Both fly (1) and bee foraging involve *for*, and PKG plays a role in the control of feeding arousal in some other invertebrates and vertebrates (18–20). This suggests that the responsiveness of *for* expression over evolutionary (flies) and ontogenetic (bees) time scales reflects aspects of a phylogenetically conserved process of regulation of feeding. We propose that evolutionary changes in food-related behaviors, including complex social foraging, are based in part on changes in the regulation of *for* and other related genes. Given the importance of gene regulation in generating biological complexity, further studies of *for* and other genes that are both under selection and subject to regulation by extrinsic factors (21, 22) should provide important insights into the influences of genes on behavior.



**Fig. 3.** *Amfor* expression in the honey bee brain. OL, optic lobes; KC, Kenyon cells. (A) Coronal section. Squares delineate regions shown magnified in (B) and (C). No labeling was seen in sections probed with a sense control (11, 12). There were no obvious spatial differences between nurses and foragers in expression patterns ( $N = 5$  brains per group); these images are from a forager brain. Results suggest that the differences in *Amfor* mRNA levels between nurses and foragers detected with qRT-PCR may represent increased expression in the same cells. Brains were sectioned from anterior (antennal lobes) to posterior (subesophageal ganglion).

### References and Notes

1. K. A. Osborne et al., *Science* **277**, 834 (1997).
2. M. B. Sokolowski, H. S. Pereira, K. Hughes, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 7373 (1997).
3. G. E. Robinson, *Am. Nat.*, in press.
4. T. D. Seeley, *Wisdom of the Hive* (Harvard Univ. Press, Cambridge, MA, 1997).
5. We used RT-PCR to isolate a fragment of the honey bee *for* ortholog *Amfor*. Total RNA was isolated from five whole bees (TRizol, Invitrogen). RT (1  $\mu$ g of total RNA) was performed with polyT<sub>(18)</sub> primer and Superscript II reverse transcriptase (Gibco BRL). PCR was done with degenerate primers designed to amplify fragments corresponding to amino acids 165 to 472 of *Amfor* (covering conserved cGMP binding and kinase domains). The full coding sequence of *Amfor* (GenBank accession number

AF469010) was obtained by probing a honey bee brain cDNA library with the positive PCR fragment. Overall similarity to *Drosophila for* (*dg2*), 87%; *Drosophila dg1*, 70%; mammalian *pkg1*, 73%; *Caenorhabditis elegans pkg*, 70%.

6. Each Northern blot lane contained 5  $\mu$ g of mRNA from heads. Hybridization was performed with a 742-base pair DIG-labeled riboprobe (corresponding to nucleotides 1561 to 2302 of *Amfor*), using Easy-Hyb buffer (Roche) at 65°C. *Ef1 $\alpha$*  loading control was as in (23).

7. To measure mRNA levels of *Amfor* in individual bee brains, we used real-time qRT-PCR with TaqMan technology (ABI). Total brain RNA from an individual brain was isolated with the RNeasy mini kit (Qiagen). Reverse transcription was performed according to protocol (TaqMan Reverse Transcription Reagents kit, ABI) with 100 ng of total RNA. PCR was performed with the default parameters of the ABI Prism 5700 sequence detector. PrimerExpress software (ABI) was used to design highly specific primers and probe for *Amfor*. Forward probe, 5'-AATATAACTCCGGTGCAACGTAT; reverse probe, 5'-CGTTTGGATCACGGAAGAAAG; TaqMan probe (ABI), 5'-FAM6-AGGCGTGCCGAGAAG-GTCCA-TAMRA. Levels of *Amfor* were quantified relative to 18S rRNA (ABI kit); there were no differences in 18S rRNA levels between nurses and foragers (17). Each sample was analyzed in triplicate, and each data point was calculated as the average of the three. Quantification was based on the number of PCR cycles (Ct) required to cross a threshold of fluorescence intensity, using the 2<sup>- $\Delta\Delta$ Ct</sup> technique (ABI User Bulletin 2) described in (24). Identification of nurses and foragers was as in (25). Bees were collected into liquid nitrogen immediately upon identification so that mRNA measurements accurately reflected gene activity under natural conditions. Bees were stored at -80°C until brain dissection.

8. One-day-old (0 to 24 hours) adult bees were obtained by removing honeycomb frames containing pupae from a typical colony in the field and transferring them to an incubator (33°C, 95% relative humidity). We marked ~1000 1-day-old bees with a paint spot on their thorax and placed them in a small beehive with a queen (unrelated to the workers), one honeycomb frame of honey and pollen, and one empty honeycomb frame (for the queen to lay eggs). Each single-cohort colony was placed in an incubator for the first 4 days and then moved outdoors.

9. Z. Y. Huang, G. E. Robinson, *Proc. Natl. Acad. Sci. U.S.A.* **89**, 11726 (1992).

10. We marked groups of 50 1-day-old bees and placed each group in a wooden cage (6 cm by 12 cm by 18 cm) placed in an incubator (33°C, 95% relative humidity) for 4 days. Bees were treated orally with a 50% sucrose solution containing 8-Br-cGMP (Sigma) at the specified dose (control bees received sucrose alone). This compound is known to increase PKG activity (26). A freshly mixed solution was given daily. On day 5, all surviving bees from each cage were counted (90 to 100% survival) and placed into a single-cohort colony with ~1000 1-day-old bees (without cGMP treatment). Observations at the hive entrance were made as in (9) to ensure that the onset of foraging was identified; observations then occurred for 4 hours per day, 2 hours in the morning and 2 hours in late afternoon—times of peak foraging activity for these colonies. All bees initiating foraging during the first 4 days of observation were marked with a second spot of paint (so they were counted just once) and recorded, and the cumulative percentage of each group that foraged precociously was calculated. Oral treatment was used because it is a noninvasive way of achieving chronic treatment; it does not allow effects to be conclusively ascribed to brain elevation, even though assays of PKG activity in the head suggest that elevation in the brain did occur.

11. Y. Ben-Shahar, A. Robichon, M. B. Sokolowski, G. E. Robinson, data not shown.

12. Brains were dissected fresh in saline. Bees were cold-anesthetized before dissection. Once dissected, brains were immediately freeze-mounted on dry ice with anterior side (identified by antennal lobes) up, and transferred to the cryostat (Bright Inst. Co., -20°C). Brains were sectioned (10  $\mu$ m) and dry-mounted on glass slides. Hybridization was performed in 50% formalde-

hyde buffer with a digoxigenin-labeled antisense RNA probe (Roche) at 60°C [same as in (6)]. Sense probe was used as control.

13. M. Heisenberg, *Learn. Mem.* **5**, 1 (1998).

14. W. Gronenberg, *J. Comp. Neurol.* **435**, 474 (2001).

15. R. E. Page Jr., J. Gadau, M. Beye, *Genetics* **160**, 375 (2002).

16. L. J. Young, M. M. Lim, B. Gingrich, T. R. Insel, *Horm. Behav.* **40**, 133 (2001).

17. M. J. B. Krieger, K. G. Ross, *Science* **295**, 328 (2002).

18. M. A. Della-Fera, C. A. Baile, S. R. Peikin, *Physiol. Behav.* **26**, 799 (1981).

19. J. E. Morley, S. A. Farr, M. D. Suarez, J. F. Flood, *Pharmacol. Biochem. Behav.* **50**, 369 (1995).

20. L. L. Moroz, T. P. Norekian, T. J. Pirtle, K. J. Robertson, R. A. Satterlie, *J. Comp. Neurol.* **427**, 274 (2000).

21. Y. Gilad, S. Rosenberg, M. Przeworski, D. Lancet, K. Skorecki, *Proc. Natl. Acad. Sci. U.S.A.* **99**, 862 (2002).

22. Y.-C. Ding et al., *Proc. Natl. Acad. Sci. U.S.A.* **99**, 309 (2002).

23. D. P. Toma et al., *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6914 (2000).

24. G. Bloch et al., *J. Biol. Rhythms* **16**, 444 (2001).

25. Y. Ben-Shahar, G. E. Robinson, *J. Comp. Physiol. A* **187**, 891 (2001).

26. S. H. Francis et al., *Mol. Pharmacol.* **34**, 506 (1988).

27. E. O. Wilson, *Sociobiology* (Belknap/Harvard, Cambridge, MA, 1975).

28. D. R. Cox, *J. R. Stat. Soc. Ser. B* **34**, 187 (1995).

29. We thank B. White for use of the ABI 5700 TaqMan machine; A. Ross and A. Cash for technical assistance in the field; S. Hartz for statistical assistance; S. E. Fahrback and R. Velarde for help with in situ hybridization; and D. F. Clayton, K. G. Ross, J. H. Willis, and members of the Robinson and Sokolowski laboratories for helpful comments on the manuscript. The "time scale perspective" on genomic responsiveness resembles Wilson's model for organismic responsiveness (27). Supported by grants from the NIH and Burroughs-Wellcome Trust (G.E.R.) and MRC and the Canada Research Chair Program (M.B.S.).

16 January 2002; accepted 20 March 2002

# A Tomato Cysteine Protease Required for Cf-2-Dependent Disease Resistance and Suppression of Autonecrosis

Julia Krüger,<sup>1</sup> Colwyn M. Thomas,<sup>1,2</sup> Catherine Golstein,<sup>1,3</sup> Mark S. Dixon,<sup>1,4</sup> Matthew Smoker,<sup>1</sup> Saijun Tang,<sup>1,5</sup> Lonke Mulder,<sup>1</sup> Jonathan D. G. Jones<sup>1\*</sup>

Little is known of how plant disease resistance (R) proteins recognize pathogens and activate plant defenses. *Rcr3* is specifically required for the function of *Cf-2*, a *Lycopersicon pimpinellifolium* gene bred into cultivated tomato (*Lycopersicon esculentum*) for resistance to *Cladosporium fulvum*. *Rcr3* encodes a secreted papain-like cysteine endoprotease. Genetic analysis shows *Rcr3* is allelic to the *L. pimpinellifolium* *Ne* gene, which suppresses the *Cf-2*-dependent autonecrosis conditioned by its *L. esculentum* allele, *ne* (*ne* necrosis). *Rcr3* alleles from these two species encode proteins that differ by only seven amino acids. Possible roles of *Rcr3* in *Cf-2*-dependent defense and autonecrosis are discussed.

Plant disease R proteins activate defense mechanisms upon perception of pathogen-derived molecules. Intracellular and extracellular race-specific elicitors are recognized by structurally distinct classes of R proteins (1, 2). Tomato *Cf*-genes confer resistance to the fungus *Cladosporium fulvum*. During infection numerous peptides are secreted into the apoplast (3), and some are products of fungal avirulence (*Avr*) genes. *Cf*- genes encode transmembrane proteins with extracellular leucine-rich repeats (LRRs) and short (23 to 36 amino acid) cytoplasmic domains (1, 2). In tomato, *Avr* peptide

recognition activates a defense reaction dependent on *Cf*- genes, the hypersensitive response (HR), which results in localized cell death and the arrest of pathogen ingress. In tobacco cells expressing *Cf-9*, elicitation with *Avr9* leads within 5 to 15 min to reactive oxygen production, protein kinase activation and novel gene expression (4). How *Cf* proteins activate defense responses is unknown.

*Cf-2* confers *Avr2*-dependent resistance to *C. fulvum*. Mutations in *Rcr3* suppress *Cf-2* function (2). *Rcr3* is unlikely to be a component shared by multiple *Cf*- signaling pathways, because it is dispensable for the function of *Cf-9* and even *Cf-5*, an ortholog of *Cf-2* (5).

We isolated *Rcr3* by positional cloning (6). *Rcr3* encodes a protein of 344 amino acids that is 43% identical to papain from *Carica papaya* (Fig. 1A). *Rcr3* expressed from its own promoter restores *Cf-2*-dependent resistance to *rcr3* mutants (Fig. 1B). *Rcr3* contains conserved amino acid residues of the active site of eukaryotic thiol proteases (C<sup>154</sup>, H<sup>286</sup>, and N<sup>307</sup> (7),

<sup>1</sup>The Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, UK. <sup>2</sup>School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, UK. <sup>3</sup>Department of Biology, Indiana University, Bloomington, IN 47405, USA. <sup>4</sup>School of Biological Sciences, University of Southampton, Southampton SO16 7PX, UK. <sup>5</sup>Department of Biology, 108 Coker Hall, University of North Carolina, Chapel Hill, NC 27599-3280, USA.

\*To whom correspondence should be addressed. E-mail jonathan.jones@bbsrc.ac.uk