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20. The *S. cerevisiae* W301-1B control strain (*MATα, ade2, trp1, leu2, ura3, his3*) and the triple ANT mutant JL-1-3 strain (*MATα, LEU::aac1, HIS::aac2, URA::aac3, ade2, trp1, leu2, ura3, his3*) [T. Drgon, L. Sabova, N. Nelson, J. Kolarov, *FEBS Lett.* **289**, 159 (1991)] were transfected with a pJG-4-5 vector containing the Trp1 selection marker and murine Bax under the control of the Gal promoter, and the lethal effect of Bax overexpression (controlled by immunoblot) was assessed [S. Matsuyama, Q. Xu, J. Velours, J. C. Reed, *Mol. Cell* **1**, 327 (1998)]. The pJG-4-5 vector containing v-Ras instead of Bax served as a negative control.
21. Cells or organs were homogenized in H buffer (150 μM $MgCl_2$, 10 mM KCl, 10 mM tris-HCl, pH 7.6), followed by elimination of nuclei (3 min at 900*g*, at 4°C, recovery of supernatant), elimination of soluble proteins (10 min at 6800*g*), resuspension of the mitochondria-containing pellet in H buffer supplemented with 0.5% Triton (v:v, final concentration; 0.5 mg of protein per milliliter), addition of polyclonal rabbit antiserum (1 μg of antibody per 10 μg of protein in sample) specific for Bcl-2, Bax (Santa Cruz Biotechnology), human interleukin-4 (IL-4) (Pharmingen), or ANT [J. Gironcalle and H. H. O. Schmid, *Biochemistry* **35**, 15440 (1996)], incubation for 90 min at 37°C, addition of 10% protein A and G agarose beads (Santa Cruz Biotechnology) for 30 min at 37°C, recovery (10 min, 2000*g*) of the washed (two times in phosphate-buffered saline, pH 7.4) beads in SDS–polyacrylamide gel electrophoresis (SDS-PAGE) sample buffer, and immunodetection with monoclonal antibody (mAb) to Bax (P19, Santa Cruz), mAb to Bcl-2 (clone 124, Dako), or antiserum to ANT.
22. A human Bak cDNA without TM [J. M. Jürgensmeier *et al.*, *Mol. Biol. Cell* **8**, 325 (1997)], human Bcl-2 lacking the TM, murine Bax cDNA lacking the TM, Ras, Fas, Traf-3, or a human ANT2 cDNA fragment (amino acids 105 to 156) were subcloned in the Eco R1–Xho I sites of pGilda or pJG4-5. EGY48 cells were used for *lacZ* reporter gene assays in conjunction with pGilda (LexA DNA–binding domain) and pJG4-5 (B42 transactivation domain) and psH 18-34 reporter plasmids. Filter assays were performed for galactosidase measurement, with cells plated on either galactose- or glucose-containing minimal medium (*19*) [M. Hanada, C. Aime-Sempé, T. Sato, J. C. Reed, *J. Biol. Chem.* **270**, 11962 (1995); H. Zha, C. Aimé-Sempé, T. Sato, J. C. Reed, *ibid.* **271**, 7440 (1996)].
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36. We thank D. Andrews for Rat-1 cells; D. Brdiczka for help in ANT purification; T. Drgon for ANT-deficient yeast; H. J. Duine for BA; S. Korsmeyer for $Bax^{-/-}$ mice; N. Roy for ANT plasmid; H. Schmid for the ANT-specific antibody; A. Zweibaum for HT29 cells; and N. Larochette and D. Métivier for technical assistance. Supported by Agence Nationale pour la Recherche sur le SIDA, Association pour la Recherche sur le Cancer, CNRS, Fondation pour la Recherche Médicale, and Ligue Française contre le Cancer (to G.K.), and University of California Breast Cancer Research Program (grant number IRB-009B) and CapCure Incorporated (to J.C.R.). S.A.S. and E.L.A.V. receive fellowships from the European Commission, I.M. from the Spanish Ministry of Science, and J.M.J. from the Deutsche Forschungsgemeinschaft.

21 May 1998; accepted 12 August 1998

Dorsal-Ventral Signaling in the *Drosophila* Eye

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The development of the *Drosophila* eye has served as a model system for investigations of tissue patterning and cell-cell communication; however, early eye development has not been well understood. The results presented here indicate that specialized cells are established along the dorsal-ventral midline of the developing eye by Notch-mediated signaling between dorsal and ventral cells, and that Notch activation at the midline plays an essential role both in promoting the growth of the eye primordia and in regulating eye patterning. These observations imply that the developmental homology between *Drosophila* wings and vertebrate limbs extends to *Drosophila* eyes.

Patterning and growth of the *Drosophila* wing depend on signaling between cells in different compartments (*1*, *2*). This signaling establishes specialized cells along compartment boundaries, which in turn secrete molecules that regulate wing development. The *fringe* (*fng*) gene regulates signaling between dorsal and ventral cells (*3*). Fringe protein (FNG) is produced by dorsal cells and inhibits cells' responsiveness to the Notch ligand Serrate (SER), while potentiating cells' responsiveness to the Notch ligand Delta (DL) (*4*, *5*). This results in activation of Notch along the *fng* expression border, which normally corresponds to the dorsal-ventral (D-V) compartment boundary. Local activation of Notch in the wing is both necessary and sufficient for long-range effects on the fate and proliferation of wing cells (*1*, *2*).

Strict compartment boundaries have not

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been detected in the eye (*6*, *7*). Another distinguishing feature of eye development is a wave of differentiation, marked by the morphogenetic furrow, that sweeps across the disc (*7*). Behind the morphogenetic furrow cells are recruited into photoreceptor-containing clusters, the ommatidia (*8*). Photoreceptor clusters are asymmetrical, and cells in dorsal and in ventral ommatidia are arranged in distinct chiral shapes. The two forms meet at the D-V midline, termed the equator. Ommatidial chirality is thought to be influenced by signals associated with the progression of the morphogenetic furrow (*9*), and by signals emanating either from equatorial cells or from cells at the dorsal and ventral periphery of the eye disc (*10*–*13*).

During early eye development, *fng* is expressed by ventral cells (Fig. 1A). This expression appears to be complementary to that of the dorsally expressed gene *mirror* (*mrr*) (Fig. 1B) (*12*). During early to mid–third instar, additional expression of *fng* appears in the posterior of the eye disc (Fig. 1C). This line of posterior *fng* expression is just in front of the morphogenetic furrow and moves across the eye ahead of the furrow (Fig. 1D).

In the wing disc, DL and SER induce each other's expression, and so become up-regulated along the D-V border where they can productively signal (*2*, *5*). DL and SER are also preferentially expressed along the D-V midline during early eye development (Fig. 1, E and F). SER expression is, like *fng* expression, complementary to that of *mrr*, whereas DL expression partially overlaps that of *mrr*. The spatial relations among *fng*, SER, and DL expression in the eye are thus similar to that in the wing, although their expression is inverted with respect to the D-V axis.

The *four-jointed* (*fj*) gene is expressed in a gradient during early eye development (Fig. 2A) (*14*, *15*), with a peak of expression along the D-V midline, and so together with SER and DL serves as a molecular marker of midline cell fate. Ubiquitous expression of FNG during early eye development, generated by placing *fng* under the control of an *eyeless* enhancer, eliminated detectable expression of SER and DL along the midline (Fig. 2, B and C). Conversely, misexpression of FNG in clones of cells, generated by use of the Flip-out system (*16*, *17*), can result in ectopic expression of SER and *fj* that is centered along novel borders of FNG expression in the dorsal eye (Fig. 2, D and E). Ectopic SER and *fj* expression can also be detected along the borders of *fng* mutant clones in the ventral eye (Fig. 2, F and G). These observations show that FNG expression borders play an essential and instructive role in establishing a distinct group of cells along the D-V midline of the developing eye.

Animals with reduced *fng* activity have small eyes (Fig. 1H). Moreover, ubiquitous *fng* expression also results in a dramatic loss of eye tissue (Fig. 1I). Tissue loss is detectable in the developing imaginal disc, before the morphogenetic furrow moves across the eye (Fig. 2, B and C). Moreover, eye loss is observed when *fng* is ectopically expressed during early development (*eyeless-Gal4 UAS-fng*), but not when *fng* is ectopically expressed behind the furrow (*GMR-Gal4 UAS-fng*). These observations indicate that a FNG expression border is required for eye growth specifically during early eye development.

Clones of cells ectopically expressing DL can induce SER expression in ventral, FNG-expressing cells, but not in dorsal cells (Fig. 3A). FNG alone can induce SER expression in dorsal cells, but only near the D-V midline

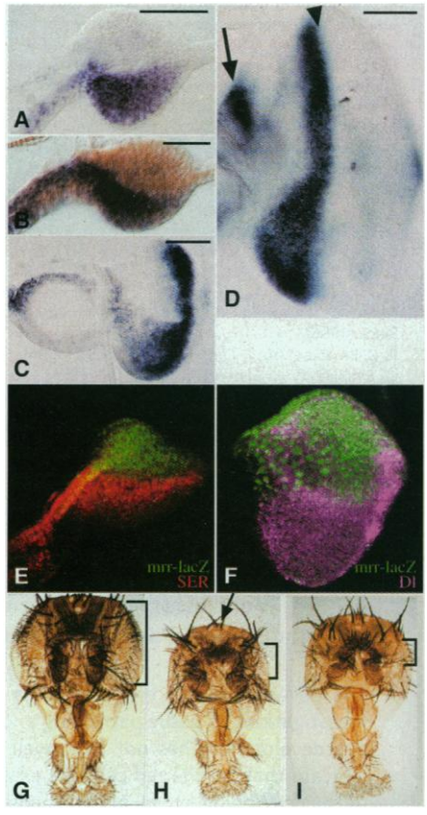

Fig. 1. Expression of *fng*, SER, and DL (*31*). (**A** to **F**) Eye imaginal discs. In all figures, these are oriented with ventral down and anterior left. (**G** to **I**) Heads, with eye tissue bracketed. (A) Early third instar; *fng* expression is ventral. (B) Early third instar; *fng* (blue) and *mrr-lacZ* (brown) expression appear complementary. (C) Mid–third instar; *fng* is expressed ventrally and in front of the furrow. (D) Late third instar; *fng* is expressed in front of the furrow (arrowhead) and in the ocellar region (arrow). Bar: 50 μm in (A), (C), and (D); 25 μm in (B). (E) Early third instar; SER (red) and *mrr-lacZ* (green) are complementary. (F) Mid–third instar; DL (purple) and *mrr-lacZ* (green) are partially overlapping at the D-V midline. (G) Wild type. (H) fng^{52}/fng^{13} (*3*). Arrow points to ocellar region, in which tissue loss occurs. (I) *eyeless-Gal4 UAS-fng27*, 29°C. Most eye tissue is lost. To view each expression pattern presented in (E) and (F) separately, see supplementary Web material for Fig. 1 at www.sciencemag.org/feature/data/982658.shl.

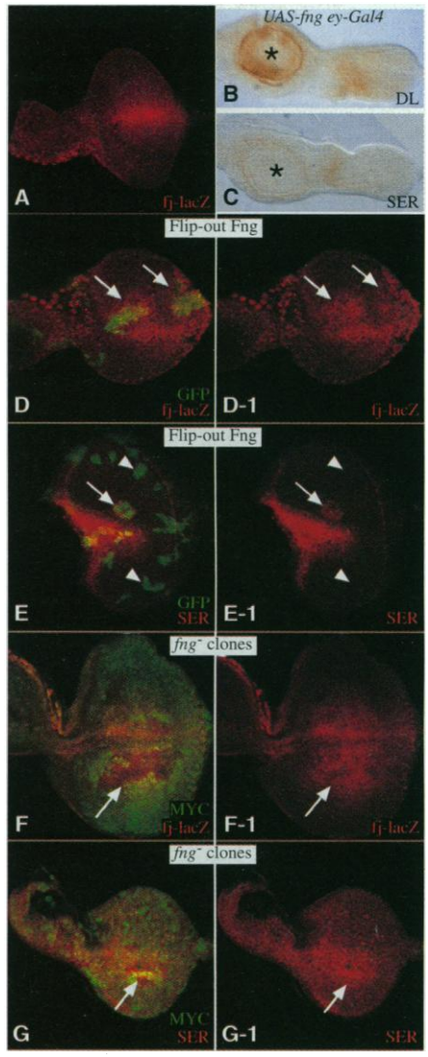

Fig. 2. Influence of *fng* on D-V midline formation (*32*). Early– to mid–third instar eye discs. White arrows point to clones that induce altered expression, white arrowheads point to clones without effect. (**A**) Wild-type *fj* expression. (**B** and **C**) *UAS-fng ey-Gal4*. The eye region (right) is reduced compared to the antennal region (left, asterisk), and D-V midline expression of DL (B) and SER (C) is absent. (**D** and **E**) Discs in which clones of cells ectopically expressing FNG have been induced, marked by coexpression of green fluorescent protein (GFP, green), and stained (red) for expression of *fj* (D) and SER (E). In contrast to the wing (*5*), in the eye SER is induced along both sides of ectopic *fng* expression borders, as evidenced by red staining in (E) (arrow). (D-1) The expression of *fj* alone; (E-1) the expression of SER alone. (**F** and **G**) Discs with *fng* mutant clones, marked by the absence of MYC staining (green), and stained for expression of *fj* (F) and SER (G). (F-1) The expression of *fj* alone; (G-1) the expression of SER alone.

(Fig. 2E). When FNG and DL are co-misexpressed, SER expression can be induced in dorsal cells even when the clones are far from the D-V midline (Fig. 3B). Clones of cells ectopically expressing SER are able to induce increased expression of DL in dorsal cells, but not in ventral, FNG-expressing cells (Fig. 3C). However, if SER is ectopically expressed in *fng* mutant animals, then it can induce DL expression in ventral cells (Fig. 3D). These observations show that FNG differentially modulates the action of Notch ligands in the eye just as it does in the wing.

The ability of SER and DL to induce each other's expression indicates that their expression is a marker of Notch activation in the eye. SER and DL expression are also induced by a constitutively activated form of Notch (Fig. 3, E and G) (*5*, *16*), as is *fj* (Fig. 3F). Because all three genes expressed with respect to the D-V midline in the early eye respond to Notch activation, local activation of Notch appears to be sufficient to specify D-V midline cell fate. Moreover, SER expression appears reduced in early third instar eye discs when *Notch* function is decreased with a conditional allele and is lost from *Notch* mutant clones (Fig. 3H). Thus, *Notch* function is also necessary for normal D-V midline cell fate.

Analysis of loss-of-function mutants of *Notch* and its ligands, as well as ectopic expression studies, indicate that Notch activation also regulates eye growth. Ectopic activation of Notch can induce overgrowths that are evident in the eye imaginal disc (Fig. 3E). Conversely, reduction of *Notch* or *Dl* function during early larval development, through the use of temperature-sensitive mutations, reduces the size of the eye (*18*). *Ser* mutant animals also have small eyes (*19*). Although local elimination of Notch signaling by production of clones of cells mutant for *Ser* or *fng* does not lead to obvious reductions in eye size (*20*), several observations indicate that the D-V midline is the focus of Notch activation required for growth. The restricted expression of Notch ligands (Fig. 1), of genes that respond to Notch activation (*Dl*, *Ser*, and *fj*), and of a synthetic marker of Notch activation (*21*) indicate that Notch is specifically active at the midline when it is required for growth. Moreover, the midline corresponds to a *fng* expression border, which is essential for growth and modulates Notch signaling during early eye development. Because local activation of Notch has long-range effects on growth and *fj* expression, we infer that Notch induces the expression of a diffusible growth factor at the midline. The lack of obvious effect of mutant clones on eye growth may thus be explained by the nonautonomous, early, and transient requirement of Notch signaling at the midline for growth, as it is difficult to generate large clones of mutant tissue at early stages of development.

As the D-V midline has been implicated in influencing ommatidial chirality later in eye development, we examined the effect of Notch activation on chirality. *fng* mutant clone borders within the ventral eye can be associated with reversals of ommatidial chirality (Fig. 4A), whereas mutant clones that cross the D-V midline disrupt the normal equator. Bar expression distinguishes the chiral forms of ommatidia in the developing eye disc (*22*). Reorientations of ommatidia are revealed by altered Bar expression along the equatorial sides of clones with ectopic Notch activation (Fig. 4, C to E). Within cells in which Notch is activated, Bar staining is

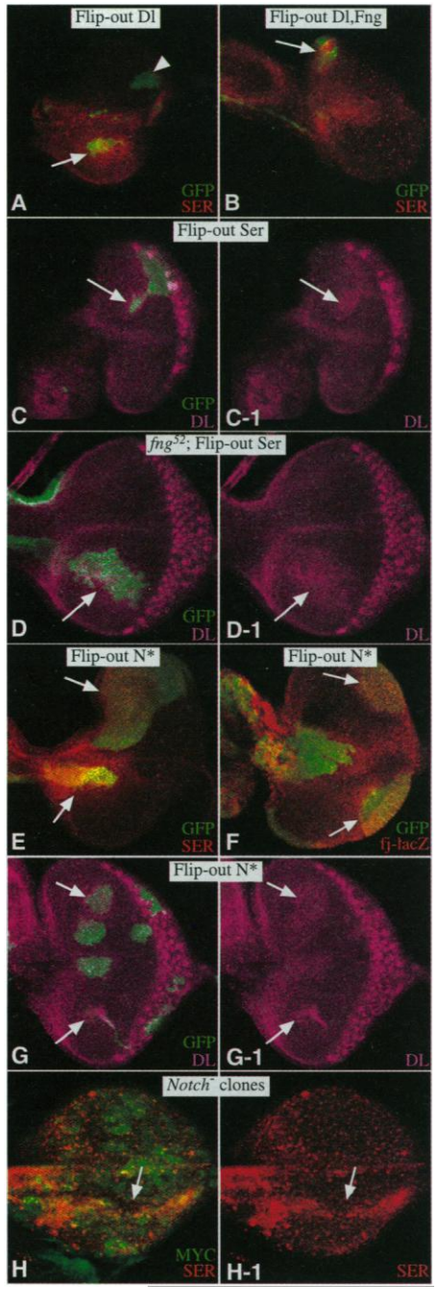

Fig. 3. Regulation and consequences of Notch signaling. Early to mid–third instar eye discs. Clones of cells ectopically expressing (Flip-out) DL, SER, FNG, or constitutively activated Notch (N*) are marked by coexpression of GFP (green). (**A**) *Dl* induces SER (red) in ventral cells, but not in dorsal cells. (**B**) Coexpression of *Dl* and *fng* can induce SER in dorsal cells. (**C**) *Ser* induces DL (purple) in dorsal cells but not in ventral cells (not shown). (**D**) In fng^{52}/fng^{13} mutant animals, *Ser* can induce DL in ventral cells. (C-1 and D-1) The expression of DL alone. (**E** to **G**) Activated Notch can induce expression of SER (E), *fj* (F), and DL (G) in both dorsal and ventral cells. Enlargement of the dorsal half of the disc is apparent in (E). (G-1) The expression of DL alone. (**H**) A $Notch^{55e11}$ mutant clone (marked by the absence of MYC, arrow) lacking SER expression. (H-1) The expression of SER alone. To view the target gene expression patterns for (A), (B), (E), and (F) separately, as well as the induction of *fj* expression by ectopic SER, see supplementary Web material for Fig. 3.

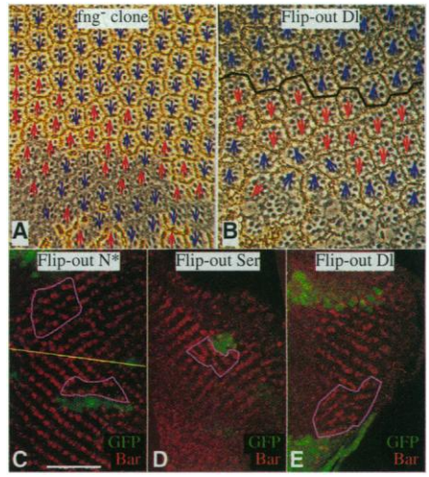

Fig. 4. Influence of Notch on chirality. Dorsal is up. Blue and red arrows identify the two chiral shapes of ommatidia (*11*). (**A**) Ventral section through a right eye, a *fng* mutant clone is marked by the absence of pigment. Normal chirality here is blue, reversals of chirality appear to be induced by both edges of the clone and can be nonautonomous. (**B**) Section through a left eye, including the normal equator (black line). The location of cells ectopically expressing *Dl* can be inferred from the inhibition of photoreceptor differentiation. Normal chirality in ventral cells here is red, reversals of chirality appear equatorial to the clone. (**C** to **E**) Late third instar to pre-pupal eye discs, stained for expression of Bar (red). A scale bar for these panels (25 μm) is at the bottom of (C). The rows of Bar-expressing cells in the dorsal and ventral halves of the eye point toward the equator (yellow line). Ommatidia with altered chirality (outlined in purple) are visible along the equatorial sides of clones (marked by coexpression of GFP, green) ectopically expressing activated Notch (C), *Ser* (D), or *Dl* (E). To view (A) and (B) without the superimposed chirality arrows, as well as the influence of ectopic SER on chirality, see supplementary Web material for Fig. 4.

repressed, as expected, because Notch activation inhibits photoreceptor differentiation (*23*). Similarly, in the eyes of adult flies in which clones of cells ectopically expressing SER or DL have been induced, scars of tissue lacking ommatidia occur. Reversals of ommatidial chirality occur along the equatorial sides of these Notch signaling–induced scars (Fig. 4B). Although these observations show that Notch activation influences ommatidial chirality, the absence of D-V midline expression of SER or DL behind the morphogenetic furrow, together with the inhibition of photoreceptor development associated with Notch activation behind the furrow, indicate that this influence must be indirect. The equatorial bias in the influence of ectopic Notch activation implies that the equator is the normal source of a Notch-dependent, chirality-determining signal (*11*).

D-V signaling in the eye shares many similarities with D-V signaling in the wing (*2*). In both cases an initial asymmetry is set up by Wingless expression. They then go through a distinct intermediate step, as in the wing Wingless represses the expression of a positive regulator of *fng*, Apterous (*3*, *24*), whereas in the eye Wingless promotes the expression of *mrr* (*13*), which encodes a negative regulator of *fng* (*25*). They then share a FNG-SER-DL-Notch signaling cassette to effect signaling between dorsal and ventral cells and establish Notch activation along the D-V midline. Local activation of Notch leads to production of diffusible, long-range signals that direct growth and patterning, which in the wing include Wingless, but in the eye remain unknown. At least one downstream target of D-V midline signaling is also conserved, as *fj* is also expressed in the wing (*14*, *15*), and its expression there is indirectly influenced by Notch (*26*).

fng-related genes appear to play analogous roles in D-V signaling during the development of vertebrate limbs (*27*). This conservation, together with conservation of other molecules involved in D-V patterning (*2*, *28*), and the similar deployment of Hedgehog- and Decapentaplegic-related proteins to pattern the anterior-posterior axes of *Drosophila* and vertebrate limbs, led to the suggestion that a common ancestor used these signaling cassettes to control patterning and growth in some appendage or tissue (*28*). Notably, Decapentaplegic and Hedgehog signaling are also required for the growth and patterning of the developing eye along its anterior-posterior axis (*29*). Thus, three disparate structures, *Drosophila* wings, *Drosophila* eyes, and vertebrate limbs, use the same signaling pathways along both anterior-posterior and D-V axes to effect patterning and growth. Although a role for D-V signaling in vertebrate eye development has not yet been described, genes that specify eye fate in *Drosophila* and vertebrates are homologous (*30*). The similarity between the molecular mechanisms that control eye and appendage development may reflect their descent from a common ancestral organ. Alternatively, the entire patterning system may have been co-opted by one of these organs from the other. In either case, the similarity seems beyond coincidence, and our results imply an evolutionarily relationship between eyes and appendages.

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21. Evidence for Notch activation along the D-V midline has been observed in eye discs of UAS-GFP larvae expressing a modified version of the Notch^{+}-Gal4-VP16 (N^{+}-GV3) protein described in G. Struhl and A. Adachi, *Cell* **93**, 649 (1998) (G. Struhl and A. Adachi, unpublished observations).
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31. *fng* expression was detected by in situ hybridization. Immunostaining was performed as described (*5*), except that SER was detected with a rat antisera (diluted 1:1000) (produced by T. Correia). *mrr* expression was detected with an enhancer trap (*12*).
32. Ectopic *fng* expression was generated by use of the Flip-out system to produce clones of cells expressing *Gal4* in animals that also carried *UAS-GFP* and *UAS-fng* transgenes (*17*). Clones of cells mutant for *fng* were produced by mitotic recombination between a *fng*^{13} FRT80B chromosome (*3*) and FRT80-3. *fj* was detected by an enhancer trap (*14*), Myc was detected with a rabbit antiserum (A14, Santa Cruz) diluted 1:100.

33. We thank E. Wieschaus for assistance during the initial phases of this project; H. McNeill and G. Struhl for communication of unpublished observations; Y. Hiromi, D. Doherty, E. Knust, U. Walldorf, H. McNeill, H. Stellar, T. Laverty, G. Struhl, and the Bloomington stock center for *Drosophila* stocks; E. Knust, M. Muskavitch, S. Artavanis-Tsakonas, K. Saigo, and T. Correia for antibodies; and R. Padgett for comments. C.R. is a Foundation for Advanced Cancer Studies Fellow of the Life Sciences Research Foundation. This work was supported by NIH grant GM-R01-54594 to K.D.I.

20 May 1998; accepted 24 August 1998

The Evolution of Agriculture in Ants

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Cultivation of fungi for food by fungus-growing ants (Attini: Formicidae) originated about 50 million years ago. The subsequent evolutionary history of this agricultural symbiosis was inferred from phylogenetic and population-genetic patterns of 553 cultivars isolated from gardens of "primitive" fungus-growing ants. These patterns indicate that fungus-growing ants succeeded at domesticating multiple cultivars, that the ants are capable of switching to novel cultivars, that single ant species farm a diversity of cultivars, and that cultivars are shared occasionally between distantly related ant species, probably by lateral transfer between ant colonies.

Fungus farming by ants of the tribe Attini originated in the early Tertiary (*1*, *2*) and thus predates human agriculture by about 50 million years (*3*). During its extensive evolutionary history, this symbiosis between "attine" ant farmers and their fungal cultivars has acquired an astonishing complexity, involving secretion of antibiotic "herbicides" to control weed molds and elaborate manuring regimes that maximize fungal harvests (*4*, *5*). Of the over 200 known