A Gene for an Extended Phenotype

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Many parasites and pathogens manipulate host behavior to enhance transmission to new victims. For example, *Ophiocordyceps* fungus–infected arboreal ants are manipulated into "zombies," inducing them to position themselves in the canopy where the microenvironment is ideal for sporulation and dispersal to new hosts, held there by clamping down with jaws on a leaf vein (*1*). In a similar manner, baculoviruses have been known to induce climbing behavior in their caterpillar hosts for over 100 years, coined as "Wipfelkrankheit" (tree top disease; Fig. 1A) (*2*). Until recently, determining the evolutionary basis for these altered behaviors has proven difficult in the absence of a mechanistic explanation. We investigated the genetic basis of manipulation of climbing behavior by a baculovirus (*Lymantria dispar* nucleopolyhedrovirus or LdMNPV) that causes natural epizootics in the European gypsy moth *L. dispar*. Just before death, infected larvae climb to the top of their host trees to die (Fig. 1A), liquefy, and release millions of infective virus particles, with dispersal facilitated by rainfall (*3*). In contrast, healthy *L. dispar* hide in bark crevices or climb down the tree to the soil during the day to avoid predation from birds, climbing back out on the leaves to feed at night.

We hypothesized that tree top disease is induced by expression of the baculovirus gene ecdysteroid uridine 5′-diphosphate (UDP)–glucosyltransferase (*egt*) [supporting online material (SOM) text]. The *egt* gene encodes an enzyme (EGT) that inactivates the molting hormone 20-hydroxyecdysone (20E) by transferring a sugar moiety from a nucleotide sugar donor to a hydroxyl group on 20E (*4*). To test our hypothesis, we inoculated newly molted fourth instar *L. dispar* with one of six different constructs of LdMNPV. The six viruses tested for their impact on climbing behavior of moribund larvae were two wild-type isolates [122b (*5*) and A21 (*6*)], two recombinants in which *egt* was disrupted by either the β-galactosidase gene (*LacZ*) (*7*) or the human transferrin gene (*htf*) (*8*) (EGT–/LacZ+ and EGT–/Htf+, respectively), and two plaque-purified isolates in which wild-type *egt* was reinserted in the egt locus after removing *LacZ* (ResEGT+A and ResEGT+B) (*9*). An equivalent number of mock-inoculated larvae were injected with media only. Larvae were placed individually in tall plastic bottles containing artificial diet in the bottom for food and lined with a fiberglass screen for larvae to climb on.

Both wild-type viruses containing the intact *egt* gene produced larval death at elevated positions, whereas deletion of *egt* eliminated this behavior (Fig. 1B). Moreover, rescue of *egt* restored climbing behavior (Fig. 1B). Larvae infected with wild-type virus or *egt*-deletion viral strains behaved similarly during early and middle phases of viral infection. However, during the late stage of infection *egt*-deletion–infected insects returned to the container bottoms and died there, whereas wild-type infected larvae stayed in elevated positions and died. Because infection with wild-type LdMNPV reduces 20E levels in gypsy moths (*10*), it is reasonable to conclude that *egt* is responsible for manipulating host behavior in this system. In addition, abnormally low levels of 20E in wild-type–infected insects could have other effects, such as reduced morbidity, which would enable infected larvae to continue to seek foliage at a later point during the infection process compared with *egt*-deletion–infected larvae.

These results provide a genetic basis for the extended phenotype whereby a gene in one organism (the parasite) has phenotypic effects on another organism (the host) (*11*).

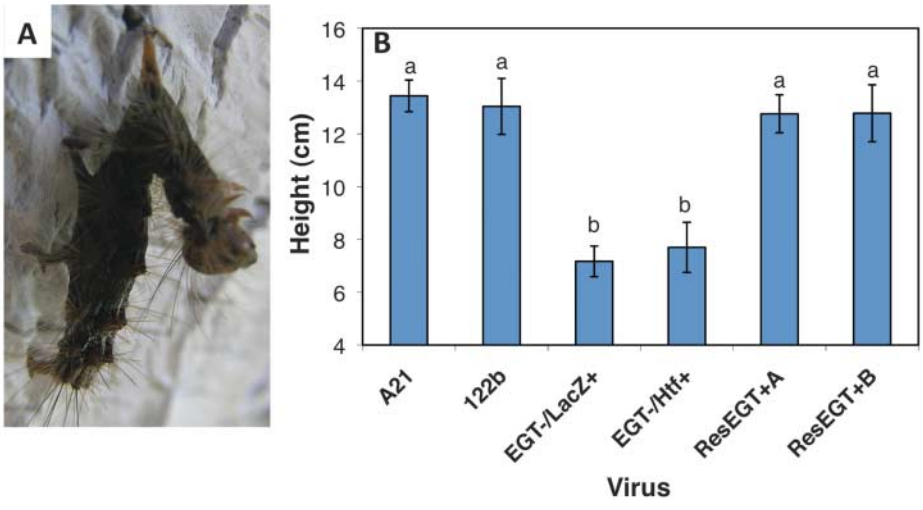

Fig. 1. (**A**) Appearance of tree top disease in the gypsy moth. [Photo credit: V. Martemyanov, Novosibirsk, Russia] (**B**) Influence of the *egt* gene on the height of *L. dispar* larvae when they died from infection by LdMNPV. Viruses contained the wild-type *egt* gene naturally (A21 and 122b) or by re-insertion (ResEGT+A and ResEGTB+) or had a functionally disrupted *egt* gene (EGT–/LacZ+ and EGT–/Htf+). Letters over the bars (±SE) represent means that are not significantly different from each other.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/333/6048/1401/DC1
Materials and Methods
SOM Text
References (*12*–*15*)

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