



Supporting Online Material for

A Gene for an Extended Phenotype

Kelli Hoover,* Michael Grove, Matthew Gardner, David P. Hughes, James McNeil, James Slavicek

*To whom correspondence should be addressed. E-mail: kxh25@psu.edu

Published 9 September 2011, *Science* **333**, 1401 (2011)
DOI: 10.1126/science.1209199

This PDF file includes:

Materials and Methods
SOM Text
References

On-line Abstract

Manipulation of host behavior by parasites and pathogens has been widely observed but the basis for these behaviors has remained elusive. Gypsy moths infected by a baculovirus climb to the top of trees to die, liquefy, and “rain” virus on the foliage below to infect new hosts. The viral gene that manipulates climbing behavior of the host was identified, providing evidence of a genetic basis for the extended phenotype.

Supplemental online material:

Basis for hypothesis: Inactivation of 20-hydroxyecdysone (20E) disrupts hormonal regulation of several physiological processes such as molting and pupation, stopping the period of feeding arrest prior to every molt (4). Once EGT titers become high enough in virus-infected larvae to inactivate 20E, the molting cycle is disrupted, inducing larvae to remain up in the tree in a feeding state rather than crawling down to hide from predators during the day. As they become more moribund, larvae remain at elevated positions and die, which enhances dispersal of progeny virus to the foliage below. In contrast, deletion of *egt* permits molting cycles to continue with their requisite periods of feeding arrest, eliminating the virus-induced climbing behavior since the insect is no longer in a continuous feeding state. Thus, we hypothesized that manipulation of host behavior to die at elevated positions is induced by *egt*, which is a conserved gene among all lepidopteran NPVs and some granuloviruses

Materials and Methods:

Inoculation of larvae: Egg masses of *L. dispar* were obtained from the USDA-Forest Service Insectary (Buzzard Bay, MA) and stored at 4-5°C until needed. Larvae were reared to the fourth instar as described previously (12). To determine larval height at death, newly molted 4th instars were injected into the base of the 2nd proleg with an equal budded virus dose (3.8 TCID₅₀ ml⁻¹) of one of six viral constructs (all viruses equally potent) using a microapplicator (Burkhard Scientific, Uxbridge, UK) fitted with a 32-gauge sharp needle. Larvae were placed individually in clean 20 oz. plastic bottles (19 cm tall x 6.5 cm wide). Data deposited in the Dryad Repository: Data deposited in the Dryad Repository: <http://dx.doi.org/10.5061/dryad.36530>. Heights at death were compared by one-way ANOVA, showing significant differences among treatments (F=18_{5, 458}, p<0.0001). Means were separated by Tukey’s HSD. N=20 larvae per treatment per trial; trials repeated 3-6X. There was no mortality in mock-inoculated controls and they showed no consistent pattern in their movements during the experiment in contrast to infected insects. Mortality among virus treatments ranged from 98-100%.

Construction of *egt* rescue viruses: ResEGT+A and ResEGT+B (strains A1D and B1D) are LdMNPV strain 122bEGT-LacZ+ rescued by replacement of the *egt* gene and removal of LacZ. To generate strain 122bEGT-LacZ+ a 4833 bp *HindIII/NheI* band containing the *egt* gene of LdMNPV-122b was cloned into vector pUC18 using standard techniques to generate pUC18-5*egt* (13). All restriction enzymes were obtained from New England Biolabs (Ipswich, MA). pUC18-5*egt* was digested with *BstEII*, dropping-out a 972 bp region within the coding sequence of the *egt* gene. The remaining plasmid was treated with Klenow to create blunt ends and was ligated with a 3715 bp fragment containing hsp70LacZ generated by digestion of clone pAcDZ1(14) with *BamHI/XbaI* to generate pMB298.1. This clone, pMB298.1, was co-transfected with viral isolate 122b into Ld-652y cells using Insect GeneJuice® (Novagen, EMD Chemicals, Inc., San Diego, CA) reagent to generate viral strain 122bEGT-LacZ+. The

transfection supernatant was plaque-purified as described previously (6) and blue plaques were selected. Viral DNA was prepared from selected LacZ positive virus and restriction endonuclease digestion profiles were analyzed to confirm the insertion of LacZ at the *egt* gene locus. To remove the hsp70LacZ gene from the genome of 122bEGT-LacZ+ and reintroduce the *egt* gene, clone pNP-8.6 that contains the *egt* gene was transfected with 122bEGT-LacZ+ DNA into Ld652Y cells, the resulting BV was isolated and plaque purified, and white plaques were selected for further analysis. DNA analysis of budded virus from two of the white plaques confirmed the complete removal of the hsp70LacZ gene and repair of the *egt* gene region, and the viral isolates were termed ResEGT+A and ResEGT+B. pNP-8.6 was generated by digestion of cosmid clone p313 (13) with *Bam*HI/*Hind*III and the 7837 bp band was gel purified using GeneClean Spin Kit® (MP Biochemicals LLC, Solon, OH) and ligated into pUC18 vector digested with *Bam*HI/*Hind*III using standard techniques. The resulting clone was designated as pNP-8.6, and contains an intact *egt* gene. All viruses were propagated in Ld652Y cells as described previously ().

Supplemental References

1. S. B. Andersen *et al.*, The life of a dead ant: The expression of an adaptive extended phenotype. *Am. Nat.* **174**, 424 (2009). [doi:10.1086/603640](https://doi.org/10.1086/603640) [Medline](#)
2. O. Hofmann, *Insektenötende Pilze mit besonderer Berücksichtigung der Nonne* (P. Weber, Frankfurt, 1891).
3. V. D'Amico, J. S. Elkinton, *Environ. Entomol.* **24**, 1144 (1995).
4. D. R. O'Reilly, M. R. Brown, L. K. Miller, Alteration of ecdysteroid metabolism due to baculovirus infection of the fall armyworm *Spodoptera frugiperda*: Host ecdysteroids are conjugated with galactose. *Insect Biochem. Mol. Biol.* **22**, 313 (1992). [doi:10.1016/0965-1748\(92\)90069-Q](https://doi.org/10.1016/0965-1748(92)90069-Q)
5. J. M. Slavicek, N. Hayes-Plazolles, M. E. Kelly, Identification of a *Lymantria dispar* nucleopolyhedrovirus isolate that does not accumulate few-polyhedra mutants during extended serial passage in cell culture. *Biol. Control* **22**, 159 (2001). [doi:10.1006/bcon.2001.0969](https://doi.org/10.1006/bcon.2001.0969)
6. J. M. Slavicek, N. Hayes-Plazolles, M. E. Kelly, Rapid formation of few polyhedra mutants of *Lymantria dispar* multinucleocapsid nuclear polyhedrosis virus during serial passage in cell culture. *Biol. Control* **5**, 251 (1995). [doi:10.1006/bcon.1995.1031](https://doi.org/10.1006/bcon.1995.1031)
7. J. M. Slavicek, H. J. R. Popham, C. I. Riegel, Deletion of the *Lymantria dispar* multicapsid nucleopolyhedrovirus ecdysteroid UDP-glucosyl transferase gene enhances viral killing speed in the last instar of the gypsy moth. *Biol. Control* **16**, 91 (1999). [doi:10.1006/bcon.1999.0734](https://doi.org/10.1006/bcon.1999.0734)
8. O. Choi *et al.*, N-glycan structures of human transferrin produced by *Lymantria dispar* (gypsy moth) cells using the LdMNPV expression system. *Glycobiology* **13**, 539 (2003). [doi:10.1093/glycob/cwg071](https://doi.org/10.1093/glycob/cwg071) [Medline](#)
9. Materials and methods are available as supporting material on *Science* Online.

10. E. J. Park, J. P. Burand, C. M. Yin, The effect of baculovirus infection on ecdysteroid titer in gypsy moth larvae (*Lymantria dispar*). *J. Insect Physiol.* **39**, 791 (1993). [doi:10.1016/0022-1910\(93\)90055-V](https://doi.org/10.1016/0022-1910(93)90055-V)
11. R. Dawkins, *The Extended Phenotype* (Oxford Univ. Press, Oxford, 1982).
12. K. Hoover, M. Grove, S. Su, Systemic component to intrastadial developmental resistance in *Lymantria dispar* to its baculovirus. *Biol. Control* **25**, 92 (2002). [doi:10.1016/S1049-9644\(02\)00041-5](https://doi.org/10.1016/S1049-9644(02)00041-5)
13. C. I. Riegel, C. Lanner-Herrera, J. M. Slavicek, Identification and characterization of the ecdysteroid UDP-glucosyltransferase gene of the *Lymantria dispar* multinucleocapsid nuclear polyhedrosis virus. *J. Gen. Virol.* **75**, 829 (1994). [doi:10.1099/0022-1317-75-4-829](https://doi.org/10.1099/0022-1317-75-4-829) [Medline](#)
14. D. Zuidema *et al.*, Expression of cauliflower mosaic virus gene I in insect cells using a novel polyhedrin-based baculovirus expression vector. *J. Gen. Virol.* **71**, 2201 (1990). [doi:10.1099/0022-1317-71-10-2201](https://doi.org/10.1099/0022-1317-71-10-2201) [Medline](#)
15. J. M. Slavicek, H. J. R. Popham, The *Lymantria dispar* nucleopolyhedrovirus enhancins are components of occlusion-derived virus. *J. Virol.* **79**, 10578 (2005). [doi:10.1128/JVI.79.16.10578-10588.2005](https://doi.org/10.1128/JVI.79.16.10578-10588.2005) [Medline](#)