Firefly genomes illuminate the origin and evolution of bioluminescence in beetles

Timothy R. Fallon\*,1,2 Sarah E. Lower\*,3 Ching-Ho Chang,4 Manabu Bessho-Uehara,5 Gavin Martin,6 Adam J. Bewick,7 Megan Behringer,8 Humberto Julio Debat,9 John C. Day,10 Antony Suvorov,6 Robert J. Schmitz,7 David Nelson,11 Sara Lewis,12 Shuji Shigenobu,13 Seth Bybee,6 Amanda Larracuente,4 Yuichi Oba,14 Jing-Ke Weng1,2

1 Whitehead Institute for Biomedical Research, 9 Cambridge Center, Cambridge, Massachusetts 02142, USA

2 Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA

3 Department of Molecular Biology & Genetics, Cornell University, Ithaca, New York 14850, USA

4 Department of Biology, University of Rochester, Rochester, New York 14627, USA

5 Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Aichi 464-8601, Japan

6 Department of Biology, Brigham Young University, Provo, Utah 84602, USA

7 Department of Genetics, University of Georgia, Athens, GA

8 Department of Biology, Indiana University, Bloomington, Indiana 47405, USA

9 Center of Agronomic Research National Institute of Agricultural Technology, Córdoba, Argentina

10 Centre for Ecology and Hydrology (CEH) Wallingford, Wallingford, Oxfordshire, UK

11 Department of Microbiology Immunology and Biochemistry, University of Tennessee HSC, Memphis, 38163 USA

12 Department of Biology, Tufts University, Medford, Massachusetts 02155, USA

13 NIBB Core Research Facilities, National Institute for Basic Biology, Okazaki 444-8585, Japan

14 Department of Environmental Biology, Chubu University, Kasugai, Aichi 487-8501, Japan

Email: tfallon@mit.edu , ses425@cornell.edu

Website: <https://experiment.com/projects/illuminating-the-firefly-genome>

**ABSTRACT.** Fireflies represent one of the most widely appreciated-examples of bioluminescence. Despite long-term interest in the biochemistry, neurobiology, evolution and biotechnological applications of firefly flash signals, only a limited number of genes related to this complex trait have been described. To investigate the genetic basis of firefly bioluminescence, we generated a high-quality reference genome for *Photinus pyralis*, the North American species from which laboratory luciferase is derived, using long-read (PacBio), short-read (Illumina), and Hi-C sequencing technologies. To facilitate comparative genomics, we also generated short-read genome assemblies for *Aquatica lateralis*, a Japanese firefly of conservation interest, and *Ignelator luminosus*, a bioluminescent click-beetle relative. Analyses of these datasets provide new insights into the evolution of beetle bioluminescence. In particular, we reveal a physical clustering of firefly lantern-associated luciferase (Luc1) with several tandemly duplicated long chain fatty acyl-CoA synthetases, supporting its origin at this locus via gene duplication followed by neofunctionalization. We report the presence of the luciferase paralog Luc2 in *P. pyralis*, which is located on a separate chromosome from the Luc1 cluster. We anticipate the genomes presented here will serve as a valuable resource for future investigations of the molecular mechanisms underlying the origin and evolution of firefly bioluminescence.

**Key words:** Photinus pyralis, Aquatica lateralis, Lampyridae, Pyrophorus, luciferase, de novo genomics, de novo transcriptomics