

Lighting the way: Forces driving the diversification of bioluminescent signalling in sea fireflies

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Understanding the drivers of diversification and processes that maintain biodiversity remains a central theme of evolutionary biology. However, these efforts are often impeded due to disparities across species and environments and the genetic complexity underlying many traits. The factors driving biodiversity can be more readily understood by focusing on the genetics of diversification, of one or few genes shared across species, with large influence over an organism's phenotype (Templeton, 1981; Wright, 1984). In this pursuit, previous studies often focus on the selective pressures that impact phenotypic diversity (Brawand et al., 2014; Yokoyama et al., 2015), often overlooking the contribution of neutral processes (i.e., genetic drift). In this issue of *Molecular Ecology*, Hensley et al. (2020) use an integrative approach, including RNA sequencing, in vitro protein expression and spectral measurements, to explore the drivers behind the diversification of bioluminescent signalling in cypridinid ostracods (Figure 1). Typical bioluminescent reactions primarily include an enzyme (luciferase) and substrate (luciferin). By focusing on a single gene, this study traces the molecular evolution of (c)luciferase in sea fireflies, elucidating diverse signatures of selection, drift and constraint to decipher the link between genotype and phenotype of their bioluminescent emissions.

Ostracods, known as sea fireflies, in the family Cypridinidae serve as an exemplar system to study the factors underlying diversification as they have distinct bioluminescent signals involved in antipredator (Figure 1b) and/or courtship displays (Figure 2; Cohen & Morin, 2010; Gerrish & Morin, 2016; Morin, 2019). These unique courtship displays, or bioluminescent pulses, have evolved exclusively among Caribbean species (males), show interspecific variation (Cohen & Morin, 2010; Morin, 2019) and can differ in brightness, kinetics (e.g., duration), and colour (Harvey, 1924; Hensley et al., 2019). Bioluminescent phenotypes among ostracods therefore show variation in both behavioural (e.g., location, angle, onset and/or tempo of pulse signalling) and biochemical parameters (enzymatic reaction of luciferase with a conserved luciferin substrate). Due to the overlap in species' ranges, behavioural modifications in signalling (e.g., microhabitat or emission angle) and modifications to emission signalling (i.e., through enzyme kinetics) may evolve more readily than changes in colour, due to stronger (sexual) selection pressures imposed by mating (e.g., Ellison et al., 2011). This research by Hensley et al.,

(2020) elucidates how underlying genetic variation in a single gene (c-luciferase) is associated with the variation in sea firefly phenotypes and how differences at the molecular level are shaped by both natural and sexual selection as well as neutral processes or drift.

While selection acts at the phenotypic level, it is the underlying genetic mutations that influence an organism's characteristics or phenotype. In this study, the authors aimed to decipher the genetic underpinnings or genotype that ultimately gives rise to cypridinid bioluminescent phenotypes, and the functional roles of the phenotype with regard to signalling in courtship displays. This study reports the emission spectra ("colour") of bioluminescence for 21 cypridinid species, in addition to 13 new c-luciferase genes extracted from de novo transcriptomes – four of which have confirmed function using in vitro protein assays. Notably, Hensley et al. (2020) pinpoints important functional sites in this gene that impact bioluminescent phenotypic diversity. Phylogenetic analysis and tests of selection suggest some amino acid sites linked to enzyme kinetics and colour evolved under positive selection. Comparative analysis of

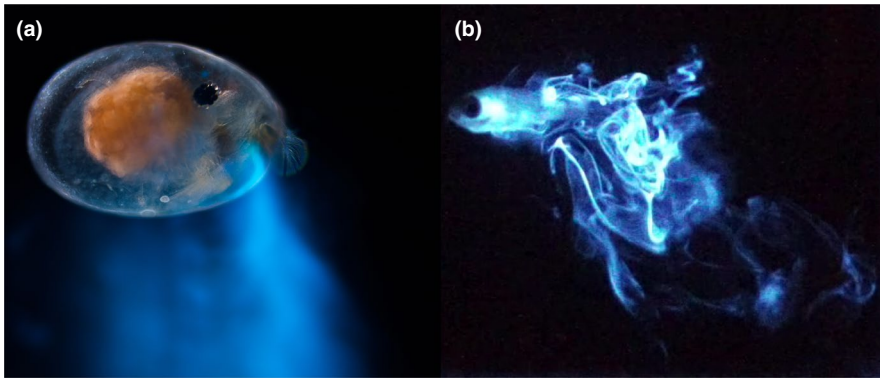


FIGURE 1 Ostracod bioluminescence (a) Photo credit: Elliot Lowndes, (b) Photo credit: Trevor Rivers and Nicholai Hensley

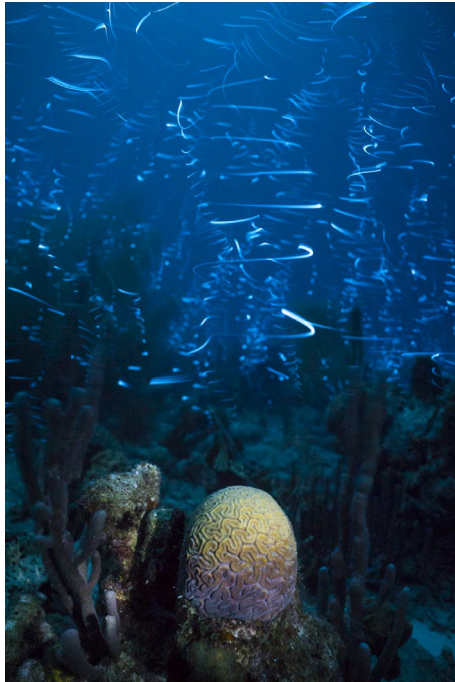


FIGURE 2 "Chorus" of lights, time-lapse photo of ostracod courtship displays above coral in Bonaire. Photo credit: Kyle McBurnie

c-luciferases revealed at least one site that plays a role in determining the rate of light decay evolves under episodic diversifying selection. If positive selection is driving biochemical variation in c-luciferases as these data suggest, it is of particular interest as decay rates can vary widely among species and influence the duration of courtship pulses (Cohen & Morin, 2010; Hensley et al., 2019). However, studies are still lacking at the organismal level to determine how light decay rates affect species' fitness. Even so, the authors hypothesize that pulse duration plays an important role in mate recognition and/or antipredation.

This study identifies additional sites that appear to have evolved neutrally or under purifying selection, potentially leading to the observed colour variation in bioluminescent signals across Cypridinidae. Building from previous mutagenesis experiments (Kawasaki et al., 2012), the authors were able to identify a site in c-luciferase (178) that strongly influences the λ_{\max} (wavelength of

maximum absorbance which dictates colour) of sea firefly emissions. This bioluminescent phenotype (colour) is thought to have minimal impact on cypridinid fitness and the results presented by Hensley et al., (2020) indicate this site may have evolved neutrally due to genetic drift. In addition to site 178, the authors found three additional sites correlated with changes to λ_{\max} that appear to be under positive selection suggesting multiple forces (selection and drift) may play a role in the diversification of emission colour. The role of phylogeny becomes evident as the authors present λ_{\max} with respect to noncourtship and courtship species and find small evolutionary shifts in λ_{\max} indicating a colour change across groups. They speculate as to whether the colour may be adaptive and linked to differences in their environment and/or visual predators, and present alternate arguments that could explain colour variation across species.

A noteworthy concept underwritten by the findings of Hensley et al., (2020) is how genetic diversity can constrain and influence the pathways of evolution (Bonner, 1982; Maynard-Smith et al., 1985; Wake, 1991). One site in c-luciferase (160) is particularly interesting as it has influence over λ_{\max} and is also correlated with light decay. Mutations at this site therefore appear to be affecting multiple phenotypes at once. The authors hypothesize that underlying biochemical constraints, including pleiotropy, may therefore be influencing c-luciferase evolution.

Pleiotropy, in which a single gene can produce two or more unrelated phenotypic effects (He & Zhang, 2006), is a common phenomenon that can force compromise among adaptations of different traits as genetic change beneficial to one trait may be deleterious or neutral to another (Barton, 1990; Otto, 2004). This can potentially lead to fitness tradeoffs (MacLean et al., 2004) and result in nonadaptive traits. In this study an inconsistent relationship between λ_{\max} and light decay is revealed across different species of Cypridinidae, especially across the genus *Photeros*, where all species have a similar λ_{\max} , but differ in light decay rates.

The authors elude to the notion that constraint is not static, but rather dynamic over evolutionary time which is both intriguing and logical. During evolution, changes to the underlying biochemical network of a species' genome, for example through gene deletions or insertions, can modify the functional capacity of a cell and/or enzyme and possibly result in phenotypic changes (Price et al., 2004). The authors suggest that variability in the constraints

acting on emission spectra over time may have allowed for the uncoupling of λ_{max} and light decay in modern lineages of *Photeros* in contrast to their ancestors. In this sense, phenotypic diversification could be facilitated by this uncoupling, allowing wavelength and pulse duration to change independently of each other across this genus.

The authors further discuss how epistatic interactions among c-luciferase sites may be genetically constraining or structuring phenotypes between species. To support this theory, Hensley et al. (2020) found a significant interaction (ANOVA) between at least two c-luciferase sites. They propose that as the bioluminescent phenotypes evolved, mutations at one site constrained the magnitude of functional change upon future enzymatic mutations at the other site. Patterns of amino acid replacement at these interacting sites are discussed between noncourtship and courtship-signalling cypridinids with respect to putative epistatic effects on protein function in select species. The authors propose that these site interactions may have also changed over the course of c-luciferase evolution as observed for other proteins (Ortlund et al., 2007; Yokoyama et al., 2014) and further exploration of site-by-site interactions could provide powerful insights into signal phenotypes.

By characterizing the luciferases and mapping the genetic differences back to the observed phenotypic variation, the authors provide a better understanding of how multiple evolutionary forces influenced the diversification of sea firefly bioluminescence. The results of this study will enable future investigations of bioluminescence in closely related systems, though the authors caution direct comparisons as the molecular characterization of these enzymatic reactions are unknown or limited (Bessho-Uehara et al., 2020) in other species. Together these data suggest the molecular evolution of c-luciferase has contributed significantly to signal diversity in cypridinids and that multiple modes of selection, in conjunction with genetic drift, can act on a single gene to drive diversification. This knowledge provides useful insight towards our understanding of the processes maintaining biodiversity, and can be used to explore the evolution of other genes and phenotypes across environments. Moreover, the authors provide compelling arguments for the role of constraint in the phenotypic diversity of bioluminescent emissions, "lighting the way" for future investigations.

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REFERENCES

- Barton, N. H. (1990). Pleiotropic models of quantitative variation. *Genetics*, 124(3), 773–782.
- Bessho-Uehara, M., Francis, W. R., & Haddock, S. H. D. (2020). Biochemical characterization of diverse deep-sea anthozoan bioluminescence systems. *Marine Biology*, 167(8), 114. <https://doi.org/10.1007/s00227-020-03706-w>
- Bonner, J. T. (1982). Evolutionary strategies and developmental constraints in the cellular slime molds. *The American Naturalist*, 119(4), 530–552.
- Brawand, D., Wagner, C. E., Li, Y. I., Malinsky, M., Keller, I., Fan, S., Simakov, O., Ng, A. Y., Lim, Z. W., Bezault, E., Turner-Maier, J., Johnson, J., Alcazar, R., Noh, H. J., Russell, P., Aken, B., Alföldi, J., Amemiya, C., Azzouzi, N., ... Di Palma, F. (2014). The genomic substrate for adaptive radiation in African cichlid fish. *Nature*, 513, 375–381.
- Cohen, A. C., & Morin, J. G. (2010). Two new bioluminescent Ostracode genera, *Enewton* and *Photeros* (Myodocopida: Cypridinidae), with three new species from Jamaica. *Journal of Crustacean Biology*, 30(1), 1–55. <https://doi.org/10.1651/08-3075.1>
- Ellison, C. K., Wiley, C., & Shaw, K. L. (2011). The genetics of speciation: Genes of small effect underlie sexual isolation in the Hawaiian cricket *Laupala*. *Journal of Evolutionary Biology*, 24(5), 1110–1119. <https://doi.org/10.1111/j.1420-9101.2011.02244.x>
- Gerrish, G. A., & Morin, J. G. (2016). Living in sympatry via differentiation in time, space and display characters of courtship behaviors of bioluminescent marine ostracods. *Marine Biology*, 163(9), 190. <https://doi.org/10.1007/s00227-016-2960-5>
- Harvey, E. N. (1924). Studies on bioluminescence. XVI. What determines the color of the light of luminous animals. *The American Journal of Physiology*, 70, 619–623. <https://doi.org/10.1152/ajplegacy.1924.70.3.619>
- He, X., & Zhang, J. (2006). Toward a molecular understanding of pleiotropy. *Genetics*, 173(4), 1885–1891.
- Hensley, N. M., Ellis, E. A., Gerrish, G. A., Torres, E., Frawley, J. P., Oakley, T. H., & Rivers, T. J. (2019). Phenotypic evolution shaped by current enzyme function in the bioluminescent courtship signals of sea fireflies. *Proceedings. Biological Sciences: Biological Sciences*, 286(1894), 20182621. <https://doi.org/10.1098/rspb.2018.2621>
- Hensley, N. M., Ellis, E. A., Leung, N. Y., Coupert, J., Mikhailovsky, A., Taketa, D. A., Tessler, M., Gruber, D. F., De Tomaso, A. W., Mitani, Y., Rivers, T. J., Gerrish, G. A., Torres, E., & Oakley, T. H. (2020). Selection, drift, and constraint in cypridinid luciferases and the diversification of bioluminescent signals in sea fireflies. *Molecular Ecology*. <https://doi.org/10.1111/mec.15673>
- Kawasaki, K., Morita, Y., Ohgiya, S., Ohmiya, Y., & Ohyama, Y. (2012). *Mutant luciferase* (USPTO Patent No. 8147842). US Patent (No. 8147842). <https://www.google.com/patents/US8147842>
- MacLean, R. C., Bell, G., & Rainey, P. B. (2004). The evolution of a pleiotropic fitness tradeoff in *Pseudomonas fluorescens*. *Proceedings of the National Academy of Sciences of the United States of America*, 101(21), 8072–8077.
- Morin, J. G. (2019). Luminaries of the reef: The history of luminescent ostracods and their courtship displays in the Caribbean. *Journal of Crustacean Biology*, 39(3), 227–243.
- Ortlund, E. A., Bridgham, J. T., Redinbo, M. R., & Thornton, J. W. (2007). Crystal structure of an ancient protein: Evolution by conformational epistasis. *Science*, 317(5844), 1544–1548
- Otto, S. P. (2004). Two steps forward, one step back: the pleiotropic effects of favoured alleles. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 271(1540), 705–714.
- Price, N. D., Reed, J. L., & Palsson, B. Ø. (2004). Genome-scale models of microbial cells: Evaluating the consequences of constraints. *Nature Reviews Microbiology*, 2(11), 886–897.
- Smith, J. M., Burian, R., Kauffman, S., Alberch, P., Campbell, J., Goodwin, B., Lande, R., Raup, D., & Wolpert, L. (1985). Developmental constraints and evolution: a perspective from the Mountain Lake conference on development and evolution. *The Quarterly Review of Biology*, 60(3), 265–287.
- Templeton, A. R. (1981). Mechanisms of speciation – A population genetic approach. *Annual Review of Ecology and Systematics*, 12(1), 23–48. <https://doi.org/10.1146/annurev.es.12.110181.000323>
- Wake, D. B. (1991). Homoplasy: the result of natural selection, or evidence of design limitations? *The American Naturalist*, 138(3), 543–567.

- Wright, S. (1984). *Evolution and the genetics of populations, Volume 3: Experimental results and evolutionary deductions*. University of Chicago Press.
- Yokoyama, S., Altun, A., Jia, H., Yang, H., Koyama, T., Faggionato, D., Liu, Y., & Starmer, W. T. (2015). Adaptive evolutionary paths from UV reception to sensing violet light by epistatic interactions. *Science Advances*, 1, e1500162.
- Yokoyama, S., Xing, J., Liu, Y., Faggionato, D., Altun, A., & Starmer, W. T. (2014). Epistatic adaptive evolution of human color vision. *PLoS Genetics*, 10(12), e1004884. <https://doi.org/10.1371/journal.pgen.1004884>

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