



BIOLOGICAL SCIENCES

Evolutionary mechanisms for camouflage in *Cladomorphus phyllinus* (Phasmatodea): A reflection on the role of evidence for hypotheses proposition

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Abstract: We address and discuss some of the many flaws exhibited by Costa et al. (2019) which tried to explain the twig-like camouflage of *Cladomorphus phyllinus*. Given the lack of both empirical and theoretical underpinnings in Costa et al. (2019), we call into question the validity of their conclusions, in particular, that horizontal gene transfer is a causal mechanism for the camouflage in *C. phyllinus*.

Key words: Hemiptera, lateral gene transfer, stink bug, walking-stick insect.

INTRODUCTION

“Extraordinary claims require extraordinary evidence.”
– Carl Sagan

In a recent paper, Costa et al. (2019) [from now on CEA] offer a report on the predation of a species of predatory stink bug [*Supputius cincticeps* (Stål, 1860) (Hemiptera, Pentatomidae, Asopinae)] over a walking-stick insect [*Cladomorphus phyllinus* Gray, 1835 (Phasmatodea, Phasmatidae, Cladomorphinae)]. The paper presents natural history observations on this interaction but further proposes an astonishing claim: that the twig aspect of this species of Phasmatodea could have arisen through horizontal gene transfer (HGT) of a morph plant gene via the stink bug. Given that very little is known about the mechanisms of morphogenesis in animals, and in insects in particular, any hypothesis of evolutionary camouflage mechanism in walking-stick insects invoking a morphogene should be adequately substantiated. Here, we present some arguments that doubt the validity of CEA

statements and strongly reject the notion of these statements being scientific hypotheses.

DISCUSSION

In their paper, CEA claimed that their observations are the first report of predation between Asopinae over Phasmatodea; nonetheless records of predator-prey interaction between Asopinae and Phasmatodea go back more than a century (e.g., Kirkland 1898), and interactions between Asopinae species and their prey are opportunistic (de Clercq 2000). The interaction documented by CEA thus seems to be casual and uncommon given that it was carried out under laboratory conditions and based on a single observation of a *S. cincticeps* nymph.

The core of CEA paper presented an alternative idea on how camouflage could have evolved in Phasmatodea, different from the evolution of masquerade camouflage (Skelhorn et al. 2010, Dias Lima & Kaminski

2019). The authors weave assumptions related to zoophytophagy, HGT, and camouflage to speculate a causality link between the predation of *S. cinctipes* over *C. phyllinus*, and the particular phenotype of this species of Phasmatodea. With such a great claim, the reader could expect a careful exposition of theories and correct use of methods and techniques designed to explore in-depth the morphological, behavioral, and molecular data involved in this predator-prey system (e.g., Lin et al. 2016, Gao et al. 2018). Unfortunately, this was not the case. Instead, CEA relied on non-supported ideas to substantiate their unusual claim. CEA invoked HGT from a plant species to a Phasmatodea via a Pentatomidae predator with nonexclusive phytophagous habit. This argumentation gravely suffers from various issues. The first is that they argue that *S. cinctipes* need to feed on plants to complete its life-cycle. Nonetheless, the results of experimental research using only animal prey (e.g., *Tenebrio molitor* L., *Musca domestica* L.), comparing longevity, fertility, and other biological parameters (e.g., Beserra et al. 1995, Zanuncio et al. 1997, 2005, Oliveira et al. 2003) do not support this claim. *Supputius cincticeps* is indeed a generalist predator that may have an advantage in longevity when supplementing their diet with plant tissue (Zanuncio et al. 2004), although plant feeding is not necessary for its development. Furthermore, *S. cinctipes*, as well as all other asopines, are predatory species that cause the death of their prey during its feeding (Martínez et al. 2016, Walker et al. 2016), not fulfilling the goal of HGT process, which is the transfer of genetic material to another non-related organism and inserting these elements permanently. Therefore, the probability of *S. cinctipes* being a vector responsible for any HGT is very low.

HGT is the acquisition of genes from organisms other than a direct ancestor (Crisp

et al. 2015). These transfers are common within Bacteria, Archaea, and between them (e.g., Ochman et al. 2000, Gophna et al. 2004). HGT involving Eukarya is more uncommon than compared to prokaryotes (Syvanen 2012). Although it is a highly complex process, given that the transmitted genes must be introduced in germline cells (Blaxter 2007), successful HGT cases have been documented in Animalia (e.g., Moran & Jarvik 2010, Walsh et al. 2013), Plantae (e.g., Baidouri et al. 2014), and Fungi (e.g., Novikova et al. 2010). However, even genes successfully transmitted and integrated into an organism are not necessarily transcribed in the recipient cell (Nikoh et al. 2008). A few instances of HGT involving retroelements of plants and arthropods are known, involving genomic elements from arthropods to spermatophytes (Lin et al. 2016, Gao et al. 2018), as well as from plants or fungi into arthropods (e.g., Moran & Jarvik 2010, Altincicek et al. 2012, Zakharov 2016). In all instances of HGT involving arthropods, there is no consensus as to how the process was achieved (e.g., Wybouw et al. 2012), but always involved genes only with particular metabolic functions (Grbić et al. 2011, Wybouw et al. 2012, Nováková & Moran 2012). Thus, it is assumed that HGT in arthropods will correspond to very specific gene functions.

The most critical and flawed argument of CEA paper is that the camouflage exhibited by *C. phyllinus* is the result of HGT “of plant-derived genetic material leading to development of a form resembling a tree stem”. This assumption is highly problematic in several respects. First, very little is known about the control of plant morphology. A fundamental question in plant biology is how different plant phenotypes arise based on particular genetic information, and how the environment interacts with this information to produce distinct phenotypes. Although this question is just starting to be answered (e.g.,

Schlichting & Pigliucci 1993, Yang et al. 2014, Casacuberta et al. 2016, Gaudinier & Brady 2016, Honkanen et al. 2016), it is clear, as recent research suggests, that plant phenotypes are the result of polygenic control (Ogura & Busch 2016, Bucksch et al. 2017), and thus must be assumed that several loci are responsible in producing particular phenotypes on specific parts of plants. CEA did not provide a plausible explanation as to how all these genetic elements were transferred and inserted into the insect. This is also highly problematic because they failed to provide a theoretical underpinning that might help explain how the whole suite of genes involved in plant morphogenesis might adequately function in another organism with radically different genetic control mechanisms. Thus, the failure is twofold, lacking both empirical evidence and theoretical support that might allow other researchers to test these ideas.

Finally, CEA implicitly expand the HGT process to other Phasmatodea, wrongly assuming that all species in the order are twig-like, which is not the case. Phasmatodea exhibit various body types, resembling various plant forms such as twigs, leaves, or moss (Bradler & Buckley 2020), and thus are probably the result of various evolutionary selective pressures, as evidenced by the various phylogenetic patterns recovered (Whiting et al. 2003, Bradler et al. 2014, Robertson et al. 2018). If HGT is responsible for the twig-like appearance of *C. phyllinus*, then it should have been explained how other species of Phasmatodea also present similar plant-looking body types, another idea that was never properly discussed in their paper.

CONCLUSIONS

A single observation of *S. cinctipes* feeding to *C. phyllinus*, lacking additional empirical and

theoretical support, prevents to postulate HGT as the driving mechanism explaining the camouflage in this phasmatodean species. In a broad sense, scientific evidence is something that gives a scientist a good reason to consider a hypothesis true (Achinstein 2008), being this evidence filtered through a personal judgment and then interpreted as strong, weak, incomplete, redundant, inconclusive, plausible, and so on (Schum 2001). Scientific evidence gains its value not from using empirical data alone, but from how the evidence was produced, and to which theories the conclusions based on this evidence are compared to (Bogen 2017). The zenith of evolutionary biology is the building of narratives based on evidence. However, the mere concatenation of evidence in a coherent sequence attributing causality between them is not subject to confirmation or disconfirmation (Abbott 1992, Mink 1987). Regrettably, CEA paper lacks both empirical data and theoretical postulates to properly advance a scientific theory with regard to Phasmatodea camouflage. Finally, all this argumentation calls for a stronger peer-review process of our ideas submitted to scientific journals, in order to produce better science.

REFERENCES

- ABBOTT A. 1992. From causes to events. Notes on narrative positivism. *Sociol Method Res* 20: 428-455.
- ACHINSTEIN P. 2008. Evidence. In: Psillos S & Curd M (Eds), *The Routledge companion to philosophy of science*. London: Routledge, p. 337-348.
- ALTINCICEK B, KOVACS JL & GERARDO NM. 2012. Horizontally transferred fungal carotenoid genes in the two-spotted spider mite *Tetranychus urticae*. *Biol Letters* 8(2): 253-257.
- BAIDOURI MEL ET AL. 2014. Widespread and frequent horizontal transfers of transposable elements in plants. *Genome Res* 24(5): 831-838.
- BESERRA EB, ZANUNCIO TV, ZANUNCIO JC & SANTOS GP. 1995. Desenvolvimento de *Supputius cincticeps* (Heteroptera,

- Pentatomidae) alimentado com larvas de *Zophobas confusa*, *Tenebrio molitor* (Coleoptera, Tenebrionidae) e *Musca domestica* (Diptera, Muscidae). *Rev Bras Zool* 12: 725-733.
- BLAXTER M. 2007. Symbiont genes in host genomes: fragments with a future? *Cell Host Microbe* 2: 211-213.
- BOGEN J. 2017. Theory and observation in science. In: *The Stanford Encyclopedia of Philosophy* (Summer 2017 Edition), Zalta EN (Ed), Available at: <https://plato.stanford.edu/archives/sum2017/entries/science-theory-observation/>.
- BRADLER S & BUCKLEY TR. 2020. Biodiversity of Phasmatodea. In: Foottit RG & Adler PH (Eds), *Insect Biodiversity: Science and Society*, Hoboken: John Wiley & Sons, New Jersey, USA, p. 281-313.
- BRADLER S, ROBERTSON JA & WHITING MF. 2014. A molecular phylogeny of Phasmatodea with emphasis on Necrosiinae, the most species-rich subfamily of stick insects. *Syst Entomol* 39: 205-222.
- BUCKSCH A ET AL. 2017. Morphological Plant Modeling: Unleashing Geometric and Topological Potential within the Plant Sciences. *Front Plant Sci* 8: 900.
- CASACUBERTA JM, JACKSON S, PANAUD O, PURUGGANAN M & WENDEL J. 2016. Evolution of plant phenotypes, from genomes to traits. *G3: Genes, Genomes, Genetics* 6: 775-778.
- COSTA J, TORRES L, PROVANCE JR DW, BRUGNERA R & GRAZIA J. 2019. First report of predation by a stink bug (*Supputius cincticeps* Stål) on a walking-stick insect (*Cladomorphus phyllinus* Gray), with reflections on evolutionary mechanisms for camouflage. *Acta Biol Par* 48: 5-15.
- CRISP A, BOSCHETTI C, PERRY M, TUNNAcliffe A & MICKLEM G. 2015. Expression of multiple horizontally acquired genes is a hallmark of both vertebrate and invertebrate genomes. *Genome Biol* 16(1): 50.
- DE CLERCQ P. 2000. Predaceous Stinkbugs (Pentatomidae: Asopinae). In: Schaefer CW & Panizzi AR (Eds), *Heteroptera of Economic Importance*, Boca Raton: CRC Press, Florida, USA, p. 759-812.
- DIAS LIMA L & KAMINSKI LA. 2019. Camouflage. In: Vonk J & Shackelford T (Eds), *Encyclopedia of Animal Cognition and Behavior*, Springer, Cham, Switzerland, p. 1-9.
- GAO D, CHU Y, XIA H, XU C, HEYDUK K, ABERNATHY B, OZIAS-AKINS P, LEEBENSACK JH & JACKSON SA. 2018. Horizontal transfer of non-LTR retrotransposons from arthropods to flowering plants. *Mol Biol Evol* 35(2): 354-364.
- GAUDINIER A & BRADY SM. 2016. Mapping Transcriptional Networks in Plants: DataDriven Discovery of Novel Biological Mechanisms. *Annu Rev Plant Biol* 67: 575-594.
- GOPHNA U, CHARLEBOIS RL & DOOLITTLE WF. 2004. Have archaeal genes contributed to bacterial virulence? *Trends Microbiol* 12: 213-219.
- GRBIĆ M ET AL. 2011. The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature* 479(7374): 487-492.
- HONKANEN ET AL. 2016. The mechanism forming the cell surface of tip-growing rooting cells is conserved among land plants. *Curr Biol* 26: 3238-3244.
- KIRKLAND AH. 1898. The species of *Podisus* occurring in the United States. *Rept Mass St Bd. Agr* 45: 412-439, Appendix: 112-138.
- LIN X, FARIDI N & CASOLA C. 2016. An ancient transkingdom horizontal transfer of Penelope-like retroelements from arthropods to conifers. *Genome Biol Evol* 8(4): 1252-1266.
- MARTÍNEZ LC, FIALHO MDCQ, BARBOSA LCA, OLIVEIRA LL, ZANUNCIO JC & SERRÃO JE. 2016. Stink bug predator kills prey with salivary non-proteinaceous compounds. *Insect Biochem Mol Biol* 68: 71-78.
- MINK LO. 1987. Narrative form as a cognitive instrument. In: Fay B, Golub EO & Vann RT (Eds), *Historical understanding*, Ithaca: Cornell University Press, New York, USA, p. 182-203.
- MORAN NA & JARVIK T. 2010. Lateral transfer of genes from fungi underlies carotenoid production in aphids. *Science* 328(5978): 624-627.
- NIKOH N, TANAKA K, SHIBATA F, KONDO N, HIZUME M, SHIMADA M & FUKATSU T. 2008. *Wolbachia* genome integrated in an insect chromosome: evolution and fate of laterally transferred endosymbiont genes. *Genome Res* 18(2): 272-280.
- NOVÁKOVÁ E & MORAN NA. 2012. Diversification of Genes for Carotenoid Biosynthesis in Aphids following an Ancient Transfer from a Fungus. *Mol Biol Evol* 29: 313-323.
- NOVIKOVA O, SMYSHLYAEV G & BLINOV A. 2010. Evolutionary genomics revealed interkingdom distribution of Tcn1-like chromodomain-containing Gypsy LTR retrotransposons among fungi and plants. *BMC Genomics* 11(1): 231.
- OCHMAN H, LAWRENCE JG & GROLSMAN EA. 2000. Lateral gene transfer and the nature of bacterial innovation. *Nature* 405: 299-304.
- OGURA T & BUSCH W. 2016. Genotypes, networks, phenotypes: moving toward plant systems genetics. *Annu Rev Cell Dev Biol* 32: 103-126.

OLIVEIRA I, ZANUNCIO JC, SERRÃO JE & PEREIRA JMM. 2003. Reproductive potential of the predator *Supputius cincticeps* (Heteroptera: Pentatomidae) affected by female body weight. *Acta Sci Biol Sci* 25: 49-53.

ROBERTSON JA, BRADLER S & WHITING MF. 2018. Evolution of oviposition techniques in stick and leaf insects (Phasmatodea). *Front Ecol Evol* 6: 216.

SCHUM DA. 2001. The evidential foundations of probabilistic reasoning. Evanston: Northwestern University Press, 545 p.

SCHLICHTING CD & PIGLIUCCI M. 1993. Control of phenotypic plasticity via regulatory genes. *Amer Naturalist* 142: 366-370.

SKELHORN J, ROWLAND HM, SPEED MP & RUXTON GD. 2010. Masquerade: Camouflage Without Crypsis. *Science* 327(5691): 51.

SYVANEN M. 2012. Evolutionary implications of horizontal gene transfer. *Annu Rev Genet* 46: 341-358.

WALKER AA, WEIRAUCH C, FRY BG & KING GF. 2016. Venoms of heteropteran insects: a treasure trove of diverse pharmacological toolkits. *Toxins* 8(2): 1-32.

WALSH AM, KORTSCHAK RD, GARDNER MG, BERTOZZI T & ADELSON DL. 2013. Widespread horizontal transfer of retrotransposons. *PNAS* 110(3): 1012-1016.

WHITING M, BRADLER S & MAXWELL T. 2003. Loss and recovery of wings in stick insects. *Nature* 421: 264-267.

WYBOUW N, BALABANIDOU V, BALLHORN DJ, DERMAUW W, GRBIĆ M, VONTAS J & VAN LEEUWEN T. 2012. A horizontally transferred cyanase gene in the spider mite *Tetranychus urticae* is involved in cyanate metabolism and is differentially expressed upon host plant change. *Insect Biochem Mol Biol* 42: 881-889.

YANG W ET AL. 2014. Combining high-throughput phenotyping and genome-wide association studies to reveal natural genetic variation in rice. *Nat Commun* 8(5): 5087.

ZAKHAROV IA. 2016. Horizontal gene transfer into the genomes of insects. *Russ J Genet* 52: 702-707.

ZANUNCIO JC, LACERDA MC, ZANUNCIO JUNIOR JS, ZANUNCIO TV, SILVA AMC & ESPINDULA MC. 2004. Fertility table and rate of population growth of the predator *Supputius cincticeps* (Heteroptera: Pentatomidae) on one plant of *Eucalyptus cloeziana* in the field. *Ann appl Biol* 144: 357-361.

ZANUNCIO JC, TORRES JB, BERNARDO DL & DE CLERCQ PD. 1997. Effects of prey switching on nymphal development of four species of predatory stink bugs. *Mededelingen Faculteit Landbouwkundige en Toegepaste Biologische*

Wetenschappen Universiteit Gent (Belgium) 62(2): 483-490.

ZANUNCIO JC, BESERRA EB, MOLINA-RUGAMA AJ, ZANUNCIO TV, PINON TBM & MAFFIA VP. 2005. Reproduction and longevity of *Supputius cincticeps* (Het.: Pentatomidae) fed with larvae of *Zophobas confusa*, *Tenebrio molitor* (Col.: Tenebrionidae) or *Musca domestica* (Dip.: Muscidae). *Braz Arch Biol Technol* 48(5): 771-777.

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