

REPLY TO SOLEY:

DNA methylation marks are stably transferred across generations in honey bees

Boris Yagound^{a,b,1}, Emily J. Remnant^a, Gabriele Buchmann^{a,c}, and Benjamin P. Oldroyd^{a,d}

Soley (1) argues that our study (2) does not prove that “transgenerational [epigenetic] inheritance serv[es] an adaptive role in evolution” in honey bees. This view misrepresents our intent. Our main findings are that in honey bees 1) DNA methylation profiles are much more similar between fathers and daughters than between unrelated males and females of different generations and 2) DNA methylation profiles are conserved across somatic and germline tissues (2). We are agnostic as to whether DNA methylation marks are caused by environmental or genetic factors. Rather, we stress that DNA methylation patterns are conserved across generations.

Soley misrepresents our main findings by stating that “only a fraction of the methylated regions was considered to have been thus transferred to the following generation” (1), referring to “the proportion of methylated CpGs that were inherited specifically from fathers to daughters [being] between 18 and 29%.” These figures are correct, yet they only refer to a small portion of the methylome that has informative polymorphisms for which it is possible to ascertain parental origin. In fact, $85 \pm 3\%$ of DNA methylation sites have an identical status across generations (2).

Soley argues that sequence polymorphism is probably the key determinant of the DNA methylation state in honey bees (1). We concur, as already highlighted in some of our previous papers (3, 4).

Soley further questions the absence of epigenetic reprogramming in honey-bee embryos (1). We agree that our findings leave open the possibility that DNA methylation marks are erased and reapplied in a genotype-specific manner each generation. Yet, recent studies have clearly shown that there is no remodeling of DNA methylation during development in honey bees (5, 6). We thus think that epigenetic reprogramming is highly unlikely in honey bees, and probably in many other nonmammalian organisms (7).

Finally, we welcome Soley’s points on future directions needed to fully ascertain the presence of transgenerational epigenetic inheritance in bees (1), as we state in our own conclusions: “Whether [transgenerational epigenetic inheritance] processes are adaptive or able to influence macroevolution remains unclear. . . . It is thus more than ever necessary to uncover the molecular consequences of DNA methylation if we are to understand its evolutionary implications” (2).

- 1 F. G. Soley, Still no evidence for transgenerational inheritance or absence of epigenetic reprogramming in the honey bee. *Proc. Natl. Acad. Sci. U.S.A.*, 10.1073/pnas.2108608118 (2021).
- 2 B. Yagound, E. J. Remnant, G. Buchmann, B. P. Oldroyd, Intergenerational transfer of DNA methylation marks in the honey bee. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 32519–32527 (2020).
- 3 B. Yagound, N. M. A. Smith, G. Buchmann, B. P. Oldroyd, E. J. Remnant, Unique DNA methylation profiles are associated with cis-variation in honey bees. *Genome Biol. Evol.* **11**, 2517–2530 (2019).
- 4 E. J. Remnant et al., Parent-of-origin effects on genome-wide DNA methylation in the Cape honey bee (*Apis mellifera capensis*) may be confounded by allele-specific methylation. *BMC Genomics* **17**, 226 (2016).
- 5 X. Xu et al., Evolutionary transition between invertebrates and vertebrates via methylation reprogramming in embryogenesis. *Natl. Sci. Rev.* **6**, 993–1003 (2019).
- 6 K. D. Harris, J. P. B. Lloyd, K. Domb, D. Zilberman, A. Zemach, DNA methylation is maintained with high fidelity in the honey bee germline and exhibits global non-functional fluctuations during somatic development. *Epigenetics Chromatin* **12**, 62 (2019).
- 7 A. de Mendoza, R. Lister, O. Bogdanovic, Evolution of DNA methylome diversity in eukaryotes. *J. Mol. Biol.* **432**, 1687–1705 (2019).

^aBehaviour, Ecology and Evolution Laboratory, School of Life and Environmental Sciences, University of Sydney, Sydney, NSW 2006, Australia;

^bEvolution and Ecology Research Centre, School of Biological, Earth and Environmental Sciences, University of New South Wales, Sydney, NSW 2052, Australia; ^cCentre for Plant Genome Engineering, Institute of Plant Biochemistry, Heinrich-Heine-Universität Düsseldorf, 40225 Düsseldorf, Germany; and ^dWissenschaftskolleg zu Berlin, 14193 Berlin, Germany

Author contributions: B.Y., E.J.R., G.B., and B.P.O. wrote the paper.

The authors declare no competing interest.

Published under the [PNAS license](#).

¹To whom correspondence may be addressed. Email: boris.yagound@sydney.edu.au.

Published July 6, 2021.