

No evidence for extensive horizontal gene transfer from the draft genome of a tardigrade

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Through a draft genome sequencing of a tardigrade *Hypsibius dujardini*, Boothby et al. (1) report that “Approximately one-sixth of the genes in the tardigrade genome were found to have been acquired through horizontal transfer, a proportion nearly double the proportion of previous known cases of extreme horizontal gene transfer (HGT) in animals.” However, the authors also state that the “cultures are not axenic,” which means that they are highly prone to contamination. Koutsovoulos et al. (2) and Delmont and Eren (3) quickly responded, by careful review of the published sequence data, that there are significant amounts of bacterial contamination in the draft genome reported by Boothby et al., and that the rate of HGT is therefore exaggerated. Even a quick inspection of the *k*-mer distribution of the sequenced reads of Boothby et al. reveals that the graph actually shows two peaks, which is suggestive of a nonnegligible level of contamination.

We have been sequencing the same strain of *H. dujardini* using ultralow input library preparation technologies to overcome such problems of contamination. With our protocol, we can obtain about 50 pg of genomic DNA and about 100 pg of total RNA from a single individual of *H. dujardini*, which is sufficient for highly sensitive library preparation protocols (4, 5). All animals are starved and incubated with penicillin/streptomycin antibiotic mixture for 48 h to remove gut contents and microbes, and are

further washed multiple times and checked for the lack of microbes on the surface of animals under a microscope before sampling.

By mapping our single-individual genome sequence reads and 35 RNA-Seq data (active and tun states of adults, first 1 d, 2 d, 3 d, 4 d, and 5 d of eggs after laying, first 1 d, 2 d, 3 d, 4 d, and 5 d after hatching, encompassing various developmental stages), we found that as many as 7,135 contigs (31.7% of all contigs), including the longest 11 contigs, are contaminated under a rather conservative estimate (less than $\times 1$ coverage in genomic reads, or without even a single hit of RNA-Seq reads from any of the 35 datum). Only 1,771 putative HGT genes remain after removing these contigs, and their percentage within the genome, 4.47%, is in line with other eukaryotic genomes.

H. dujardini is a hygrophilic and hydrophilic tardigrade, which is not exclusively aquatic but is often found in freshwater habitats. Thus, the anhydrobiotic capabilities and extremotolerance of this species are not very significant compared with other anhydrobiotic animals. Therefore, extensive incorporation of foreign genes in the process of anhydrobiosis is also doubtful from such a perspective.

The rapid counterarguments presented by Koutsovoulos et al. and Delmont and Eren are perfect examples of the success of open science. The availability of the sequencing data allowed the rapid reanalysis.

- 1 Boothby TC, et al. (2015) Evidence for extensive horizontal gene transfer from the draft genome of a tardigrade. *Proc Natl Acad Sci USA* 112(52):15976–15981.
- 2 Koutsovoulos G, et al. (2016) No evidence for extensive horizontal gene transfer in the genome of the tardigrade *Hypsibius dujardini*. *Proc Natl Acad Sci USA* 113(18):5053–5058.
- 3 Delmont TO, Eren AM (2016) Identifying contamination with advanced visualization and analysis practices: Metagenomic approaches for eukaryotic genome assemblies. *PeerJ* 4:e1839.
- 4 Rykalina VN, et al. (2014) Exome sequencing from nanogram amounts of starting DNA: Comparing three approaches. *PLoS One* 9(7): e101154.
- 5 Picelli S, et al. (2014) Full-length RNA-seq from single cells using Smart-seq2. *Nat Protoc* 9(1):171–181.

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Data deposition: All of the data used for the analysis stated in this letter are also deposited in public repositories (DDBJ-DRA: DRA004455; Figshare: https://figshare.com/articles/Validation_of_assembled_contigs_of_Hypsibius_dujardini_genome_of_Boothby_et_al_using_ultra_low_input_sequencing/2300251/1, https://figshare.com/articles/K_mer_distribution_plot_of_Boothby_et_al_Illumina_short_reads_500bp_insert_/2300053/1).

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