Specific responses to Arakawa, 2016

We thank Dr. Arakawa for contributing his time, expertise, and data to this scientific discussion. Below we provide feedback on and discussion of the approaches and interpretations presented by Dr. Arakawa in the hopes that they can help move this research forward.

Indeed, analysis of k-mer distribution as shown in (1) results in a curve with 2 peaks. However, this pattern, two peaks with one of approximately half the multiplicity of the other, is traditionally viewed as evidence of heterozygosity (*e.g.* SNPs), not as a sign of contamination (2, 3) (Fig. 7). Gene duplication and/or repetitive regions can also contribute to such a pattern. Indeed, an inspection of mapped reads from Dr. Arakawa's, the Blaxter group's, and our sequencing project reveals that there are many loci within the *H. dujardini* genome with clear signs of heterozygosity (Fig. 8). While Arakawa's dataset (generated from a single tardigrade, not a population) also identifies some of these SNPs, it does not identify all of them (Fig. 8). We are thankful to Dr. Arakawa for providing his datasets, which allowed us to distinguish between individual and population level heterozygosity.

Arakawa also questions our speculation that desiccation-rehydration cycles may play a role in the acquisition of foreign genes, stating that he believes this species does not have a very significant anhydrobiotic capability. However, since 1989 the field has known that *H. dujardini* – like all other anhydrobiotic tardigrades – tolerates desiccation at low relative humidity only after preconditioning (4), a fact reconfirmed in a recent study (5). This species also survives other abiotic stresses such as freezing and irradiation (6, 7). As sequencing resources from other tardigrade species become available, it will be interesting to assess the degree to which HGT correlates with the propensity of different species' native habitats to undergo drying as has been seen in rotifers (8).

We acknowledge (9) there is contamination in our assembly. However, we disagree that lack of RNAseq read mapping alone is sufficient to identify contaminants. It is well documented in the literature that many HGT genes are expressed at low levels or in some cases not expressed at all. For example, it is known that essentially the entire *Wolbachia* genome has been transferred into the genome of *Drosophila ananassae,* yet the same study found that only ~2% (28/1206) of these horizontally acquired genes are transcribed at detectable levels (10). Follow-up investigations confirmed the extensive HGT into the nuclear genome of this *Drosophila* species, but failed to detect biologically relevant expression of any foreign genes (11, 12). Thus, while identification of an expressed foreign gene could be viewed as evidence in favor of HGT, lack of expression alone is not a criterion for disproving HGT. While we speculated that HGT might be important for tardigrade biology, such analysis was beyond the scope of our original paper (13), and thus our analysis was restricted to examination of HGT rather than functional HGT. New datasets from the community are now allowing us to assess the potential function of these genes. There are many scaffolds in our assembly that Dr. Arakawa excludes on the basis of lacking RNAseq data that show coverage with his genomic reads. If one sums the number of scaffolds from our postfiltered assembly without genomic and RNAseq coverage (based on Arakawa's analysis), one identifies 419 (2.6%) scaffolds containing 382 foreign genes, as opposed to the 7,135 (31.7%) scaffolds and 4,892 foreign genes initially proposed by Dr. Arakawa.

Importantly, mapping of Dr. Arakawa's datasets against two independent *H. dujardini* genome assemblies independently confirms the majority of scaffolds containing foreign genes within those assemblies (Fig. 5). In these independent assemblies, the levels of foreign genes identified using 3 different metrics are elevated compared with 'typical' animals (Figs. 1&2).

We are grateful to Dr. Arakawa for his analysis, time, and shared resources. Mapping his reads, which were generated from single tardigrades treated with antibiotics, starved, washed, and visually inspected for signs of contamination, to independent *H. dujardini* assemblies is strong evidence that the majority of foreign genes in these assemblies (Figs. 1&2) are not contaminants. The single tardigrade sequencing pioneered by Dr. Arakawa will be valuable in studying the biology of tardigrades.

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