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Comparative Genomic Analysis of 31 Siphoviridae Reveals Extensive Host-Dependent Relationships and Novel Phage Subclusters

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Bacteriophages comprise the most abundant group of biological entities on the planet. As a ubiquitous feature of the Earth’s various ecosystems, this class of organisms is estimated to outnumber all other organisms on the planet combined. If we are to fully harness the current momentum in phage research, we must continue to investigate evolutionary relatedness among phages. In this study, we hypothesized that host evolutionary processes may govern patterns in phage relatedness; as such, we predicted that phage genetic similarities resulting from coevolutionary pressures would reveal the intricate relationships which exist among phages, allowing for improved understanding of the bacteriophage pangenome. In our preliminary analysis, the *Gordonia* terrae-infecting phage Tanis was selected for genome annotation. Phage Tanis is a member of phage cluster DJ, a relatively new addition to the phage clustering system: of the 14 member phages that have been discovered since cluster DJ’s inception, 50% have been isolated in the past year alone. Thus, it is unsurprising that 76.9% of genes identified during manual annotation possessed no known function. Comparative genomic analyses were performed using 21 additional *Siphoviridae* and their hosts (phages were isolated on hosts belonging to 2 phyla, 3 genera, and 11 species of bacteria). Phage and host phylogenies confirmed expected patterns of phage- and host-relatedness. Codon usage analyses revealed that phages with similar codon biases tended to infect hosts belonging to the same genera. Dot plots illustrating phage nucleotide similarity also revealed extensive regions of conserved nucleotide content, with increased statistical noise among phages infecting the same host; specifically, Actinobacteriophages exhibited genetic similarities distinct from other phage groups. Trends in average nucleotide identity (ANI) further supported the relationships observed during phage dot plot analyses; however, various patterns in ANI values challenged the current organization of several phage clusters, prompting an expanded investigation which included all known cluster DJ phages. Notably, our expanded ANI and core gene analyses provided evidence for the subdivision of cluster DJ, potentially calling for the creation of up to 4 novel DJ subclusters. Overall, the predominance of genes with unknown function in the Tanis genome demonstrated the need for continued research on phage biology. Initial comparative analyses supported a mechanism for phage-host coevolution, wherein the evolutionary distance between phages was directly proportional to the evolutionary distance between hosts. ANI analysis supported qualitative measures of phage relatedness and suggested the reorganization of cluster DJ phages into discrete subclusters. In sum, our individual and comparative genomic analyses provided insight into the complexity of this growing field, while demonstrating the need for ongoing reevaluations of phage relatedness.