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The Mystery of the Disappearing Arthrobacterphage and the Analysis of Chipper1996 and Zorro, New Members of the AR and AK Clusters

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During the fall of 2016 the Bucknell University Phage Hunters were happily isolating and purifying bacteriophage using *Arthrobacter* sp (ATCC 21022) as the host bacterium when all of a sudden the bacterial cultures became resistant to infection with any of the phage that had been identified. After trying various media formulations, growth temperatures and growth conditions to no avail, a new *Arthrobacter* sample was purchased from ATCC. Even the new cells appeared to be resistant to infection with all of the phage that had been isolated except Chipper1996 and Cheddar. Genomic DNA was purified from these two phage, along with Zorro which had been isolated in fall 2015, and submitted for sequencing. Genome sequences of Chipper1996 and Cheddar were identical and presumably represent contamination of phage between lab groups. Chipper is in the *Myoviridae* family, AR cluster and has a genome of 70090 bp. It is one base shorter than Tophat, which was isolated in summer 2015 at Bucknell. This one base deletion causes a frameshift mutation and results in gene 60 being split into two open reading frames, suggesting the protein product of this gene is not essential for lytic infection. Chipper encodes 112 genes with one block of five reverse genes. Several of these genes encode proteins with putative DNA modification functions, such as methylases and endonucleases. Zorro is in the *Siphoviridae* family, AK cluster and has a genome of 43562 bp. It has 7 base differences when compared to Dino, also isolated at Bucknell in fall 2015. These 7 differences, which are mostly purine transitions, change four amino acids in four separate genes. The less conservative changes are in genes near the end of the genome and are likely to code for non-essential functions. While the 43 genomes in the AK cluster are relatively similar to each other, 25 contain three genes at the 5’ end not found in the remaining 18 genomes. These 25 genomes also contain a different minor tail gene than the other phage. Zorro encodes 62 putative genes with a single block of 5 reverse genes. There are several interesting genes including gp49 that codes for a putative adenylosuccinate synthetase enzyme, which is involved in nucleotide metabolism. This gene is conserved in all of the AK genomes and in the genome of Ghobes, a singleton isolated from *Gordonia*. In addition, there is a block of genes (gp46-49) that encode possible zinc binding proteins. MEME identified several DNA motifs in both genomes that may be associated with transcriptional and/or translation start sites in the genomes of these phage. Further analysis of these repeats may determine if they are significant regulatory sequences for either of these phage.