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REVIEW ARTICLE

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Circular Genome Visualization and Exploration using Genome Graph Techniques

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ABSTRACT

Genome graphs can represent genetic variation and sequence uncertainty. Aligning sequences to genome graphs is the key to many applications, including error correction, genome assembly, and genotyping of variants in a pangenome graph. Genetic mapping uses the Mendelian principles of segregation and recombination to determine the relative proximity of DNA markers along the chromosomes of an organism. Genetic markers are important developments in the field of plant breeding. The CG View (Circular Genome Viewer) software family is a popular collection of tools for generating genome maps for bacteria, organelles and viruses. In this review, we describe the capabilities of the original CG View program along with those of subsequent companion applications, including the CG View Server and the CG View Comparison Tool. We also discuss G View, a graphical user interface-enabled rewrite of CG View, and the G View Server, which offers several integrated analyses for identifying shared or unique genome regions relative to comparison genomes. In this work I present and conclude with some remarks about our current development efforts related to CG View aimed at adding new functionality while increasing ease of use.

Keywords: *Circular Genome Viewer (CGV), Genome Graph, Structural genotyping and Genetic maps.*

INTRODUCTION

Graphs provide a natural way of expressing variation or uncertainty in a genome. They have been used for diverse applications such as genome assembly. Error correction, short tandem repeat genotyping, structural variation genotyping and reference-free haplotype reconstruction. With the growing usage of graphs, methods for handling graphs efficiently are becoming a crucial requirement for many applications. What is Plant Genome Mapping? Genome mapping produces fingerprints of DNA sequences to construct a physical map of the whole genome. Recent advances in genome-mapping technology will better allow researchers to detect large (>1kbp) structural variations between plant genomes.

Genetic maps are constructed using populations that segregate for variants between loci. Consequently, the marker density of genetic maps is limited by the level of polymorphism measurable in each considered population. Whenever shared markers are identified, genetic maps can be compared and potentially merged into a consensus map. The C Map program enables common molecular markers between genetic maps to be readily identified and linked (Fang et al., 2003; Duran et al., 2010). Join Map has been developed to construct consensus maps from multiple genetic maps (Stam, 1993). Both programs were successfully used for the construction of the first consensus maps of wheat (Somers et al., 2004) and barley (Varshney et al., 2007). By bringing together collections of molecular markers, consensus maps increase the overall number of markers available for molecular breeding and genetic analysis. These maps also linked the first Restriction Fragment Length Polymorphism (RFLP) genetic maps to the more recent PCR marker maps, which are more suitable for high through-out screening of germplasm in breeding programs. Consensus maps can also be used for the analysis of QTL. The Meta QTL method uses consensus models to merge distinct genetic maps and QTL datasets (Veyrieras et al., 2007). The intra species synteny increases the probability of identifying true QTL by integrating information from various genetic sources and multiple sets of phenotypic data.

CG View (<http://wishart.biology.ualberta.ca/cgview/>) is a Java program developed in 2005 as a tool for generating high-quality, navigable maps of circular genomes (Stothard and Wishart, 2005). Originally intended for bacterial genomes, it has proven to be popular for organelle genomes as well. CG View supports a custom XML (Extensible Markup Language) input format for describing the contents and appearance of a map, which the program then converts into graphical format. Bitmap images (PNG or JPG) or vector-based output in SVG (Scalable Vector Graphics) format can be generated. The SVG format offers advantages for image editing and printing, but the file size can be problematic when complex maps are generated. The contents of a sample XML file and the resulting map generated by CG View are shown in Figure 1. Simpler input formats are supported, which allow the positions of genes to be described, but which offer less control over how the information is displayed. Also provided is a well-documented API (application program interface), which allows the CG View Java code to be used in other applications. For example, the BRIG program (Alikhan et al., 2011) uses CG View code and its associated API to generate maps displaying the results of sequence similarity comparisons between a bacterial genome of interest and other genomes.

CG View converts the contents of an XML file (A) into a graphical map (B). The XML describes general characteristics of the map (height, width and font styles and sizes, for example) as well as the features that are to be depicted. Each feature can have one or more ranges drawn on the map, the positions of which are described using 'feature Range' elements. Features are grouped by 'feature Slot' elements, which represent rings on the

graphical map. A full description of the XML format and additional examples is available on the CG View Web site.

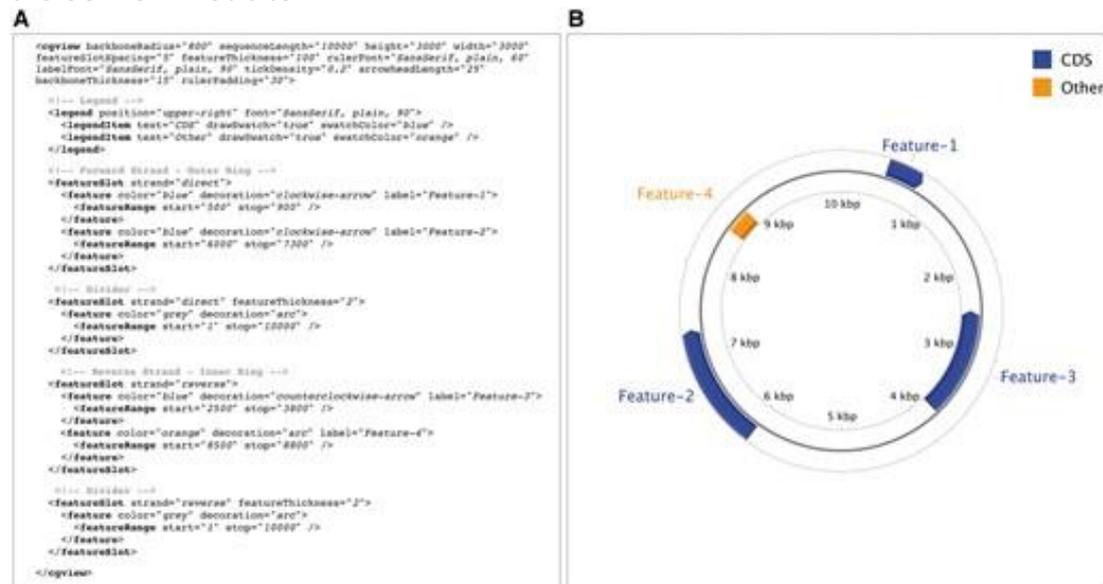


Figure 1 a.

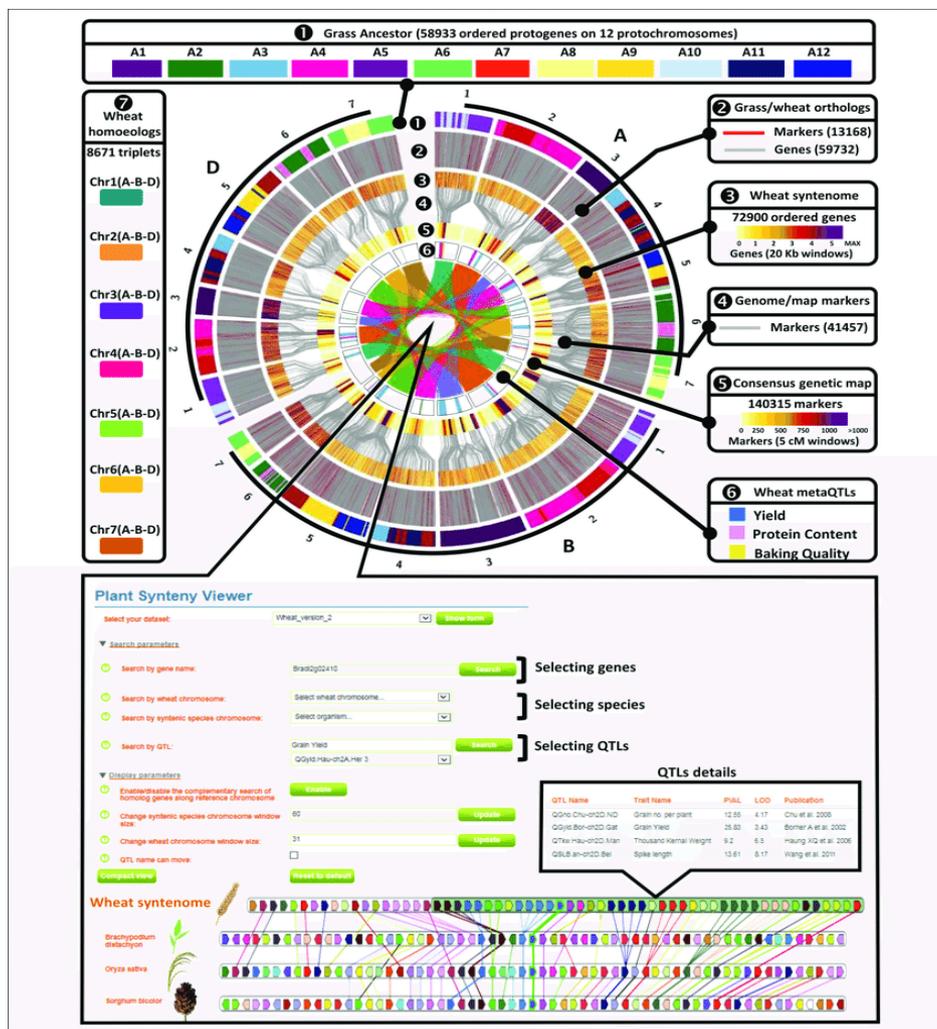


Figure 1 b.

The polyploid wheats are tetraploid (4 sets of chromosomes, $2n=4x=28$), or hexaploid (6 sets of chromosomes, $2n=6x=42$). The tetraploid wild wheats are wild emmer, T.. Genetic analysis has shown that the original hexaploid wheats were the result of a cross between tetraploid domesticated wheat, such as T.

The CG View application remains a popular genome visualization tool; however, the process of creating a map is intimidating and laborious for many users. First, CG View is a command-line tool, meaning that users must enter specific commands and options in a command-line environment. Although command-line interfaces offer important advantages, many users understandably prefer an intuitive graphical user interface provided via a Web server or stand-alone application. Second, CG View itself does not identify sequence features or perform sequence analyses. Instead, the user must use other software to identify features of interest and then provide that information in a format that CG View supports. Although the CG View application download includes documentation on how to do this as well as a script for converting Gen Bank or EMBL files into an XML file suitable for CG View, many users are likely to lack the experience or inclination to build the required input files.

Map creation simplified: the CG View Server

CG View's command-line interface and API allow it to be easily incorporated into more capable visualization tools or pipelines. One such tool is the CG View Server (http://stothard.afns.ualberta.ca/cgview_server/), a Web server released in 2008 that offers a convenient interface to CG View and has built-in analysis capabilities [10]. For example, up to three sequence data sets (protein or DNA) can be uploaded to the server, along with the primary genome sequence of interest (also termed the 'reference sequence'). BLAST is used to compare the reference sequence with each of the uploaded data sets, and the results are included on the map. If the reference sequence is uploaded in Gen Bank or EMBL format, feature information is extracted and also displayed. A variety of other feature types can be identified or calculated and displayed (open reading frames, start and stop codons, GC content and GC skew) or uploaded in a simple tab-delimited format. Internally, the CG View Server generates the map using CG View and an XML input file that it builds according to the user-supplied options, information extracted from the reference sequence and the BLAST analysis results. Users can choose to have the server return a map displaying the entire sequence, or, thanks to the zooming capabilities of CG View, a portion of the genome at an expanded size (Figure 2).

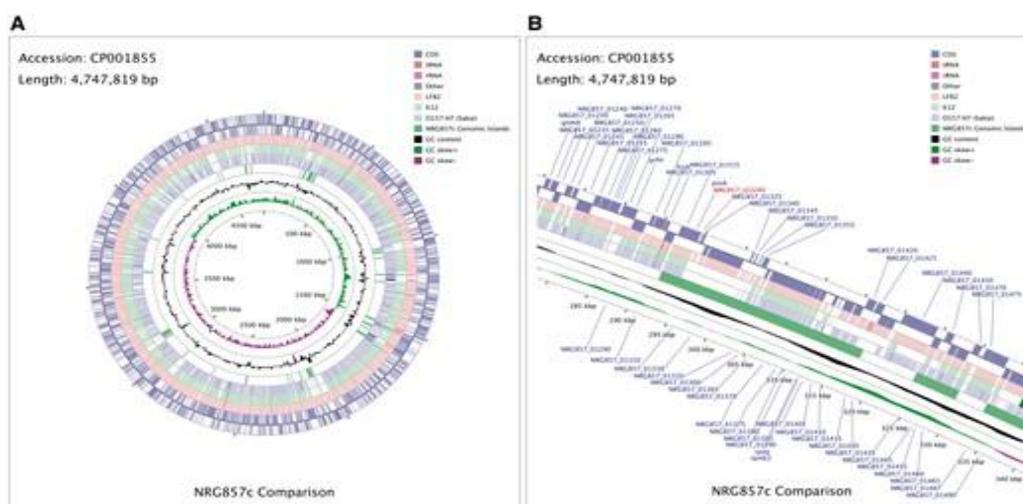


Figure 2.

A map generated using the CG View Server, showing a full view of the genome (A) and an expanded view of a region of interest (B). The contents of the feature rings (starting with the outermost ring) are as follows: Ring 1 and Ring 2 depict features from the forward and reverse strands, respectively, read from the primary sequence file (*E. coli* NRG857c; accession: CP001855); Ring 3 (*E. coli* LF82; accession: CU651637); Ring 4 (*E. coli* K12 substr. MG1655; accession: NC_000913) and Ring 5 (*E. coli* O157:H7 str. Sakai; accession: NC_002695) shows BLAST comparison results (BLASTN) with the primary sequence; Ring 6 shows putative NRG857c genomic islands indicative of horizontal gene transfer [11]; Ring 7 shows GC content; and Ring 8 shows GC skew. The BLAST comparison results are drawn at partial opacity—darker regions indicate the presence of multiple hits to the corresponding portion of the reference sequence.

Some notable limitations of the CG View Server include support for only three comparison sequence data sets, the absence of vector-based output and reduced control over the appearance of maps compared with when the CG View application is used directly. The sequence comparison limitation exists to reduce the workload for the server. Vector-based output, SVG in the case of CG View, is not supported because the resulting files can be too large to send by e-mail even when compressed (the CG View Server sends the final map to the e-mail address supplied by the user). Instead, all maps are 3000 × 3000 pixels in PNG format. Finally, the reduced control over map appearance is the result of providing a simplified Web interface for controlling map appearance that does not provide the full flexibility of the XML format used by CG View.

Comparing thousands of genomes using the CG View Comparison Tool

Following the release of the CG View Server, we frequently received requests from users of the server who wanted maps to be modified. Typical requests included the addition of further comparison data sets, the changing of font sizes or feature colors, the creation of larger maps and the labeling of specific genes or features of interest. Over time, we developed a software pipeline that we used to handle these requests. This pipeline consists of a variety of scripts to build or modify complex maps potentially involving thousands of sequences. Eventually, we released this pipeline as the CG View Comparison Tool (CCT) (<http://stothard.afns.ualberta.ca/downloads/CCT/>) (Grant et al., 2012).

Although CCT is a command-line tool, its use is simplified through wrapper scripts that automate the map building process—creating a map can involve a few simple commands. For example, the map comparing *Escherichia coli* O157:H7 str. Sakai with 100 additional *E. coli* genome sequences (Figure 3) required four simple commands to generate (one to download the primary sequence; one to start a map project; one to download the comparison sequences; and one to complete the map). The last command generates several maps automatically, differing in terms of size and level of detail, as well as in terms of how the BLAST comparisons are done (at the nucleotide level or at the level of translated coding sequences). The maps depicting translated coding sequence comparisons also, by default, display COG (Cluster of Orthologous Groups) classifications, generated through the use of a COG sequence database [Tatusov et al., 2003]. The contents and appearance of the maps can be changed from the default settings using a simple configuration file present in the map project directory, and by using command-line options. Additional custom feature types can be shown, as described in the tutorials section of the CCT Web site.

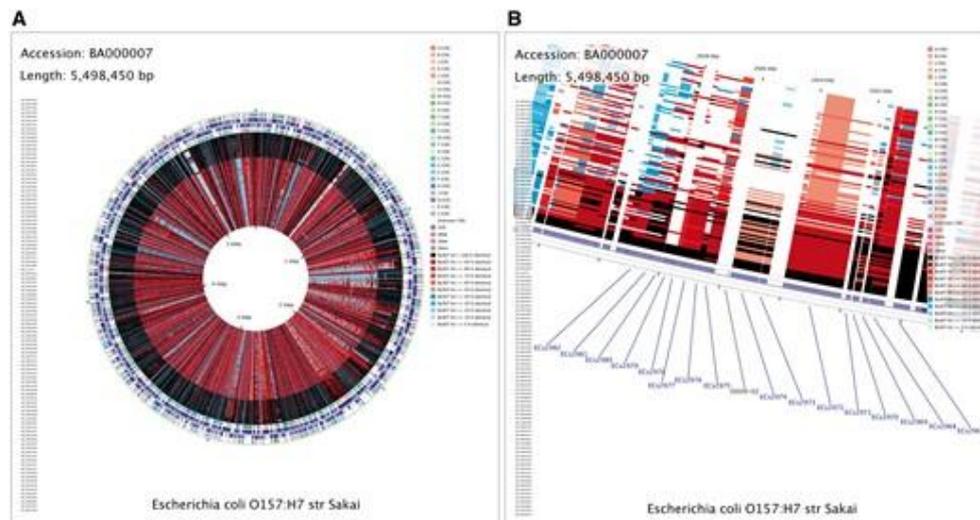


Figure 3.

CCT map comparing *E. coli* O157:H7 str. Sakai (accession BA000007) with 100 additional *E. coli* genome sequences. A full-genome view (A) and zoomed view (B) are shown, with the latter centered on Shiga toxin I subunit A and B genes, labeled as ECs2974 and ECs2973, respectively. The contents of the feature rings (starting with the outermost ring) are as follows: Ring 1: COG functional categories for forward strand coding sequences; Ring 2: forward strand sequence features; Ring 3: reverse strand sequence features; Ring 4: COG functional categories for reverse strand coding sequences. The next 100 rings show regions of sequence similarity detected by BLAST comparisons conducted between CDS (coding DNA sequence) translations from the reference genome and those from 100 *E. coli* comparison genomes.

CCT is well suited to the analysis of large collections of complete or partial genomes generated through high-throughput sequencing, as thousands of comparison genomes can be displayed on a single map simply by placing each of their respective contig files (in Gen Bank, EMBL or FASTA format) in the 'comparison genomes' directory of a CCT project. Metagenomes can be included as comparison genomes, in which case the map will serve to indicate which portions of the reference genome are similar to sequences in the metagenomes. In some scenarios, it may be of interest to generate many different maps, using a variety of reference genomes. CCT, by virtue of its command-line interface, can be run repeatedly from a script to generate separate maps for a group of reference sequences of interest. Alternatively, users can use the included 'build_blast_atlas_all_vs_all.sh' script. This script generates a separate map for each genome in a group of completed genomes, comparing the genome to all the others in the collection. The advantage of generating multiple maps in this manner is that the non-conserved regions of each genome can be found as regions lacking BLAST hits when the genome serves as the reference.

G View: CG View reloaded

CG View, the CG View Server and CCT are popular research and visualization tools, as they provide the means to generate the kinds of circular genome maps most often desired by the research community while largely shielding the end user from the computational complexity required for their construction. At the time of its original development, the available processing power in most commodity computing hardware limited CG View to the generation of static output, thus limiting its interactivity. Over time, the processing power in

most personal computers had improved to the point that the development of CG View capable of user interaction, such as panning and zooming, seemed plausible. This motivated us to redevelop CG View as a stand-alone Java application that could quickly render and allow the end user to interact with even large genome maps in real time.

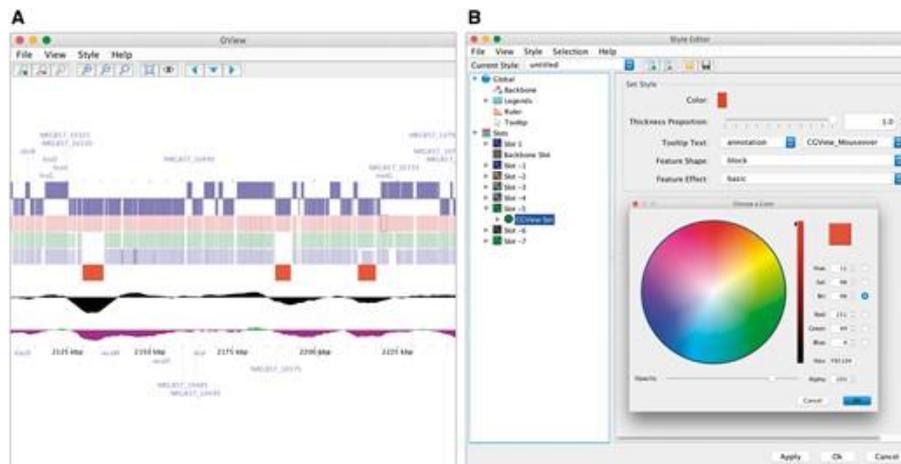


Figure 4.

A genome map (A) drawn by G View from an XML file created by the CG View Server. The G View Style Editor (B) allows numerous properties of the map to be adjusted interactively.

Table 1. Genome comparison analyses supported by the G View server.

G View analysis	Description
BLAST Atlas ^a	Displays the regions of similarity between a reference genome and one or more query genomes
Core Genome ^a	Displays only the regions of similarity shared among all genomes (reference plus query genomes)
Accessory Genome ^a	Displays regions found within the reference and query files, but absent from the core genome
Unique Genome ^a	Displays regions unique to the reference genome, i.e. displays only the regions in the reference genome that are not found any of the query genomes
Signature Genes ^a	Displays genes that are unique to a user-defined inclusion genome group (including the reference genome) in comparison with the user-defined exclusion genome group
Pan-genome ^a	Displays the full genomic content of a group of genomes. Regions absent from the reference genome but present in any of the other genomes are grouped and appended to the end of the reference sequence, with no attempt to preserve synteny
Reciprocal BLAST ^b	Displays possible orthologous regions between two genomes
Custom Analysis ^c	Performs logical operations on a set of BLAST results between a two or more genome sequences and displays the results

G View (<https://www.gview.ca>) is a complete rewrite of the CG View program that preserves the aesthetic of the original and many of its most popular features [Petkau et al., 2010]. G View fully supports the CG View XML format, has a public API that facilitates its incorporation into other Java programs and has a command-line interface that allows it to be run from scripts. G View also introduces a number of new features that address the main limitations of CG View. Aside from its real-time interactivity, the most notable new feature is the addition of linear maps. The linear map view is often used to depict zoomed-in regions of the genome, where the features are clearly defined, and can be helpful to convey the comparative synteny of those features in multiple genomes. Another major enhancement is the addition of the Style Editor (Figure 4). This utility allows users to customize nearly every aspect of their maps including the backbone, legends, ruler, features, labels and plots. Customizations can be separately saved as a 'genome style sheet', similar in concept and design to the Cascading Style Sheets format used to apply styles to Web pages. Other features implemented in G View include parallel processing capability, a 'birds-eye-view' to keep track of zoomed in views relative to the full map, stabilized label placement when zooming in and out of maps and compatibility with the Gen Bank, EMBL and GFF3 genomic file formats standardly used to record information about protein-coding sequences, ribosomal RNA, transfer RNA and other genomic features that are commonly displayed on circular genome maps.

Like CG View, G View has a companion Web application, the G View Server (<http://server.gview.ca>), to assist users in performing commonly desired comparative genomics analyses and rendering the corresponding genome maps (Figure 5). Users supply sequence files corresponding to a reference genome and optional comparison genomes, and an analysis type. The available analysis types are listed and described in Table 1. The G View Server then guides the user through the process of performing the specified analysis and customizing the initial style for the map. In-line help and tooltips are provided for nearly all the analysis parameters and other options involved in the procedure. The completed request is sent to a queue for processing and the end user provided a job identifier, which can be used to check on the status of the analysis. A simple analysis involving one or a few genomes is typically processed in under a minute, but G View does not place restrictions on the number of genomes that can be submitted for an analysis, and large numbers of genomes can sometimes require many hours of processing time. The G View Server can e-mail users when their large jobs are complete. Completed jobs are presented to the user as an HTML report with a snapshot of the maps in both circular and linear layouts. Users can download the data sets for execution in their stand-alone G View application, or they can use Java Web Start to download and launch G View and the data directly from the Web application. Most of the analysis results are also available as a separate downloadable spreadsheet file.

CONCLUSION

CG View can be used to generate visually impressive graphical maps of bacterial genomes. CG View's custom XML format, command-line interface, API and zooming ability have led to its incorporation into a variety of other programs created by us and by others. For example, the CCT package, which can be used to generate massive maps comparing thousands of genomes, relies on CG View for map drawing. The newer G View program offers similar features to CG View along with support for linear maps and interactive editing. The companion servers (CG View Server and G View Server) provide the simplest means to

access the functionalities of CG View and G View, respectively, and include additional analysis tools. The G View Server in particular provides several helpful analysis pipelines for comparative genomics.

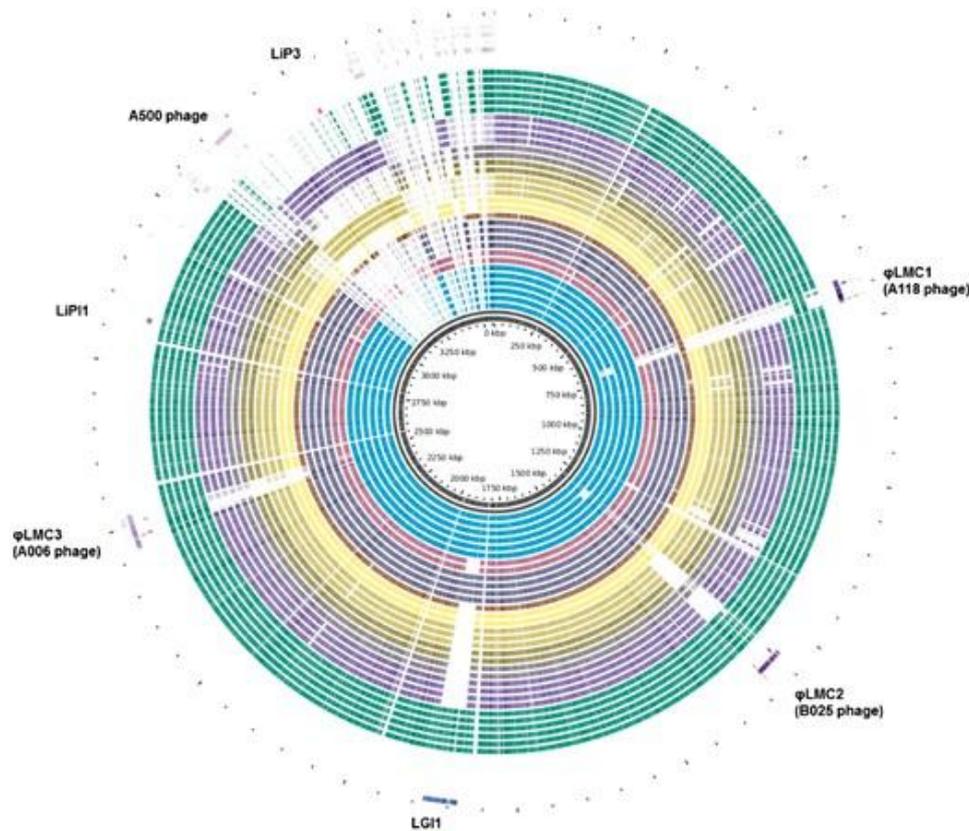


Figure 5.

The democratization of high-throughput sequencing has resulted in the generation of genome databases of staggering size. Bioinformaticians have had to rethink the way they design their software using ‘big data’ approaches, where the software now must be able to operate on remote centralized data sets using modern APIs and Web technologies. New advances in Web-based visualization technologies, such as the D3 Java Script library and HTML 5 Canvas are creating exciting new opportunities for genome visualization systems that can operate on remote data while providing the interactivity historically available in stand-alone systems. The nature of the analyses is also beginning to change, for example the existence of large data sets of closely related bacterial genomes is making possible the identification of subtle differences among genomes, and the correlation of these differences with biological traits [Desjardins et al., 2016]. This new analysis will influence the way that we view and interact with microbial genomic sequence data. We are currently prototyping the third generation of our circular genome map viewer technology using cloud computing and Web-based visualization technologies. However, there is still much work to be done in the area of API development, better support for high-throughput sequence data and innovative new ways to explore and visualize the ever-growing repositories of genomic sequence data. Although the challenges are significant, so is the reward; we are excited to be in a position to continue to develop the advanced technologies that will facilitate the next generation of microbial genome.

A pan-genome map of 40 *Listeria monocytogenes* genomes isolated from foodborne disease outbreaks in Canada from 1980 to present, generated with the G View Server. The locations of several pathogenicity-associated mobile elements (phage and genomic islands) are highlighted.

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