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Developing genomics tools for the western corn rootworm – progress and promise

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Abstract
The Diabrotica Genetics Consortium was organized in 2003 as a way to enhance and encourage communication among scientists in North America and Europe conducting research on all aspects of rootworm genetics (Sappington et al. 2006). The initial impetus for organizing ourselves was the mutual discovery that five laboratories in the US and France were simultaneously engaged in, or about to engage in, development of microsatellite DNA markers for population genetics studies of the western corn rootworm, Diabrotica virgifera virgifera. Development of microsatellites is a costly and rather time-consuming endeavor. We agreed that we could all benefit from more efficient use of our resources by coordinating our efforts and freely sharing information and markers, thus reducing needless duplication of effort and unproductive competition. We further agreed to open membership in the Consortium to anyone working in the field of genetics of any Diabrotica species, as well as to anyone whose research was impacted by those fields. The response and interest were overwhelming. The Consortium has since grown to include more than 40 laboratories in seven countries in North America, Europe, and Australia. It also has expanded far beyond the relatively narrow field of population genetics.

Keywords
microsatellite DNA markers, Diabrotica, genome sequencing

Disciplines
Agronomy and Crop Sciences | Entomology | Plant Breeding and Genetics

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Article

Developing Genomics Tools for the Western Corn Rootworm — Progress and Promise

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The *Diabrotica* Genetics Consortium was organized in 2003 as a way to enhance and encourage communication among scientists in North America and Europe conducting research on all aspects of rootworm genetics (Sappington et al. 2006). The initial impetus for organizing ourselves was the mutual discovery that five laboratories in the US and France were simultaneously engaged in, or about to engage in, development of microsatellite DNA markers for population genetics studies of the western corn rootworm, *Diabrotica virgifera virgifera*. Development of microsatellites is a costly and rather time-consuming endeavor. We agreed that we could all benefit from more efficient use of our resources by coordinating our efforts and freely sharing information and markers, thus reducing needless duplication of effort and unproductive competition. We further agreed to open membership in the Consortium to anyone working in the field of genetics of any *Diabrotica* species, as well as to anyone whose research was impacted by those fields. The response and interest were overwhelming. The Consortium has since grown to include more than 40 laboratories in seven countries in North America, Europe, and Australia. It also has expanded far beyond the relatively narrow field of population genetics.

Early on, the idea of eventually obtaining a genome sequencing project through leveraging the expertise and collective influence of the Consortium membership was discussed as something of a dream for the distant future. But with rapid advances in sequencing technology, a precipitous drop in the cost of sequencing, and concerted efforts within the Consortium to generate genomics tools and resources, the day of sequencing the genome is now upon us (Miller et al. 2010). In November 2008, members of the *Diabrotica* Genetics Consortium and other interested scientists convened a Western Corn Rootworm Genome Sequencing Workshop at the Annual Meeting of the Entomological Society of America in Reno, Nevada. The purpose of the Workshop was to review the genomic assets already available to the rootworm scientific community, identify which resources were still needed, and generate a plan to coordinate the development of the needed resources. The idea was to position ourselves as an international research community to submit one or more viable grant proposals within the following two years to fund a genome sequencing project for *D. v. virgifera*. Nick Miller (now an Assistant Professor in the Department of Entomology, University of Nebraska) summarized the workshop and strides forward in a talk at the Second International Conference on *Diabrotica* Genetics, held in conjunction with the 23rd IWGO Conference in Munich in April 2009, which was published in more detail
(Miller et al. 2010) as an article in the special IWGO issue of the Journal of Applied Entomology that came out in June 2010 (134:420-428). Considerable progress was made in the year after the Conference, and two large grant proposals were submitted in spring and summer 2010. One contained sequencing the genome as an objective (Hugh Robertson, PI), and the other involved sequencing the transcriptome (Thomas Guillemaud, PI). Both proposals were funded, and Nick Miller and Thomas Guillemaud presented goals and updates of the two projects at the Third International Conference of Diabrotica Genetics held in conjunction with the recent 24th IWGO Conference in Freiburg. In the remainder of this article, I will briefly summarize some of the background preparatory work that helped make these landmark projects possible from a technical standpoint, and where we are now.

Among the assets already available at the time of the 2008 workshop in Reno was the Diabrotica Genetics Consortium itself, which represents an organized international community of interested scientists. This is important not only for facilitating a coordinated effort to generate genomics tools and resources, but just as importantly for demonstrating a significant number of laboratories whose future research will utilize and benefit from the sequenced transcriptome and genome. We also have available a bacterial artificial chromosome (BAC) library, constructed by Blair Siegfried at the University of Nebraska, which consists of 110,592 clones containing long inserts of D. v. virgifera DNA. Two expressed sequence tag (EST) libraries have been constructed, one from the midgut (Siegfried et al. 2005) and one from the head (Knolhoff et al. 2010). ESTs are generated from genes transcribed during a particular stage, in a particular tissue, and under prevailing environmental conditions. We also had an estimate of D. v. virgifera genome size made by Spencer Johnston at Texas A&M University of 2.5 Gbp, but there was some uncertainty as to precision of the estimate. This was because no one expected the genome to be so large, and the estimate fell outside the largest size standard. Brad Coates (USDA-ARS, Ames, Iowa) has now confirmed the astonishingly large size of the D. v. virgifera genome as 2.58 Gbp (manuscript submitted).

Among the needed resources identified at the workshop was development of an inbred line of D. v. virgifera. Such an inbred strain is necessary to reduce the amount of variation in the genome due to alleles at a given locus. In model organisms like Drosophila, inbred lines are commonplace, but it was not an easy proposition to develop one in D. v. virgifera because of the difficulties inherent in rearing this insect. Wade French of USDA-ARS took on the challenge at the North Central Agricultural Research Laboratory rootworm rearing facility in Brookings, South Dakota. His team started several lines of D. v. virgifera from single-pair matings drawn from the partially inbred non-diapause colony, then nursed a number of families through seven generations of single-pair sibling matings. He now has an inbred line that is maintained by mass matings within the line. This is a major achievement that is critical to the success of sequencing and reassembling the genome, and these lines will be valuable for many other future applications as well. Both the transcriptome and genome sequencing projects are utilizing this inbred colony.
The BAC library is being used to provide information on the structure of the *D. v. virgifera* genome. Because of its large size, we anticipate the genome contains much repetitive DNA, which can complicate reassembly of the genome sequence. DNA preparations representing the entire library, organized into "pools" and "superpools," were screened for genes of interest by PCR. Each superpool contains DNA from 4,608 clones corresponding to twelve 384-well plates of library clones, giving a total of 24 superpools for the entire library. For each superpool, there is an associated pool plate containing 12 pools of DNA from each 384-well plate, 16 pools from each row of all the plates, 24 pools from each column of all the plates and 24 diagonal pools. This kind of arrangement allows efficient screening for particular genes of interest so that a BAC clone can be identified and sequenced in its entirety. Twenty-eight clones have been identified and selected by University of Nebraska and USDA-ARS (Iowa) scientists for sequencing because screening revealed they contain genes coding for important proteins such as receptors and enzymes that are often involved in insecticide resistance. DNA from these clones has been extracted, and is now being purified and prepared for complete sequencing. Seventy additional clones will be selected based on their relative position in the genome according to the recently completed linkage map.

A linkage map will be critical in assembling the genome sequence, as well as in identifying genes that are responsible for, or are associated with, phenotypic traits of interest. In a large ongoing project involving collaborators from University of Illinois, University of Nebraska, and USDA-ARS in Iowa, next-generation genomic sequencing technology (Illumina GoldenGate assay) was used to identify and verify large numbers of single nucleotide polymorphisms (SNPs) as molecular genetic markers from *D. v. virgifera*. Of 2,222 candidate SNPs that were determined to be potentially useful, 1,536 were chosen for further testing at the University of Illinois W. M. Keck Center for Comparative and Functional Genomics. Verified SNPs were integrated by Brad Coates (USDA-ARS, Iowa) into 10 consensus linkage groups of about 35 markers per group. The BAC library is being screened further with the validated SNPs, and 72 clones will be strategically selected for full sequencing in addition to the 28 already targeted as mentioned above. Many of the SNPs are already being used in population genetics studies as well.

A mechanism for sharing data through an integrated database of genetic maps, nucleic acid sequences, and related data will be a powerful catalyst to accelerate research progress on *D. v. virgifera*. The bioinformatics Co-PIs on the various grants are actively discussing the best way to integrate our efforts to make the oncoming flood of genomics data most useful to the research community. The genetic maps and associated molecular and other data generated by large-scale sequencing projects are generally similar in both content and scope to those in other genetic/genomic databases. The two leading possibilities being considered at this time for housing *D. v. virgifera* data are AphidBase, maintained by Denis Tagu and Fabrice Legai of INRA, Rennes, France, and SoyBase, the home of soybean genomics data developed and maintained by David Grant of USDA-ARS in Iowa.
One of our biggest needs has been to generate more EST sequence data, which are very valuable for assembling and annotating the genome sequence. The large international project being led by Thomas Guillemaud of INRA (Sophia-Antipolis, France) to generate a reference transcriptome of *D. v. virgifera* fulfills this need. The data generated will be based on 454 Titanium pyrosequencing of pooled libraries from whole bodies of all developmental stages, under both optimal rearing conditions and a variety of stressful environmental conditions (e.g., heat shock, sublethal doses of insecticide, entomopathogenic nematode challenge, etc.). The reference transcriptome will also be of great value in its own right as a resource for gene discovery, gene expression, and characterization of sequence variation.

All of these resources and the organized research Consortium make *D. v. virgifera* the most logical translational genomics model for species of underground insect pests of grasses proposed as biofuel crops, such as *Miscanthus* and switchgrass. Translational genomics is a field wherein genome sequence information in a model species is leveraged to solve practical problems in related, but genetically uncharacterized, species. Corn already serves as the translational genomics model of biofuel grasses. Protection of biofuel crops is a new priority of the USDA Agriculture and Food Research Initiative grant program. It is difficult to obtain funding solely to sequence an insect genome, but we were able to make the case in a multi-institution proposal led by Hugh Robertson at the University of Illinois that sequencing the *D. v. virgifera* genome will allow identification of genes involved in successful attack of roots by this species. With the decreasing cost of sequencing, we were able to embed an objective for genome sequencing within a larger project that will exploit that information to discover genes involved in colonization of roots by coleopteran larvae. The western corn rootworm will likely be a direct pest of *Miscanthus* (Spencer and Raghu 2009), but the role of *D. v. virgifera* as a translational genomics model for other insect pests will make the results of this study of even broader value.

These are exciting times for entomologists studying *D. v. virgifera* because of the amazing research tools rapidly becoming available. Whether involved directly with genetics research or not, all of us who study this pest and who seek ways to mitigate the damage and misery it causes will benefit from the new advances being made in unlocking the secrets of its genome. One of the most rewarding and exciting aspects of working on this insect is the extensive international cooperation that continues to make such rapid advances possible (Moeser and Guillemaud 2009).

**References Cited**


