Coordinated *Diabrotica* Genetics Research: Accelerating Progress on an Urgent Insect Pest Problem

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ABSTRACT. Corn rootworms, *Diabrotica* spp., represent the most destructive pest complex of continuous corn (*Zea mays*) in North America, and the western corn rootworm (*D. virgifera virgifera*) (WCR) is now posing a major and spreading risk to corn in Europe since it was first detected in the early 1990s. Corn rootworms are a very difficult pest to manage because they have developed resistance to both chemical insecticides and cultural management systems, such as crop rotation. The recent deployment of transgenic *Bt* corn in the U.S. for controlling *Diabrotica* pests has raised concerns that rootworms will develop resistance to this technology as well unless appropriate insect resistance management strategies are employed. The evolution and spread of insecticide and behavioral resistance to crop rotation, the introduction and spread of the western corn rootworm in Europe, and the recent large-scale commercial deployment of rootworm-active *Bt* corn in North America have converged to generate a sense of urgency among scientists involved in rootworm research. Of particular importance to all these issues is an increased understanding of rootworm genetics. Over the last two years, a group of more than 35 scientists from 21 different institutions in North America and Europe have organized to form the *Diabrotica* Genetics Consortium. The main role of the Consortium is to facilitate communication among those working on genetics-related problems, both to coordinate efforts and to reveal opportunities for synergism through cooperation/collaboration. Members of the Consortium organized and convened the first International Conference on *Diabrotica* Genetics Research in December 2004 to exchange information on ongoing research, to explore potential collaborations, and to discuss areas of needed research. This is a highly energized group of scientists with a broad range of expertise, uniquely motivated to cooperate on large issues of rootworm genetics. Such issues cannot be tackled in a timely fashion under the conventional paradigm of fragmented research funded by individual grants to one or a few investigators at a time. With a targeted infusion of funding, this Consortium is in a unique position to significantly accelerate progress in critical areas of *Diabrotica* genetics through efficient large-scale cooperative research.

URGENT CHALLENGES POSED BY *DIABROTICA*

Diabrotica spp. (western, northern, and Mexican corn rootworms) represent the main pest complex of continuous field corn, *Zea mays* (L.), in North America (Levine and Oloumi-Sadeghi 1991; Levine et al. 2002). The western corn rootworm (WCR) is also the main pest of continuous corn in Central Europe, and represents a major risk in Western Europe. Rootworm larvae feed on corn roots, and damaged plants are more susceptible to drought and disease, have decreased yield, and are prone to lodging. Costs of control and yield loss are as high as $1 billion per annum in the U.S. (Metcalf 1986). Crop rotation and chemical control have been the primary management strategies (Levine and Oloumi-Sadeghi 1991), but the western corn rootworm is becoming increasingly difficult to control due to its sequential ability to evolve resistance to virtually all management strategies that have been employed.

<u>Insecticide Resistance:</u> Many examples exist of corn rootworm adaptation to uniform, large-scale agricultural practices. Chlorinated hydrocarbons were the first synthetic insecticide class to be introduced in Nebraska for larval control in the late 1940s (Hill et al. 1948). However, as a consequence of their broadcast application and extreme persistence, resistance to cyclodienes was detected in less than a decade (Ball and Weekman 1963; Metcalf 1986). In certain areas of the Platte Valley of south central Nebraska, adult rootworm control was adopted after the development of cyclodiene resistance (Meinke 1995). Microencapsulated methyl-parathion (Penncap® M) eventually became the most commonly used insecticide formulation. Because of its low cost and relatively long persistence, Penncap® M was used consistently in some areas of the state for extended periods. Repeated applications of carbaryl followed by Penncap M® in these areas led to control failures in the early 1990s (Meinke et al. 1998) and methyl-parathion resistance has remained consistently high in some areas even after the selection pressure was removed (Parimi et al. 2005).

Crop Rotation Resistance: Until recently, crop rotation between corn and soybeans in much of the eastern Corn Belt had eliminated the need for rootworm-targeted insecticide applications to first-year corn. In areas of Indiana and Illinois, however, a behavioral variation of some western corn rootworms is responsible for the failure of these crop rotation strategies, and the spatial range of the new behavior is spreading (Onstad et al. 1999; Levine et al. 2002; O'Neal et al. 2002; Isard et al. 2004). Extended egg-diapause, which has been documented in the northern corn rootworm (Krysan et al. 1984, 1986), was initially thought to be responsible for first-year corn damage by western corn rootworm (Levine and Oloumi-Sadeghi 1996). However, it is now known that insects displaying the new adaptive behavior disperse from corn fields to feed and oviposit in soybean fields (Sammons et al. 1997; Isard et al. 1999; Levine et al. 2002), thus increasing the probability that eggs deposited in soybeans will hatch and cause damage to corn planted in that field in the following year. As a result, both soil and foliar insecticide applications are now being commonly used to prevent rootworm damage in first-year corn in these areas.

Invasion of Europe: Another challenge posed by this insect is its recent introduction into (Sivcev et al. 1994) and spread throughout Europe (Kiss et al., 2005). WCR was first detected near the Belgrade airport in Serbia (former Yugoslavia). Believed to have originated on intercontinental flights from Chicago to Belgrade, WCR has spread rapidly, infesting more than 70,000 square miles. Isolated rootworm outbreaks have been detected in several Central and Western European countries including Italy, Switzerland, the Netherlands, France, and the U.K. (Reynaud 2002, Kiss and Edwards 2004). European countries have developed extensive detection, eradication, and containment programs to prevent establishment and minimize the impact of this invasive insect. However, it seems only a matter of time before the WCR becomes established in maize growing regions throughout Europe. Until recently it was assumed that the isolated outbreak populations were leap-frogging out of the expanding Eastern Europe population. However, a population genetics study accelerated by cooperating Consortium laboratories in the U.S. and France has revealed that ongoing independent introductions from North America are responsible for many of the new outbreaks (Miller et al. 2005). This is sobering news, because the genetic variability and the probability that adaptive alleles (e.g. insecticide resistance alleles) are present in Europe may be larger than expected for a single invasion event. It also raises the spectre of eventual introduction of the rotation-resistant variant and possibly of the northern corn rootworm into Europe. This emphasizes the need to obtain information concerning the routes of introduction of the WCR into Europe and to obtain precise data using both selected and neutral population genetic markers to determine the structure of both European and North American populations.

Transgenic Corn and Insect Resistance Management: Overlaid on top of these challenges is the recent commercialization of transgenic corn in the U.S. for controlling *Diabrotica* pests (Rice 2004). Transgenic, or *Bt*, corn expressing the Cry3Bb1 protein is intended to provide protection against certain species of the corn rootworm including the western, northern, and Mexican corn rootworms. This technology offers growers a viable and attractive alternative for managing this pest; however, there is concern that large-scale introduction and widespread acceptance of the technology will lead rapidly to resistance evolution. Substantial efforts are being made to delay the development of resistance as long as possible through insect resistance management (IRM) strategies. IRM is based on the premise that the course and timing of pest evolution in response to selection via an insecticide can be predicted, monitored, manipulated, and mitigated. Our ability to successfully predict and model evolution in the corn rootworm requires a fundamental understanding of the genetics and ecology of this insect, both at the level of the individual and of the population.

SOLUTIONS THROUGH MULTI-INSTITUTIONAL COOPERATIVE RESEARCH

The *Diabrotica* Genetics Consortium: The evolution and spread of insecticide- and rotation resistance, the introduction and spread of the western corn rootworm in Europe, and the recent large-scale commercial deployment of rootworm-active *Bt* corn in North America are all complex problems, with a number of researchers in several countries working on various aspects. Beginning in the Fall of 2003, a group of more than 35 scientists from 21 institutions in the U.S., Canada, and France, came together to form the *Diabrotica* Genetics Consortium (Table 1). This Consortium has been formalized via a USDA-ARS Non-Funded Cooperative Agreement among participating institutions. Laboratories in Mexico and Germany will be joining shortly. The objective of the Consortium is to increase our knowledge in all areas of, and relevant to, corn rootworm genetics, including (but not limited to) development of molecular markers; QTL analysis of important traits; linkage mapping; identification and mapping of genes or candidate genes conferring insecticide resistance; identification, cloning, sequencing, regulation, and expression of genes; functional analysis of gene products; population genetics; molecular ecology and phylogeography; and establishment of artificially selected laboratory lines (Table 1). The role of the Consortium is to facilitate communication among those working on genetics-related problems to coordinate research activities, to avoid unnecessary overlap of effort, and to reveal opportunities for synergism through cooperation and collaboration.

Members of the Consortium organized an International Conference on *Diabrotica* Genetics that convened in Kansas City, MO in December 2004. The conference brought together Consortium participants, their students/postdocs, and other interested scientists from nine countries to facilitate information exchange, formalize collaborations, and identify research needs and directions for future research. Scientists within the Consortium have discovered that they have many research interests in common with regard to rootworm genetics. This is a highly energized group of scientists with a broad range of expertise, uniquely motivated to cooperate on large issues of rootworm genetics to fill major knowledge gaps in both applied and basic arenas:

Table 1. Participant scientists in the *Diabrotica* Genetics Consortium (listed alphabetically by and within institution), and their related areas of research expertise.

Institution	Scientist	Area(s) of Expertise [a]
Colorado State University	Michael F. Antolin	1, 3
EPA, NERL, Molecular Ecology Res Br	Mark Bagley	
	Uwe Stolz	1, 3, 4, 5
Illinois Natural History Survey	Eli Levine	
	Joseph L. Spencer	7
INRA (National Institute of Agricultural Research) – France	Denis Bourguet	1, 3, 4, 7, 8
	Sylvie Derridj	
	Arnaud Estoup	
	Thomas Guillemaud	
	Nick Miller	
Iowa State University	Jon J. Tollefson	7
Mississippi State University	Michael A. Caprio	3, 8
Pennsylvania State University	Dennis D. Calvin	7, 8
Purdue University	C. Richard Edwards	4, 7
University of Arkansas	Allen L. Szalanski	1, 3, 4
University of Guelph – Canada	K. Peter Pauls	1, 4, 6
University of Illinois	Susan T. Ratcliffe	1, 3, 6, 7
	Hugh M. Robertson	
University of Maryland	David J. Hawthorne	1, 2
University of Missouri	Thomas L. Clark	1, 3, 4, 5, 7
University of Nebraska	John E. Foster	2, 3, 4, 5, 6, 7
	Lance J. Meinke	
	Blair D. Siegfried	
University of Vermont	Rosanna Giordano	1, 3, 4
University of Wisconsin	Eileen M. Cullen	7
USDA-ARS, Corn Insects & Crop Genetics Research Unit, Ames, IA	Richard L. Hellmich	1, 2, 3, 7
	Kyung Seok Kim	
	Thomas W. Sappington	
	Douglas V. Sumerford	
USDA-ARS, Crop & Entomology Research Unit, Brookings, SD	Michael M. Ellsbury	2, 5, 7
	B. Wade French	
USDA-ARS, Plant Genetics Research Unit, Columbia, MO	Bruce E. Hibbard	2, 3, 5, 7
USDA-ARS, Insect Genetics and Biochemistry Research Unit, Fargo, ND	Richard L. Roehrdanz	1, 3, 4
USDA-ARS, Biological Research Unit, Manhattan, KS	Richard W. Beeman	6
	Brenda S. Oppert	

[a] 1: Development of molecular markers
2: Linkage mapping, QTL analysis, mapping of insecticide resistance genes
3: Population genetics, molecular ecology
4: Phylogeography
5: Selection of laboratory lines
6: ESTs; microarrays; genomics; identification, sequencing, and expression of genes
7: Ecology, dispersal behavior, pest management
8: Modelling

Resistance genetics: In the area of resistance monitoring there is a need to characterize population genetic structuring of rootworm species and then design a rational sampling plan based on that structure. Existing methods for detecting resistance alleles in populations are hindered by low sensitivity. There is a pressing need to provide molecular diagnostics to improve sensitivity. Furthermore, there are fundamental knowledge gaps in resistance risk assessment. The resistance that has already been documented among rootworm populations together with the availability of diagnostic techniques and identification of resistance genes offers an important opportunity to observe the development of resistance, examine the movement of resistance genes, and quantify the factors responsible for resistance evolution. QTL and marker assisted mapping strategies can be used to elucidate the genetic architecture of resistance traits. New technologies such as seed treatements and transgenics offer growers viable and environmentally safe alternatives for rootworm management. Although IRM is needed to extend the durability of both technologies, there are differences in EPA regulatory IRM requirements between transgenics (stringent) and neonicotinoid seed treatments (none). Research also is needed to explore the possible effects of interactions of the two technologies on the potential for resistance evolution. Linkage maps developed in this effort will open the door to study the genetic architecture of other traits of interest (Hawthorne 2003), including diapause, behavior, morphology, immunology, and host-parasitoid interactions, promising to make *Diabrotica* a model system for understanding the genetics of such traits in other insects.

Population genetics: Prediction of development and spread of resistance under various inheritance and IRM scenarios depends on robust estimates of rootworm dispersal. Traditional ecological methods for studying insect dispersal are essential and informative, but by themselves are inadequate to provide the kind of data crucial for these kinds of predictions. Population genetics tools can be brought to bear to provide necessary estimates of gene flow at different geographic scales. Recent findings by Consortium scientists of low genetic structuring on a coarse geographic scale in the U.S. (Kim and Sappington 2005) suggest that temporal methodologies will be required to obtain good estimates of gene flow. The detailed historical knowledge of WCR range expansion in the U.S. during the last century and of the ongoing range expansion in Europe, has provided a rare opportunity to follow and probe the genetics of an invasive species on the march. The chance to engage in such research in a coordinated, multi-institutional, intercontinental manner, and at the speed necessary to catch ecological and evolutionary events in real time, is even more rare and invaluable. The Consortium is in an ideal position to take advantage of this opportunity. The drive to elucidate details of the initial introduction(s) of WCR into Europe has spurred development of powerful new approximate Bayesian analytical techniques for analyzing population-genetic data by Consortium scientists (Miller et al. 2005). These and future techniques developed to investigate *Diabrotica* will be useful for scientists in any organismal discipline dealing with questions of range expansion, invasive species, colonization, and dispersal.

Phylogeography: Research across the genus *Diabrotica* will provide a better understanding of the genetics of adaptation, host plant associations, and invasiveness. Those working in classical biological control are searching for very specific parasitoids of

Diabrotica beetles for use against WCR, especially in Europe. Assessing the risks of importing a parasitoid always requires a series of experiments to establish host specificity. Unfortunately, this is not an easy task considering the hundreds of *Diabrotica* and parasitoid (e.g., *Acalymma*, *Ceratoma*, etc) species that exist and the current confusion in the phylogenies and systematics of these groups. The phylogenetic relationships within the Diabroticina tribes are not clear, especially at the genus level. The application of genetic markers and molecular genetic analyses will help resolve the current uncertainties among these taxa. The picture is complicated by the bacteria *Wolbachia*, which can cause cytoplasmic incompatibility between conspecifics and therefore impose geographic discontinuities on gene flow, a phenomenon observed in several populations of western, northern, and Mexican corn rootworms. At the same time, the pioneering work by Consortium scientists on the effects of *Wolbachia* on the population genetics and phylogeography of rootworms (Giordano et al. 1997; Roehrdanz et al. 2003; Roehrdanz and Levine 2004), combined with the expertise and genetic resources which can be applied to this topic through cooperation with other Consortium scientists, has provided an exceptional opportunity to explore these effects in depth. Thus, *Diabrotica* is poised to become a model for the many other insect species where *Wolbachia* influences their evolution and ecology.

Genomics: A preliminary estimate of the size of the *D. v. virgifera* genome indicates that it is rather large, but rapid improvements in genome sequencing efficiency, the current effort to sequence the maize genome, and the importance of this insect and its congeners to society will likely make such a project attractive in the near future. In the meantime, expressed sequence tag (EST) databases developed by Consortium scientists from the head (Ratcliffe et al., unpublished data) and from the midgut (Siegfried et al. 2005) are available for data mining and use in microarray analyses. Preliminary microarray analyses of the head-derived cDNAs by a Consortium scientist suggests promise for identifying genes involved in resistance to crop-rotation, while the midgut-derived cDNAs should be useful in identifying genes involved in insecticide resistance. Analyses of *Diabrotica* homologs of behavior-related genes in other insects may lead to development of a molecular diagnostic for distinguishing the crop-rotation variant of WCR. A Consortium member coauthored the white paper in the successful bid to sequence the *Tribolium* genome (Brown et al. 2003), and comparison of *Diabrotica* EST and other sequences with those from *Tribolium* will provide fundamental insights into gene function and regulation.

BENEFITS TO BE REALIZED FROM LARGE-SCALE COOPERATIVE *DIABROTICA* GENETICS RESEARCH

Understanding the genetics of existing instances of resistance and its spread will:

- Improve effectiveness of IRM strategies for both conventional and transgenic control tactics.
- Improve the ability to predict likely mechanisms of resistance and cross-resistance to new technologies.
- Provide information necessary to develop approaches to sustain novel control strategies.

- Lead to development of resistance-associated markers, which in turn may provide a means to differentiate among both North American and European populations.
- Allow the development of linkage maps to determine the genetic architecture of many other traits of interest.

An understanding of WCR/Bt corn interactions:

- Can serve as a model system for understanding other low-dose transgenic events.
- Will be important to seed companies, the EPA, and modelers in their attempts to develop resistance management plans for transgenic corn by providing more realistic assumptions in current mathematical models.

Estimates of gene flow through population genetics analyses will permit:

- The parameterization of models predicting geographic spread of resistance variants.
- The prediction of the spread of the WCR and the choice of the most appropriate mitigation responses to new outbreaks.
- The characterization of both intra and interspecies variation to guide choice of appropriate field-testing locations for screening efficacy of new transgenic traits and new chemistries for rootworm control.
- New analytical techniques to be developed to provide new tools for population geneticists studying other organisms.

Application of molecular genetic analyses to phylogeographic and phylogenetic questions:

- Will clarify phylogenetic relationships within *Diabrotica*.
- Will clarify phylogenetic relationships within parasitoid species such as *Acalymma* and *Ceratoma*.
- Will elucidate the complex dynamics of *Wolbachia* infections on gene flow and genetic structuring of *Diabrotica* populations and subspecies.

Genomics approaches to gene identification and characterization will help:

- Isolate genes involved in resistance to crop-rotation.
- Isolate genes involved in resistance to Bt toxins as well as conventional insecticides.
- Identify target sites for novel toxins with increased specificity and reduced impact on environmental and non-target organisms

CONCLUSION

The problems posed to corn producers by rootworms in the genus *Diabrotica* are mounting and are associated at a fundamental level with the genetics of this important pest complex. The *Diabrotica* Genetics Consortium is a large international group of scientists dedicated to communicating our research objectives and findings, sharing our expertise and genetic resources, and coordinating our activities in order to accelerate progress toward a common goal. Thus we are exceptionally motivated and uniquely positioned to address the large, complex questions of rootworm genetics that so urgently need resolution. Such questions cannot be tackled in a timely fashion under the conventional paradigm of fragmented research funded by individual grants to one or a few investigators at a time. With a targeted infusion of funding, our Consortium is in a unique position to significantly accelerate progress in critical areas of *Diabrotica* genetics through efficient large-scale cooperative research. Because of the large and diverse group focusing on this insect, such cooperation will elevate *Diabrotica* to the status of a model organism in a very short time with far-reaching benefits to those working on other species in the fields of resistance genetics, population genetics, phylogeography, and genomics.

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