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

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Western corn rootworm
feeding on corn silks.
Photo: Blair Siegfried,
University of Nebraska..

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D*iabrotica* 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, Rice 2004). The western corn rootworm, *Diabrotica virgifera virgifera* LeConte, also has become the main pest of continuous corn in Central and South-eastern Europe since its introduction near Belgrade 15–20 years ago, and it represents a major risk to Western Europe (Sivcev et al. 1994, Barčić et al. 2003, Baufeld and Enzian 2005). It has already caused economic losses in Eastern Europe, and Western countries such as France have committed large expenditures for containment and/or eradication (Anonymous 2003).

Rootworm larvae feed on corn roots, and damaged plants are more susceptible to drought and disease, have decreased yield, and are prone to lodging (Reidell 1990, Spike and Tollefson 1991, Urías-López and Meinke 2001). A recent economic analysis estimates that costs of control and yield loss are about \$1.17 billion a year in the United States (P. Mitchell, University of Wisconsin, personal communication). 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 because of its sequential ability to evolve resistance to almost all management strategies that have been used (Meinke et al. 1998, Wright et al. 2000, Levine et al. 2002). The recent deployment of transgenic *Bt* corn in the United States for controlling *Diabrotica* pests has raised

concerns that rootworms will develop resistance to this technology as well, unless appropriate resistance management strategies are developed and practiced (Onstad et al. 2001, Storer 2003, Crowder et al. 2005, Siegfried et al. 2005b).

The evolution and spread of resistance to insecticides and 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 who are involved in rootworm research. Of particular importance to all these issues is an increased understanding of rootworm genetics. Over the past 3 years, a group of more than 35 scientists from 21 institutions in North America and Europe have organized to form the *Diabrotica* Genetics Consortium. Members of the consortium organized and convened the first International Conference on *Diabrotica* Genetics Research in Kansas City, MO, 13–15 December 2004. The conference brought together 45 participants from nine countries, including members of the consortium, their students/postdocs, and other interested scientists to facilitate information exchange, formalize collaborations, and identify research needs and directions for future corn rootworm genetics research.

Contacts and negotiations facilitated by this conference have accelerated progress through collaborative research in several critical areas. For example, hyper-variable genetic markers shared among laboratories were used to identify western corn rootworms' routes of invasion into Europe

(Miller et al. 2005). An agreement on developing a core set of microsatellite DNA markers for future studies of western corn rootworms made at the conference will simplify direct comparisons between studies performed in different laboratories—a situation that is usually a problem with other organisms because of competition and lack of communication among distant scientific teams.

This energized group of scientists with a broad range of expertise is highly motivated to cooperate on large issues of rootworm genetics to fill major knowledge gaps in applied and basic arenas. A panel discussion at the conference covered the topics of creating a consortium web site, potential funding opportunities, and the merits of creating a *Diabrotica* genetics database. The organizers agreed to prepare a white paper to circulate to appropriate program leaders at USDA–CSREES, NSF, and other potential funding sources to seek support for a large multi-institution project.

This article represents in large part the white paper that emerged from the conference. It outlines the pressing issues posed by this insect, why the time is right for a coordinated push to accelerate progress in the various areas of *Diabrotica* genetics research, and the expected benefits of such a large-scale multi-institution research program. In addition, we believe that the *Diabrotica* Genetics Consortium can serve as a model for organizing cooperative endeavors in other areas of entomological research. We hope this article will form the basis of an ongoing dialogue in the entomological community; and we invite comments, suggestions, and views on any of the ideas or issues presented in this article to be posted on the *Diabrotica* genetics research Forum page accessed through the ESA's Ent-Chats website (<http://www.entsoc.org/membership/toolbox/entchats/index.htm>).

Urgent Challenges Posed by *Diabrotica*

Insecticide Resistance. Many examples exist of corn rootworm adaptation to uniform, large-scale agricultural practices. Cyclodienes 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, cyclodiene resistance was detected in less than a decade (Ball and Weekman 1962, Metcalf 1986). In certain areas of the Platte Valley of south central Nebraska, adult rootworm control was adopted after resistance rendered larval control by cyclodienes ineffective (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. 2006).

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 this behavior is spreading (Onstad et al. 2003, 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, *Diabrotica barberi* Smith and Lawrence (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). It is now known, however, that insects displaying the 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, Rondon and Gray 2004, Schroeder et al. 2005), and therefore increase the probability that eggs will hatch and cause damage to corn planted in that field in the following year. As a result, soil and foliar insecticide applications are being used commonly to prevent rootworm damage in first-year corn in these areas.

Consortium scientists in Europe and the United States using microsatellite DNA markers found no evidence for local population structuring between rotation-resistant and susceptible rootworms (Miller et al. 2006). This means that finding genetic markers diagnostic of rotation resistance will require identifying the responsible gene(s) or discovering tightly linked loci.

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). The western corn rootworm was first detected near the Belgrade airport in Serbia (former Yugoslavia). Believed to have originated on intercontinental flights from the midwestern United



Two third instar western corn rootworm larvae attacking an artificially infested plant. Photo: Stephen Thompson

States to Belgrade, the western corn rootworm has spread rapidly, infesting more than 70,000 square miles (181,300). Isolated rootworm outbreaks have been detected in several central, southeastern, and western European countries including Italy, Switzerland, the Netherlands, France, and the United Kingdom (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. Because of the apparent existence of chronic transatlantic introductions, however, it seems likely that the western corn rootworm will become established in maize-growing regions throughout Europe (Hemerik et al. 2004, Miller et al. 2005).

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 United States 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 about the routes of introduction of the western corn rootworm into Europe and to obtain precise data using selected and neutral population genetic markers to determine the structure of European and North American populations.

Transgenic Corn and Insect Resistance Management. Overlaid on these challenges is the recent commercialization of transgenic corn in the United States for controlling *Diabrotica* pests (Rice 2004). Transgenic, or *Bt*, corn expressing Cry toxins from *Bacillus thuringiensis* is intended to provide protection against certain species of the corn rootworm, including the western, northern, and Mexican corn rootworms (*Diabrotica virgifera zea* Krysan and Smith). This technology offers growers an 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 (Siegfried et al. 2005b). 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 resistance evolution in the corn rootworm requires a fundamental understanding of the genetics and ecology of this insect, at the level of the individual and of the population.

Solutions through Multi-Institutional Cooperative Research

Beginning in the fall of 2003, a group of more than 35 scientists from 21 institutions in the United States, 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, Germany, and Switzerland are expected to join 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; analysis of Quantitative Trait Loci (QTL) analysis; linkage mapping; identification and mapping of genes or candidate genes conferring insecticide resistance; identification, cloning, sequencing, regulation, and expression of genes coding essential and adaptive traits; functional analysis of gene products; population genetics; molecular ecology and phylogeography; and establishment of artificially selected laboratory lines (Table 1).

Participants need not be engaged in genetics research per se, but may be working in areas that inform, or are informed by, such research. 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 identify opportunities for synergism through cooperation and collaboration. Members are engaged in a broad range of applied and basic research.

Given the importance of this pest, it is somewhat surprising that such a consortium did not materialize before now, and that the “billion-dollar bug” is not already a major model for biology and genetics research. On the other hand, the North Central Region Technical Research Committee on Corn Rootworms (NCR-46, recently redesignated as NCCC046) has been actively involved in rootworm research since its formation in 1964 (http://www.lgu.umd.edu/lgu_v2/homepages/home.cfm?trackID=7896), and the rootworm research community has a long history of cooperation and information exchange at the regional level. Indeed, there is considerable overlap in participants among the NCCC046 Committee and the consortium. Nevertheless, we are extraordinarily late in focusing on the genetics of this insect in a coordinated way, and the quick consolidation and enthusiastic response of many research teams to forming the consortium is testimony to the need and desire for a larger effort.

Resistance Genetics. There is a need to characterize population genetic structuring of rootworm species to design a rational sampling plan to monitor for resistance development. Existing methods for detecting resistance alleles in populations are hindered by low sensitivity, so 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

Table 1. Participating institutions in the *Diabrotica* Genetics Consortium (listed alphabetically), and areas of research expertise among participating scientists. More than one scientist is often involved at a given institution.

Institution	Areas of Expertise ^a
CABI Biosciences Switzerland Centre	7
Colorado State University	1, 3
EPA, NERL, Molecular Ecology Research Branch	1, 3, 4, 5
Georg-August-University, Germany	7
Illinois Natural History Survey	7
IFIT-Colegio de Postgraduados, Mexico INRA (National Institute of Agricultural Research), France	3, 4 1, 3, 4, 7, 8
Instituto de Ecología, A.C., Mexico	4, 7
Iowa State University	7
Mississippi State University	3, 8
Pennsylvania State University	7, 8
Purdue University	4, 7
University of Arkansas	1, 3, 4
University of Guelph, Canada	1, 4, 6
University of Illinois	1, 3, 6, 7, 8
University of Maryland	1, 2
University of Missouri	1, 3, 4, 5, 7
University of Nebraska	2, 3, 4, 5, 6, 7
University of Vermont	1, 3, 4
University of Wisconsin	7
USDA-ARS, Corn Insects & Crop Genetics Research Unit, Ames, IA	1, 2, 3, 7
USDA-ARS, Crop & Entomology Research Unit, Brookings, SD	2, 5, 7
USDA-ARS, Plant Genetics Research Unit, Columbia, MO	2, 3, 5, 7
USDA-ARS, Insect Genetics & Biochemistry Research Unit, Fargo, ND	1, 3, 4
USDA-ARS, Biological Research Unit, Manhattan, KS	6

^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: Modeling

among rootworm populations, together with the availability of diagnostic techniques and identification of resistance genes, makes it possible 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 treatments and transgenics, give growers viable 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 the development and spread of resistance under various inheritance and IRM scenarios depends on two crucial parameters: the spatial scale at which individuals reproduce freely (population size) and the amount of connectivity (dispersal) between such reproductive units. Estimates for these parameters are required to quantify the balance between selection and migration and to determine whether the local evolution of adaptive traits, such as insecticide resistance, is possible (Roughgarden 1996). Traditional ecological methods for studying insect population size and dispersal are essential and informative, but by themselves are inadequate to provide the necessary data for predicting resistance evolution. Population genetics tools can be used to provide necessary estimates of population size and gene flow at different geographic scales. Recent findings by consortium scientists of low genetic structuring on a coarse geographic scale in the United States (Kim and Sappington 2005) suggest that temporal methodologies will be needed to obtain good estimates of gene flow. The detailed historical knowledge of western corn rootworm range expansion in the United States during the

past 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 the western corn rootworm 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

a phenomenon observed in several populations of western, northern, and Mexican corn rootworms (Giordano et al. 1997, Roehrdanz et al. 2003). 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 that 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 whose evolution and ecology are influenced by *Wolbachia*.

Genomics. A preliminary estimate of the size of the *D. v. virgifera* genome indicates that it is rather large (~2.5 Gbp), but rapid improvements in the efficiency of genome sequencing, 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 soon. In the meantime, expressed sequence tag (EST) databases developed by Consortium scientists from the head (Ratcliffe and others, S. T. Ratcliffe, University of Illinois unpublished data) and from the midgut (Siegfried et al. 2005a) 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, and the midgut-derived cDNAs should be useful in identifying genes involved in insecticide resistance. Analyses of *Diabrotica* homologues of behavior-related genes in other insects may lead to the development of a molecular diagnostic for distinguishing the crop rotation variant of the western corn rootworm. A consortium participant 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.



Western corn rootworms mating.
Photo:
Maurice Degrugillier,
USDA-ARS, Fargo,
ND, retired.

invasiveness. Those working in classical biological control are searching for very specific parasitoids of *Diabrotica* beetles to use against the western corn rootworm, especially in Europe (Kuhlmann and Burgt 1998). Assessing the risks of importing a parasitoid always requires a series of experiments to establish host specificity (Kuhlmann et al. 2005). Unfortunately, this is not an easy task because of the variety of Diabroticite genera/species and parasitoid (e.g., *Centistes*, *Celatoria*, etc.) species that exist (Eben 2002, Walsh et al. 2003) and the current confusion in the phylogenies and systematics of these groups. The phylogenetic relationships within the Diabroticina subtribe 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,

Benefits of 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 resistant and susceptible individuals among both North American and European populations; and
- Allow the development of linkage maps to determine the genetic architecture of many other traits of interest.

An understanding of western corn rootworm/Bt-corn interactions can serve as a model system for understanding other low-dose transgenic events, and it 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 western corn rootworm in Europe and the choice of the most appropriate mitigation responses to new outbreaks;
- The characterization of intra- and interspecies variation to guide choice of appropriate field-testing locations for screening efficacy of new biological control agents, new transgenic traits and new chemistries for rootworm control; and
- 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* genera and species; Clarify phylogenetic relationships within parasitoid taxa, such as *Centistes* and *Celatoria*; and
- 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 to conventional insecticides; and
- Identify target sites for novel toxins with increased specificity and reduced impact on the environment and nontarget organisms.

Conclusion

The problems posed to corn producers by *Diabrotica* rootworms 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. The Consortium is poised to significantly accelerate progress in critical areas of *Diabrotica* genetics through efficient large-scale cooperative research. Because of the large and diverse groups 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 insect pest species in the fields of resistance genetics, population genetics, phylogeography, and genomics.

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