GENOMICS
FOR FUTURE FORESTS

FIRST CANADIAN FOREST GENOMICS SYMPOSIUM

Symposium Report

Edited by
Anne-Christine Bonfils
and Isabelle Gamache

Natural Resources Canada
Canadian Forest Service

Ressources naturelles Canada
Service canadien des forêts

Canada

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2–3 September 2004
Ottawa, Ontario, Canada

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We thank the symposium speakers for preparing and delivering fascinating high-quality contributions, the session chairs — CFS's Ariane Plourde and Errol Caldwell, Roger Foxall of Genome BC, and Cecil Yip of the Ontario Genomics Institute — for keeping us on time and entertained, and all participants for the productive and motivating discussions during the two dialogue sessions. We look forward to their continued participation in future discussions.

We are very grateful to those responsible for the smooth running of the symposium. CFS's Diane Orr and Pina Vieira provided essential logistic support; Danielle Monette designed the symposium Web site; Sandra Abi-Aad handled the audio-visual equipment and the assembly and design of participant materials, which Bette Reid assisted in formatting; and Jennifer Goostrey audiotaped the presentations. Tina Grznar of Genome Canada arranged the distribution of promotional materials. Lyle Makosky of InterQuest Consulting facilitated the dialogue sessions and produced summary notes.

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Preface

In 2004, genomics research reached an important milestone in Canada. The federal research support program for genomics was concluding its initial phase, and the submissions for renewal were being prepared considering past performance and future priorities. It was an opportune time for Natural Resources Canada's Canadian Forest Service and Genome Canada to host the First Canadian Forest Genomics Symposium. Genomics for Future Forests, as it was titled, was held in Ottawa, Ontario, 2–3 September 2004. The symposium had two primary goals:

- to present key achievements in Canada's forest genomics research, in the format of broad strategic overviews, to the forest sector and to the biotechnology science and policy communities; and

- to provide a forum for joint discussions on the positioning of future strategic research in this area, in order to optimize benefits to Canadians.

The symposium benefited from the momentum created by the Second Canadian Plant Genomics Workshop, 29 August–1 September 2004. Hosted by Université Laval, Québec, Quebec, the workshop brought together plant genomics speakers of international calibre and Canadian leaders in genomics research. The Forest Genomics Symposium, however, opted for a more generalist approach than the workshop in order to reach the nonspecialist.

The symposium was well-attended with over 70 participants from 12 federal government departments and agencies, nine universities, two forest research institutes, two provincial forestry departments, two national forestry associations, and one forest biotechnology company. The participants who completed the evaluation form expressed a high degree of satisfaction with the event. They appreciated the excellent quality of the presentations and found the discussion sessions particularly useful. Perhaps most importantly, the Canadian forest genomics scientific community was able to take advantage of this meeting to strengthen partnerships, explore new horizons, and strategically consolidate programs to be submitted for support by the federal government through Genome Canada and the Canadian Forest Service.

With this publication, we aim to provide a written record for the symposium and to extend awareness of Canada's forest genomics research activities to a wider audience. We trust that the enthusiasm expressed by the contributors about their research can be conveyed to you, the reader.

Anne-Christine Bonfils
Chair, Genomics for Future Forests

Introduction

Forest genomics opens a whole new world of possibilities for innovative approaches in the context of responsible intensive forestry and high-yield plantations in selected areas. Considerable progress is being achieved in the fundamental understanding of the genetics related to key systems such as wood formation, pest resistance mechanisms, and coevolution of interacting organisms. Innovative applications can now be envisioned for Canada's forests, such as growing trees with desired growth and wood quality characteristics and developing tools for early detection, diagnosis, and control of forest pests and diseases.

Funding for forest genomics has moved Canadian research to a very competitive level, allowing Canada to build scientific leadership in international forestry research. Between 1999 and 2004, the federal government invested $11 million in forest genomics research, administered through Natural Resources Canada's Canadian Forest Service (CFS). The federal government also supported the creation of Genome Canada in February 2000 and over the next four years invested $435 million in this organization. Of those investments, more than $24 million financed eight innovative environment and forestry research projects across Canada. These funds were highly leveraged with federal departments' existing infrastructure and resources, academic and private sector research partners, and provincial governments.

The CFS is at the leading edge of unique technological platforms for tree and insect tissue culture, molecular diagnostics, population genetics, biological control products, genetic engineering, and functional genomics. Its research is highly complementary to that carried out in Canadian universities. As well, the research capacity of the CFS in genomics has allowed profitable synergy with Genome Canada and participation in large multidisciplinary clusters.

Genome Canada is a not-for-profit organization dedicated to developing and implementing a national strategy in genomics and proteomics research for the benefit of Canadians and to ensuring that Canada becomes a world leader in those fields. It is the primary funding and information resource relating to genomics and proteomics in Canada. It has established five centres across the country (Atlantic, Quebec, Ontario, Prairies, and British Columbia).

This publication reports on presentations delivered at Genomics for Future Forests, the First Canadian Forest Genomics Symposium, hosted by the CFS and Genome Canada, 2 – 3 September 2004. The presentations covered the following subject areas:

- Genomics of wood formation and pest resistance in forest trees;
- Comparative and functional genomics in forest trees;
- Genomics of forest insect pests and their associated viral pathogens;
- Spin-off applications for bio-active molecules;
- Genomic approach to identifying fungal enzymes for the pulp and paper industry;
- Genomics of host–pathogen interactions; and
- Molecular markers for diagnosis of pathogens.

The symposium also featured two keynote speakers describing some of the major Canadian research involvements in international programs. Presentations are grouped under three subject headings in the symposium report — tree genomics, genomics of insect pests and their viral pathogens, and fungal genomics — reflecting how they were presented at the symposium.

Two discussion periods took place during the symposium. They covered future priorities for forest genomics research, particularly as they apply to the CFS and Genome Canada, and the role of the scientific community in addressing a range of environmental, ethical, economic, and social issues related to research. Key ideas from these sessions are summarized near the end of the publication. A poster session held during the symposium is not reported on here.

Welcoming Addresses

Brian Emmett | Assistant Deputy Minister, Canadian Forest Service, Natural Resources Canada

On behalf of the Canadian Forest Service, Natural Resources Canada, welcome to the first Canadian Forest Genomics Symposium.

The Canadian Forest Service (CFS) provides the science base that allows decision makers, such as forest managers and policy makers, to make sound decisions on our country's sustainable forest management. This is particularly challenging when the decision making involves a field that requires highly specialized knowledge such as genomics.

The CFS is the largest Canadian organization involved in forest biotechnology. The CFS mandate is the sustainable management of Canada's forests. Put simply, this means paying attention to the economic, environmental, and social implications of decision making in this area. Linked to this mandate is a need to continually improve, to keep pushing back boundaries so that society can make broader decisions, more-informed decisions. Genomics research is a key part of accomplishing this. It enables Canada to compete internationally and provides society with decision-making tools for natural resources.

At this symposium, you will hear about significant accomplishments in forest biotechnology by Canadian scientists; over the past 15 years, they have created a vision and built momentum for genomics research in this country. These accomplishments have allowed Canada to take a leadership position and to develop key technology platforms — tissue culture, somatic embryogenesis, genetic engineering, and biological control. Without these platforms, we would not have been able to make such tremendous advances in the field of forest genomics.

Genomics research helps us chart future uses of forests. It has the potential to strengthen our ability to compete successfully in the global forest economy and to support and improve the quality of life of Canadians, hence promoting the goal of sustainable management. Increasing tree growth rates, improving tree resistance to diseases and insects, enhancing the quality of wood fiber, and reducing exploitation pressures on forests are not only good for business but also for society. Everyone will benefit from the increased productivity, the employment opportunities created, and the reduction in environmental impact.

Forest genomics research in Canada has the following objectives:

- to advance the understanding and practice of sustainable development,
- to provide factual, unbiased forest science to support sound decision making, and
- to improve forest productivity and the standard of living of Canadians.

Genomics research is fascinating, but it is complex and therefore often misunderstood. Significant challenges exist that must be addressed to achieve our objectives. A basic underlying problem with genomics research is public perception; people are worried about it. They ask, should we be messing with nature in this way? This question is worth considering. The first victims of the Draft Riots that broke out in New York City during the American Civil War were the new mechanized street-cleaning machines, which had replaced the unskilled laborers doing the work before the machines were introduced. There has always been a reaction against advances in technology. However, the question about what we are doing with nature is a bit different. It is more ethically based, expressing concern about what path we are on as a society and as human beings.

As researchers and policy makers, we must understand that we do not operate in a bubble, isolated from the public. Reporting research findings within the forest science community only takes us so far. We have to relate to the public as we make scientific improvements and effectively address the policy challenges and concerns it raises. Just as foresters need a licence to harvest trees, we need a "social licence" to do our research. Only then will we obtain the funding and political support we seek. We have to take up the challenge to communicate what we are doing and why we are doing it. We must explain that forest genomics research is not just science-driven but is strategically designed to respond to priority public policy issues for potential users. We need to talk about our deep motivation — to do science to expand the choices society can make and to contribute to sustainable development. We must probe the political and social sensitivities underlying public concerns. The main thing Canadians want to know from us about genomics is whether its applications are safe. Genomics offers numerous exciting research opportunities, but we must assure Canadians that their health and safety are our first concern.

Public awareness of biotechnology — its applications and the issues surrounding it — often goes with the ebb and flow of public events and the media coverage of new developments. Nevertheless, public opinion research shows that for the

most part Canadians appreciate the potential benefits of biotechnology, particularly with respect to health. There remain information gaps and concerns, however, particularly with respect to broader ethical, social, and environmental issues. Canadians in general have confidence in their national regulatory system, but they often do not know how it applies to biotechnology practices and services. Our job is to bridge this gap in understanding. Let's identify misconceptions and transmit correct information about genomics in such a way that it reaches those outside the scientific community. My test of this is, can I explain it to a concerned citizen like my mom? We have to clearly link our research objectives to public policy priorities since the research is funded by taxpayers' money. The forest research community needs to communicate effectively to ministers so they can report to Cabinet the choices available to them and the trade-offs they are confronted with. We always have to be aware of the political and social context in which we practise forest genomics research.

I wish you every success for a fruitful discussion and a successful symposium.

Martin Godbout | President and CEO, Genome Canada

Genome Canada is proud to host the first Canadian Forest Genomics Symposium with Natural Resources Canada's Canadian Forest Service. We are also pleased that over 80 participants registered for the symposium. Welcome.

When Genome Canada was established four years ago, no one in Ottawa could even spell the word genomics! And yet, today, we have come here from universities, industry, and government laboratories to share our thoughts on how we can have large-scale projects in genomics and proteomics that can be competitive internationally. Quite an accomplishment in such a short period of time.

Most Canadians are aware of the human health implications of genomics; seldom do they consider the applications of genomics to agriculture, forestry, fishery, and the environment. Actually, the applications for genomics are endless. Although work in forest genomics began in Canada only a few years ago, it is internationally recognized. This came about not only because Canada has vast forests but also because genomics was seen to offer opportunities for improving the everyday life of Canadians.

Canada's reputation in genomics also owes much to the leadership provided by a special breed of scientists with a desire to understand the fundamental genetics of trees. Trees differ from other organisms that geneticists more traditionally have studied. Trees remain stationary throughout their entire lives. They do not have immune systems, yet some live for a hundred years or more without being destroyed by insects or pathogens. Trees deal with drought and nutrients in unique ways. We are only at the beginning of understanding intricate tree functions. To accelerate tree breeding requires a comprehensive knowledge of the genetic blueprint of a tree. At the same time, people want more forests for the benefit of the global ecology, for recreation, and for industrial use. Forests provide socioeconomic benefits for every Canadian. For these reasons, this symposium is important.

Over the next two days, some of the leaders in the field of forest genomics will discuss their projects. Genome Canada supports these scientists. To date, over $29 million has been invested in four large-scale forestry projects across Canada and, through international partners, in other parts of the world. For example, in 2003, Canadian and Swedish forest researchers began collaborating on a project involving the genetic responses of poplar to abiotic and pest-related stresses and to the environment. That announcement was made at a scientific workshop held here in Ottawa, which was attended by the Prime Minister of Sweden, Göran Persson, during his official visit to Canada. The former Secretary of State for Science, Research and Development, Dr. Rey Pagtakhan, was also there. This type of collaboration demonstrates the importance this field has to many nations. In the spring [2005], we will also hold our GE^{3}LS (Genomics Ethical, Environmental, Economic, Legal, and Social Issues) Symposium, which will focus on ethical issues related to agriculture and forestry. As this field continues to grow, we expect to engage in more collaborations and discussions. We hope this first Canadian Forest Genomics Symposium and the recent announcement of Genome Canada's Competition III will foster more collaborations and provide an opportunity to discuss the socioeconomic benefits that could be derived from the research projects presented today.

Setting the Scene

Geoff Munro | Director General, Science and Programs Branch, Canadian Forest Service, Natural Resources Canada

Most Canadians live in urban centers, yet Canada is often called a forest nation. Canada has 402 million ha of forest and other wooded land, of which 46% is considered commercially viable, meaning it grows relatively fast, produces trees large enough to be cut and used, and can be replaced or maintained. At present, only a small fraction (0.3%) of the forest is harvested, but this harvest generates an industry worth $80 billion per year and ensures the viability of about 300 communities. Canada is a forest nation because Canadians depend in part on the forest for their economic, social, and environmental well-being.

Forestry in this country has gone through a number of stages over the last two hundred years. The industry started in earnest with the harvesting of eastern white pines for the ship masts of the British Royal Navy. At the time, Canada's forest resources seemed inexhaustible. Later, when it became apparent that this was not the case, forest managers adopted a sustainable supply philosophy and tried to control the amount of forest cut so that there was always more available. Over time, the population began to see forests not only as a source of timber, but also to appreciate them for a range of other values including their aesthetics. These values were still seen as putting a constraint on harvesting. This attitude has recently matured into an approach called sustainable forest management — management that favors the economic development of the forest and social well-being of present and future generations of Canadians, without damaging forest ecosystems beyond their resilient capacities.

There are several major forest management challenges for Canada. First, Canada must generate the same economic benefits on an ever-smaller land base (the "much wood–little land" bottom line) to respond to public pressures. For example, in Ontario, growing public concern for the conservation of lands in their natural state led to the "Lands for Life" debate in the late 1990s and resulted in the establishment of a series of parks and protected areas. Second, Canada must manage the effects of a more extreme or changing climate on the country's forests. Finally, Canada must find innovative solutions, through initiatives such as Canada's Innovation Strategy, to increase forest productivity in general.

Research in forest biotechnology, and particularly in genomics, can help find ways to continue to sustainably manage Canada's forests in the face of the above-mentioned challenges. Various existing techniques can be used to produce faster-growing trees that provide a higher yield of pulp and timber per surface area of land, hence promoting intensive forest management. Other techniques (for example, phytoremediation of contaminated sites) can use the knowledge of forest ecosystem processes and resilience stemming from current ecological research to help maintain or restore biodiversity. Forest biotechnology also has the potential to make the wood pulping process more energy-efficient and to reduce the need for hazardous chemicals to control pests and diseases.

The Canadian Forest Service (CFS), Natural Resources Canada (NRCan), is actively involved in a number of key "horizontal" initiatives involving partnerships within and across governments and with universities, industry, the public, and other stakeholders. Forest 2020's Plantation Demonstration and Assessment, for instance, is a CFS-led initiative aimed at exploring the capacity of fast-growing hardwood tree plantations to mitigate the effects of climate change. NRCan's Program of Energy Research and Development is looking for ways to produce large quantities of inexpensive, sustainable energy. Above all, the CFS plays a major role in the Canadian Biotechnology Strategy (CBS), forestry being a key sector for biotechnology research. Canada's biotechnology agenda, of which genomics research is an essential part, has its origins in the 1983 National Biotechnology Strategy (NBS). Over the following years, the federal government made different commitments with respect to biotechnology. In 1998, after broad-based and sector-specific consultations, the NBS was renewed as the CBS; its vision is "to enhance the quality of life of Canadians in terms of health, safety, the environment, and social and economic development by positioning Canada as a responsible world leader in biotechnology."[1] A permanent secretariat and advisory committee were established, along with a results-based accountability framework. The years 1999 and 2000 saw major investments in forest genomics initiatives and the creation of Genome Canada. The scope of genomics projects has continued to enlarge over the years, resulting in stronger cooperation among governments, universities, and industry.

The CFS is the largest science-based forest research and policy-coordination agency in the country. Part of the challenge is harmonizing the policies developed at national

[1] Industry Canada, Canadian Biotechnology Strategy Secretariat. 1998. *The 1998 Canadian Biotechnology Strategy: An Ongoing Renewal Process*. Available online: http://strategis.ic.gc.ca/cbs

headquarters in Ottawa with the research taking place in the five CFS centers across the country. Through its centers and various partnerships, the CFS is at the leading edge of forest biotechnology research. Techniques that have been developed to address critical issues such as forest regeneration and forest protection include conventional tree improvement or biological control methods, cell and tissue culture, and advanced molecular biology techniques. Large investments continue to be made in tree improvement and the advances in this area have been considerable. Biotechnology increasingly enables Canada to regenerate and protect the forest and supports its sustainable forest management agenda. The current CFS Biotechnology Strategic Plan aims to provide knowledge-based technologies to contribute to enhanced tree productivity. Before this occurs, the CFS must ensure that the products derived from these technologies are safe and effective and that appropriate regulations and strategies for deployment are in place. The CFS must continue to foster the responsible development of forest biotechnology.

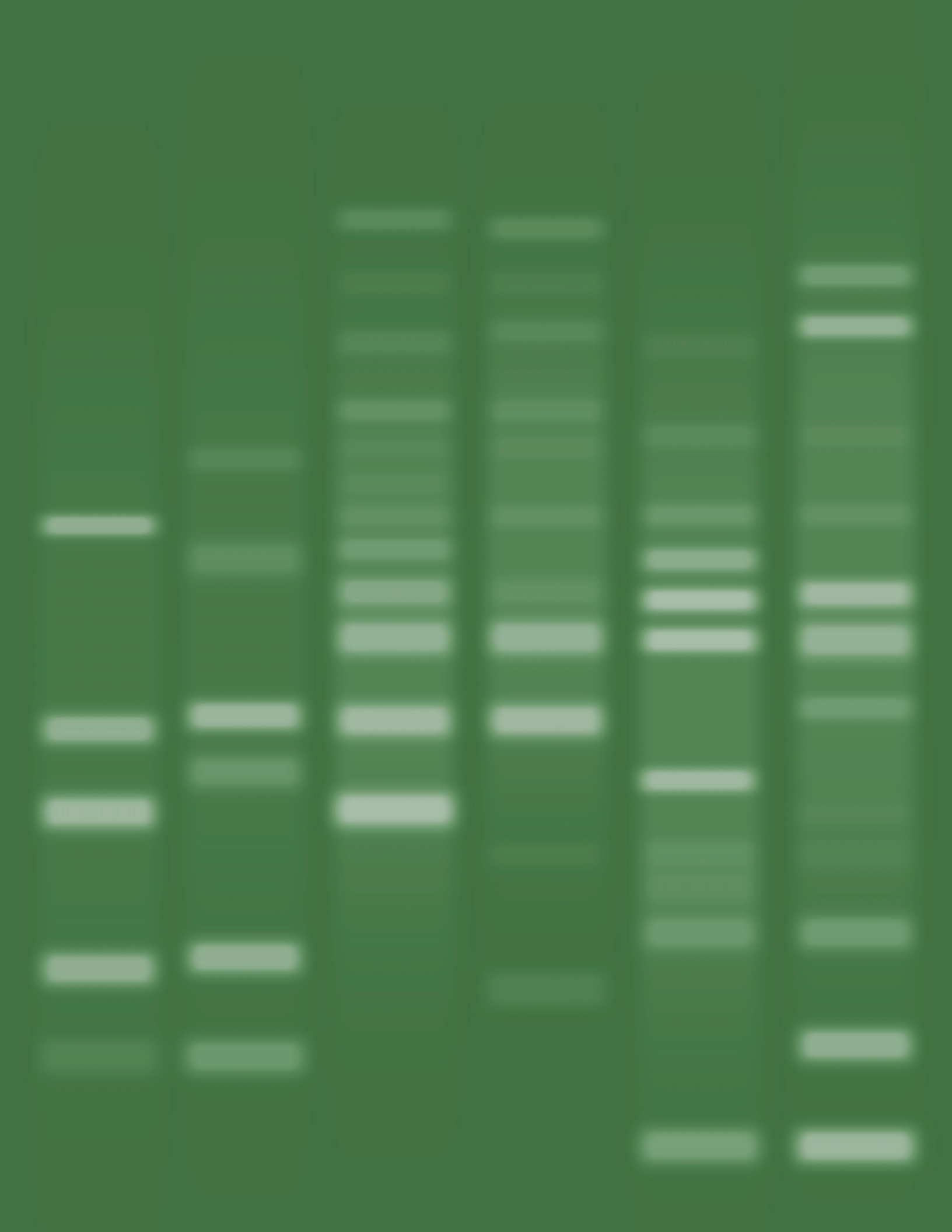
To move towards a 21st-century economy, the federal government has drawn up a blueprint for biotechnology in Canada. CFS research and development activities contribute greatly to meeting government objectives of innovation, stewardship, and commercialization in an international context, while moving Canada towards the "bioeconomy." Investments in biotechnology are still significant and are granted mainly through the CBS Fund, the funding of Genome Canada, and intramural genomics funds. The Treasury Board Secretariat has recently reviewed the management of expenditures on biotechnology by the government. The review called for more coordination of the research and policy development between government departments, universities, ant the private sector. The government is currently looking at realigning how the money is spent and how efficiently the research results are delivered to the community at large. In addition, the Treasury Board review highlighted interdepartmental and governance issues and called for a better definition of respective responsibilities among government departments.

Six departments/agencies — the National Research Council of Canada, Agriculture and Agri-Food Canada, Health Canada, Environment Canada, Fisheries and Oceans Canada, and NRCan — participate in the federal genomics research and development initiative, with a common framework for accountability. Each phase of the initiative lasts three years, and the second phase will be completed in 2005. The CFS has received about $11 million to date for forest genomics initiatives. It has established a management structure and a competitive process. Yearly budget allocations are subject to milestone reviews. The CFS genomics initiative is built by program, and the projects selected must deal with one of the following: forest tree production and protection systems; the design of molecular markers for diagnosis, monitoring, and early selection; the production of genetically improved trees; or the production of environmentally acceptable forest protection methods.

According to a multicriteria analysis of the number and impact of specialized scientific publications from Science-Metrix, a company that evaluates science, technology, and innovation, Canada led other countries in forest genomics research between 1992 and 1997, but fell behind France, Sweden, and Australia from 2001 to 2003. It is still not clear whether the other countries simply caught up to Canada or whether there was a significant decrease in scientific productivity here. It is worth noting that the Science-Metrix measurements were based on scientific publications produced, not on the number of biotechnology companies established or products at the commercialization phase. Other, perhaps more relevant, measures may be better in evaluating the success of biotechnology in any country. Nevertheless, this comparative analysis can be a "heads-up": while Canada was definitely ahead of its time in the early 1990s with its NBS, it must now work harder to maintain its competitive edge in forest genomics, a field where other countries also excel. This grading can also be seen as an invitation to seek collaborations with these other players.

During this symposium, a number of projects reflecting the scientific excellence of Canadian forest genomics research will be presented. Your feedback during discussion periods will help guide the direction of the strategic plan for genomics and biotechnology in the Canadian forest sector and the leading role of the CFS.

TREE GENOMICS



Canadian Research Involvement in International Forest Tree Genomics Projects

Keynote Address Brian Ellis | Michael Smith Laboratories, University of British Columbia, Vancouver, BC

I am going to speak about Canadian involvement in international efforts in forest genomics, and more particularly about one of its best examples, the work that has been done on the poplar (*Populus*) genome. First, I will briefly explore the concept of "green genomics", that is, the genomics of plants in general; then I will explain the reasons why poplar has been selected as a model for tree genomics and focus on the poplar genome project; and finally, I will look at Canada's present status and future directions in forest genomics.

There are probably about 250 000 plant species, which clearly makes it impossible to study them all at a genomics level. Some are more important commercially than others, but typically the most important commercial species have genomes too complex to be studied effectively. It is therefore essential in plant genomics to have good model species. The model plant first selected for genomics study was the well-known plant, *Arabidopsis thaliana*.[1] In 1996, an international team of scientists was formed[2] to do the sequencing and in 2000, *Arabidopsis* became the first plant to have its genome sequenced. Plant genomes are enormous information-rich entities, and as a result, scientists can seldom say with certainty that a genome is completely sequenced. Nevertheless, the international collaboration on *Arabidopsis* genome sequencing produced a high quality product: 125 million base pairs (Mbp) of genetic information distributed over five chromosomes.

The *Arabidopsis* genome sequence created a major new resource for plant researchers and its availability and quality has galvanized plant research. Using *Arabidopsis* as a search term in the title, I have determined that the number of published papers that address *Arabidopsis* has increased steadily over the last 20 years, but even more so since the genome sequence became available in 2000.

The obvious impact of sequencing the *Arabidopsis* genome stimulated interest in finding a model organism specific for trees. Clearly, there are differences between the herbaceous annual *Arabidopsis* and these large, long-living, woody perennials. Moreover, trees are polyphyletic; that is, as a group, they do not have a single unique ancestor. Trees also typically have very large genomes; for example, the genome of any pine species is several times the size of the human genome (about 27 000 Mbp), which is much larger, in turn, than that of *Arabidopsis*. This size creates a major technical challenge for sequencing tree genomes, but fortunately, the technology and procedures used in sequencing are continually becoming faster and cheaper, which makes it easier to consider sequencing larger genomes. Nevertheless, the sequencing of pine or spruce genomes has yet to be undertaken.

Compared with most other trees, however, poplar has a relatively small genome (about 500 Mbp), well within the reach of current sequencing technology. In addition, poplar is commercially important and widely distributed and grown throughout the Northern Hemisphere. It is used as a short-rotation biomass crop and as a source of solid wood and fiber. From the point of view of effectively applying genomics technology, poplar is easily micropropagated and its DNA can be transformed to study the effects and behavior of genes of interest.

Recently, therefore, the United States Department of Energy (DOE) decided to sequence the poplar genome. The black cottonwood (*Populus trichocarpa*) tree selected as the DNA donor was a female specimen located in Washington State. The extracted DNA was provided to the Joint Genome Institute (JGI), operated by the University of California for the DOE. An international consortium of researchers interested in poplar, and in the fact that it was about to become a model tree, was formed to collaborate and build resources for the poplar genome project. I will describe some of the contributions of these international collaborators.

The JGI chose the sequencing method—whole-genome shotgun sequencing, which I will describe later—and provided the technology platform for it. JGI researchers are currently doing the assembly of sequenced DNA fragments and primary gene annotation; that is, they are applying names to the genes identified in the sequence. Oak Ridge National Laboratory (ORNL) in Tennessee, also associated with the DOE, helped map those genes, as part of the integration of the actual DNA sequence with the poplar genetic map, and also participated in annotation of the genome sequence.

Canada was also actively involved in the international consortium, both through the University of British Columbia's Treenomix project, where I was one of the four principal investigators (with my colleagues, Carl Douglas, Jörg Bohlmann, and Kermit Ritland), and through the Arborea project in Quebec.

Both the Treenomix and Arborea projects were funded by Genome Canada to foster the application of genomics to the Canadian forest resource. The Treenomix team worked with the Michael Smith Genome Sciences Centre (GSC) in Vancouver to generate a physical map of the poplar genome as an aid to genome sequence assembly. In the process, we helped create a resource for future research—a "library" of bacterial artificial chromosomes (BACs) containing large chunks of the DNA from the poplar genome. The ends of the BACs were sequenced, and the unique sequence "fingerprint"

[1] *Arabidopsis thaliana*, commonly known as wall cress or mouse ear, is a small flowering plant and a member of the mustard (Brassicaceae) family.

[2] Arabidopsis Genome Initiative (AGI).

of each BAC was collected and used to define the overlaps between different BACs. These overlapping BACs could then be assembled into a "tiling path" that together with the BAC end sequences would help define the physical structure of the genome. The Treenomix team also fully sequenced complementary DNA (cDNA) copies for thousands of poplar genes to generate a library of established verified gene sequences that have been crucial for improving the initial genome sequence assembly. Interestingly, the Treenomix project was not originally funded by Genome Canada to work on the sequencing of the poplar genome, since that effort had not yet been announced. However, Genome Canada and Genome British Columbia (BC) allowed us to divert a significant portion of our funding to collaborate on the DOE sequencing effort when it was realized how valuable our contribution would be to the success of the sequencing project.

In total, over 250 000 expressed sequence tags, or ESTs (short-sequence reads off poplar cDNA clones) have been collected in various centres. Both Sweden (Umeå Plant Science Centre) and Canada have been major contributors in this activity. In Belgium, Ghent University has used its expertise in bioinformatics to help analyze the genome organization and to associate this with patterns of biological data.

The JGI sequenced the 485 Mbp poplar genome using a whole-genome shotgun sequencing approach. Simply put, this method involves slicing the genome's DNA into pieces of various sizes, from relatively small to much bigger, and feeding randomly selected pieces through DNA sequencing machines. Each sequencing run produces about 500 bp of usable data. The fragments are then discarded and the process is repeated over and over. Eventually, every part of the genome's DNA will have been sequenced multiple times, but in a random fashion. This "shotgun" approach results in an enormous number of pieces of data that need to be linked to re-create the true genome sequence. Fortunately, the JGI has super computers and assembly software capable of comparing all the short pieces of DNA sequences and finding where they overlap. Longer, composite sequences are thus generated (contiguous stretches of DNA, or contigs). Through more computer work, these contigs are assembled into extensive regions of the genome called "scaffolds". The JAZZ software package used by the JGI has generated several thousand such scaffolds, but a small subset of these consists of major scaffolds that cover virtually all of the gene coding regions. Ideally, the number of scaffolds would collapse to 19 because poplar has 19 chromosomes, but this goal will be difficult to reach because of the many stretches of repetitive sequence that tend to confound the assembly programs. Fortunately, the ORNL recently received a National Science Foundation award to continue working on these scaffolds in order to reduce the gaps and further improve the assembly. However, based on the data generated so far, the poplar genome comprises about 485 Mbp, and the portion of the sequence reliably assembled is estimated to contain about 98% of the actual gene-coding regions and thus most of the biologically relevant information.[3]

We now have a poplar genome sequence — 485 Mbp of it — but what about the genes themselves? Three research teams (the JGI, ORNL, and Ghent University) have been working in parallel to identify and locate the genes encoded within that sequence. This computational approach tentatively identified around 45 000 gene models and gene predictions. Biologists fine-tuned the predicted gene annotation during an intensive annotation workshop called a "jamboree". During the jamboree, experts from various biological areas attempted to verify the computed gene calls, based on their knowledge of particular families of genes.[4]

With 45 000 gene models, poplar appears to be better endowed genetically than *Arabidopsis*. The number of poplar genes may be overestimated but it is also possible that poplar has evolved to make use of a more elaborate gene set than has *Arabidopsis*. Since more than 90% of the poplar full-length cDNA sequences — the readout of individual genes — are similar to the corresponding *Arabidopsis* genes, poplar and *Arabidopsis* are clearly closely related, which is not surprising. Where poplar appears to differ is in the extensive gene duplication that has taken place in many poplar gene families. However, it will require more biological research to establish whether these duplicated genes are making important new contributions to the ecological success of poplar.

The efforts to sequence the poplar genome have also resulted in the development of new research tools for the study of gene expression patterns. For example, DNA microarrays (spotted arrays of gene messages) are being built. To build cDNA microarrays, individual cDNA copies representing the output of a large part of the coding genome are placed on little glass slides at enormously high density. Current poplar cDNA microarrays have up to 25 000 genes on a single microscope slide. Affymetrix is also preparing commercial poplar microarrays based on their proprietary oligonucleotide printing technology. These are powerful resources that can be used by research groups wanting to track the expression of poplar genes under any experimental conditions of interest.

What has been accomplished? We finally have the first blueprint for a tree, thanks to a major international effort. We have new genomics tool-kits for the study of tree species. Governments are better informed about forest genomics, and the forest industry too is now more aware of genomics than it was two or three years ago. However, the forest sector remains somewhat ambivalent about genomics because it often confuses genomics with genetic engineering. We in the forest science community have a responsibility to inform

[3] On 21 September 2004, the JGI announced that it and its collaborators had completed sequencing the genome of black cottonwood.

[4] The annotation jamboree of the poplar genome took place at the JGI in December 2004.

people about the crucial differences between genomics and biotechnology.

The first tree genome sequence presents great opportunities for other initiatives in forest genomics in Canada. Forest genomics research platforms are now well developed, particularly in British Columbia and in Quebec, and Canada also has a strong plant biology research community ready to extend and apply these resources. Furthermore, the forest is a major Canadian bioresource and the excellence of Canada's accomplishments in forestry and genomics is recognized around the world.

Forest genomics in Canada, however, faces some challenges. Poplar is not a conifer and Canada is conifer country, which means that future genomics efforts will now have to be focused more on commercial species such as pine and spruce. There has also been serious erosion of the Canadian forest genetics resources that were once so well developed in this country. Loss of important breeding populations can make it difficult to extract full value from the new genomics tools. Many tools have been developed in the course of Genome Canada supported forest genomics research but they are still not widely accessible to the community. It is unclear, for example, how the thousands of poplar cDNA clones will be made available when Genome Canada projects end, or who will ensure that the results achieved from this huge investment will be effectively exploited. Overall, Canada lacks a long-term plan and national strategy for forest genomics research. Funding for forest genomics has been short-term and unstable, when this type or research really needs continuity.

Another challenge comes from the forest industry, which places more emphasis on harvesting than on the benefits it could get from tree genetic improvement or other applications of genomics. Finally, there is a challenge from interests outside the country; several foreign biotechnology companies have been performing forest genomics research longer than Canadians and are quickly capturing the associated intellectual property.

Nevertheless, Canada is in a good position to seize the opportunities created by recent research on broadleaf trees and to link genotype to phenotypes important for the Canadian landscape. A great opportunity also exists to apply knowledge gained from poplar genomics research to develop a model conifer genomics tool-kit. As pointed out previously, a long-term strategy for forest genomics is needed in Canada and should be implemented as soon as possible. Canada as a nation needs to decide whether or not it is going take advantage of these unprecedented opportunities and continue to be a leader in forest genomics research.

Functional Genomics of Regulation in Forest Trees

John MacKay | Centre d'étude de la forêt, Université Laval, Québec, QC

"Functional Genomics of Regulation in Forest Trees" is the theme of the Arborea project and the title of this presentation. Arborea's theme originates in the project's commitment to investigate the function of genes on a large scale. Four institutions have partnered to lead Arborea: Université Laval (Québec); Carleton University (Ottawa); the Laurentian Forestry Centre (Québec), Canadian Forest Service (CFS), Natural Resources Canada; and the Center for Computational Genomics and Bioinformatics (CCGB), University of Minnesota (Minneapolis). Arborea is one of the two large projects in Canada[1] delivering large-scale tree gene discovery. This is unprecedented and makes the two projects world-class. As well, these projects have assembled a unique set of experimental platforms to analyze the role of genes in wood formation and forest health.

I am the project leader of Arborea and will describe this project in detail in the latter part of my presentation. To start, I would like to give you my perspective on forest genomics. I will discuss the linkage that exists between genes and the manufacture of forest products and how that linkage is essential to the competitiveness of the forest industry in Canada.

When I was a scientist at the Institute of Paper Science and Technology in Atlanta, Georgia, Paul Kibblewhite, a world-renowned specialist in forest products from New Zealand, was invited to give a seminar. His outstanding talk was about the various properties and attributes of wood fibers (mostly the softwood fibers) and the importance of these in the paper-making process. He compared different kinds of wood fiber and then indicated that the gold standard was Canada's softwood fibers.[2] These fibers are considered so desirable because they tend to be long, imparting strength to the paper; they are thin-walled, facilitating their formation into sheets; and they are slender, adding smoothness to the paper. For many decades, Canadian softwood fiber was indeed considered the highest-quality fiber. This led to an impressive pulp and paper industry throughout Canada and especially in eastern Canada.

Industrial papermaking methods were developed in the first half of the 20th century and carried through into the latter part of the century, largely driven by the belief that there was an unlimited wood supply. The Canadian forest products industry carved out a strong niche based on a high-quality fiber supply coupled with relatively low production costs. Canada has been able to maintain or increase spruce productivity over the years and ensure a supply of fiber to industry by extensive,[3] rather than intensive, forest management. In addition, significant tree improvement through genetic selections has taken place, particularly in the case of spruce. Using estimates drawn from my experience in eastern Canada, I can show that forest productivity was improved this way, from rather low [3 m^3/ha] to quite high — at least 10 m^3/ha — with a harvest age ranging from 35 to 60 years. However, in the latter half of the last century, other models of forest management emerged in competitor nations, and Canada's competitive edge started to decrease, slowly at first, then faster and faster. For example, Brazil grows eucalyptus trees to about 30 m in six years. It has achieved this through intensive plantation management and genetic selection, taking advantage of its favorable climate and soil. Brazilian forest productivity has soared to the point [about 50 m^3/ha] of posing a significant threat to Canada's forest industry based on a low-cost–high-volume commodity model.

Canadian competitiveness in its traditional low-cost–high-volume forest product commodities, such as newsprint, market pulps, and lumber, has been significantly eroded. The forest industry in Canada has, for the most part, concentrated on ensuring its supply of raw material, without worrying about its quality or how to optimize its use. It has led by tightly controlling production costs. A good example of this is softwood lumber. Profits have been maintained over the years by increasing production and export volumes to capture market shares. This strategy is in part to blame for the imposition of tariffs by the United States on Canadian softwood lumber, which has led to the closing of mills in Canada. The strategy no longer works and the forest industry is now refocusing to remain competitive.

Some essential elements need to be changed in how Canada's forest industry operates. First, it must determine how to make the most of the quality and use of its raw material, wood. Second, it needs to invest more in the engineering and design of products; this is already occurring because of the imposition of US tariffs on softwood lumber. Finally, it has to develop appropriate business strategies based on new approaches to product innovation, customer needs, value-added products, and the like. These three elements are interconnected.

Optimization of raw wood material can strongly benefit from research in the fields of genetics and genomics. Canada needs to produce forest products that have a higher value. There are a number of low-value commodity products, for example, newsprint, from which the pulp and paper industry today does

[1] The other is the University of British Columbia's Treenomix.

[2] Specifically, those of black spruce, *Picea mariana*.

[3] Basic forest management relying largely on natural regeneration and protection of the forest resource from insects and fire.

not generate large profits and even, occasionally, sells at loss. Other pulp and paper products like tissues are highly valued and generate larger profits, but the right type of raw material is required to target this kind of product. Another approach is to develop forest products that have better performance. Here again, there is an opportunity for Canada's forest industry to carve out niches and to develop specialty products. A third approach is to reduce production cost. Reducing cost has been the forte of countries like Brazil and Indonesia — their productivity is high and their labor costs are low. For Canada, this does not seem to be an advantageous strategy anymore. Forest industry members do not agree yet on what is the winning approach here, but they have to think about the impact the chosen approaches will have on the value-recovery chain. Specifically, what are the desired qualities in trees so that tree breeders can work to improve them?

Trees are undomesticated; thus, genetic improvement has to start with wild populations, which are more variable. In traditional tree improvement, the best trees are selected and bred to obtain an improved population. This is a time-consuming process because one has to cross the trees, grow the progenies, measure them, and move on to the next step. It is a process that is slow, incremental, and generally focused on forest productivity. New and more aggressive approaches will involve clonal propagation and refocusing on the quality, performance, and value of the wood product, not just on forest productivity. As well, the trend in tree improvement worldwide is to use a combination of genomics and molecular techniques such as genetic engineering.

Linkage maps were previously developed by several research groups, including Oak Ridge National Laboratory, Tennessee, for pines, spruces, poplars, and a few other species. They can be considered an approximate representation of chromosomes and show regions of genes controlling wood density throughout the genome. Although this was a step in the right direction, scientists soon realized that each of these regions identified by mapping the phenotypic variability of phenotypes — called QTLs for quantitative trait loci — contains hundreds of genes. For QTLs to be useful in tree improvement, we would need to know which of the genes in any given region is the one that controls the underlying variability in the trait. This is especially important since as one looks at other traits of significance, more and more regions (and thus hundreds and hundreds of genes) are revealed that have a potential role in trait expression. To sort out which genes are important and then use them effectively in molecular breeding, scientists must understand the function of genes and their global regulations in the genome. I will use as an example a gene we studied at Université Laval to illustrate how gene information can be used to learn about wood properties, improve upon wood properties, and influence product manufacture and performance. This gene, which occurs naturally in pines, confers increased reactivity to wood, hence producing a more soluble lignin that is easier to pulp. Using molecular markers, we identified which progeny of a tree with the altered lignin gene also had a copy of this gene. Then we looked at pulping properties. We found that less energy (that is, shorter cooking time) was required to manufacture the pulp from the trees with the altered lignin gene and that the paper sheets produced from the pulp of these trees had better strength properties than sheets produced from the pulp of trees that did not have the gene. This probably can be attributed to the shorter cooking time required to pulp the lignin-altered trees, longer cooking being known to degrade some of the desirable pulp traits.

Having set the stage, I will now talk about Arborea. One of the objectives of the project is gene discovery and for this we are using two approaches: expressed sequence tag (EST) sequencing and activation tagging. You have already heard from Brian Ellis about how ESTs have been used in the sequencing of the poplar genome. I will discuss our use of ESTs in the sequencing of the spruce genome a little later. The second approach, activation tagging (or gain-of-function mutagenesis), is being used in our work on poplar. In activation tagging, you let the trees tell you what genes they have and what those genes are doing by changing gene expression in the trees and looking for variations in their phenotypes. Activation tagging is an important platform for discovering genes and revealing their function. It has allowed us to create a unique collection of lines that will be available (as of 2005) for continuation of work over the long term. Sharon Reagan, based at Carleton University, leads this part of the Arborea project.

Given the volume of work that has been done by the scientific community on poplar, we have focused most of our gene discovery efforts on spruce. Our results from EST sequencing of spruce are now available. We have run about 73 000 sequencing reactions on complementary DNAs (cDNAs) — which represent expressed spruce genes. Of course, these sequences have to be processed to remove low-quality sequence information, trimmed to remove vector sequences, and assembled to provide an estimate of the number of genes they actually represent. Our current estimate is 16 000 spruce genes; in reality, it is probably a little lower. We have been working on annotating a unigene set to assign functions to these genes. As sequence data become available, we are releasing them into public databases and into a project-specific publicly accessible database on the Web called BioData. The clones provide reagents for us to create microarrays to find single nucleotide polymorphisms (SNPs), match genes, and mine data regarding gene function. The information is being put in relational databases called SpruceDB and ForestTreeDB, which can be queried in multiple ways and which will be publicly accessible. The CCGB is collaborating in the development of the database. We are also committed to supporting the distribution of spruce clones derived from our project and should be able to make our collections available in their entirety by early 2005.

Another objective of Arborea is large-scale investigation of gene function and diversity. We are targeting wood development,

a complex process, and tree defence response. In particular, we are looking at the genes responsible for signaling molecules in poplar and how they are involved in the response of trees to pathogen attack. To analyze gene function and diversity, we use candidate genes expressed in transgenic trees; the goal is not to deploy transgenic trees, but to use them as an experimental platform. We can then analyze the trees at the whole transcriptome level using microarrays and subsequently identify target genes for tree improvement or assessment of tree performance. We are looking at the same genes from a gene diversity perspective with the support of the transgenic trees technology platform developed by Armand Séguin's group at the Laurentian Forestry Centre. This platform, a feature unique to Arborea's Canadian initiatives, enables us to produce and analyze transgenic spruce on a significant scale. Last winter, for example, our greenhouse contained nearly 6 000 transgenic spruce seedlings. Furthermore, we have developed first-generation microarrays with unigene sets of around 9 000 spruce genes and 3 700 poplar genes. We have established a pipeline for data management and analysis and the next step is to coordinate Arborea with Treenomix so that our second- and third-generation arrays are stronger and more coordinated at the Canadian level. Again, the arrays are part of our strategy of distributing resources to make them available to other Canadian scientists.

The transgenic approach to researching gene functions allows us to perturb the tree system to understand how it works. The knowledge gained can be useful to the forest industry. For example, when a transcription factor is overexpressed, it causes an imbalance in the ratio of xylem to phloem tissue. Trees altered for this overexpression can then be used in conjunction with our microarrays to understand how gene regulation is controlled at the global level. These data are now being used to select candidate gene markers that will be tested to discover markers of genetic selection of trees with more desirable wood properties. The genetic selection methods that we hope to develop with the use of marker-assisted selection decrease the selection time and increase selection accuracy because selection is no longer based on the phenotype, but on the gene.

In closing, I would like to acknowledge the researchers affiliated with the four partner Arborea institutions; they are making important contributions to forest genomics.

Genetically Improved Trees and Interacting Genomes in the Forest Pest Context

Armand Séguin | Laurentian Forestry Centre, Canadian Forest Service, Natural Resources Canada, Québec, QC

At the Laurentian Forestry Centre in Québec [Canadian Forest Service (CFS), Natural Resources Canada], I am a member of a team working on two projects that can be put in the overall context of CFS research activities in genomics.[1] One of these projects concerns the production of genetically improved trees and the creation of a National Tree Functional Genomics Platform for collaborators; the other deals with interacting genomes in tree defence response for the production of environmentally acceptable forest protection methods,

Just like a chemist needs a periodic table of elements to make plastic, a biologist needs information about the genetic code to understand how living organisms are made. All the genetic information is contained in DNA, which codes for proteins that are subsequently arranged to form a living organism. The genetic code of all living beings is similar to a computer program. All the information to accomplish a specific task is written in a specific programming code. However, living organisms are much more complex than computers, and their actual traits depend on interactions between the genetic code and the environment. The genetic code is encrypted in chromosomes located in the cell nucleus, the control panel of the living cell.

DNA is composed of four building blocks or bases: guanine, adenine, thymine, and cytosine (G, A, T, and C). They are the basic code or alphabet that spells out the information contained in genes. Genes are grouped to make up chromosomes. The genetic code is universal since the same language is shared by all living organisms; this allows one to compare genes from bacteria, humans, and plants. All the genetic material in the chromosomes of a particular organism (that is, its genome) is contained in every cell of that organism, and thus each cell has all the coded information to do every cellular task of the organism.

Traditional genetic crosses permit the exchange of chromosome segments carrying one or more desired genes, and this genetic mixing produces individuals that have superior characteristics for a given phenotype. However, exchanged chromosomal regions may sometimes have negative non-target effects. On another front, genetic engineering can now be used to introduce a gene conferring some beneficial trait, such as insect resistance, into a particular genotype. The result is the acquisition of a new character. The usual method involves cleaving a DNA chain at a specific location with a restriction enzyme (these enzymes serve as molecular scissors). The restriction enzyme is used to cut a plasmid, a molecule of extra-chromosomal circular DNA found in certain bacteria. The DNA fragment is inserted into the plasmid at the site of the cut. The resulting molecule is called a construct, a specific vehicle to move DNA from one organism to another. The construct can then be introduced into a recipient cell. Plasmids of *Agrobacterium tumefaciens*, a naturally occurring soil bacterium, are widely used to transfer genes to plants cells, including tree cells. During the natural processes of *Agrobacterium* infection of a plant cell, the construct will insert the new gene into the host tree cell.

Damage to trees caused by insect pests and fungal pathogens greatly reduces the volume of Canadian wood that can be harvested. At the same time, new environmental policies dictate a complete banning of chemical pesticides in natural ecosystems. Recent development of transformation techniques has made possible the improvement of crop plants by the introduction of cloned genes and offers the opportunity to engineer pest resistance. Based on the well-documented effect of the crystal proteins from *Bacillus thuringiensis (B.t.)* on insects, we genetically engineered white spruce (*Picea glauca*) with a synthetic *B.t.* toxin gene against eastern spruce budworm (*Choristoneura fumiferana*). As a result, the trees can produce this biological insecticide themselves. Transgenic trees derived from the present work have shown a significant resistance to spruce budworm in laboratory testing; total resistance has even been observed in many lines. The *B.t.* insecticidal protein has been used commercially for many years and resistance to it in open-field populations of spruce budworm has not been reported. However, several common species of other pest insects have developed resistance to the *B.t.*, both in the field and the laboratory. Alternative tools for biological control of forest pests thus need to be developed. At LFC, we are now attempting to identify genes, such as defensins and protease inhibitors, involved in tree protection from forest pests.

During the last decade, the Human Genome Project prepared the way for a sophisticated and exciting science, that of genomics. Genomics means more than working exclusively on a few selected genes; it is the science of the genome. Researchers in genomics want to decipher and understand all the genetic information contained in an organism's chromosome in order to determine the relationship between gene activities and cell functions. Genomics can be subdivided into **structural genomics** (the development of genome maps and sequences) and **functional genomics** (the discovery of the biological function of particular genes and how gene-product sets work together). In my opinion, genomics is the key to the future of the biotechnology industry. It not only applies to genetic engineering; it could also, for instance, greatly affect tree breeding.

Now that the need to protect the world's forests is well recognized, better management practices and technologies are

[1] These activities are Programs 3 and 4 of the CFS Genomics R&D Initiative.

required to conserve and better use current resources. In Canada and several other countries, selection and breeding of productive trees to be grown in tree plantations has been ongoing for a few decades. Genomics research is expected to identify DNA sequences or markers to help accelerate and improve the accuracy of tree selection and breeding. This application is referred to as molecular breeding. There is no need or intention to convert Canada's forests to transgenic plantations, monocultures, or fiber farms. Only a small fraction (about 0.1%) of the total forested area in Canada is currently replanted each year. Productive tree plantations managed intensively for wood and wood fiber are part of an overall strategy to meet growing demands for wood-based products without the need to encroach on old-growth forests and wildlife habitats. They will help ensure the long-term sustainability of Canada's forest resources.

Another part of our program is related to interacting genomes: we evaluate how tree genomics can help improve the protection of trees from pest attack. Trees have long life cycles, an indication that they have developed distinct defence mechanisms allowing long-term survival. At present, forest pest problems are largely understated, and many pathogenic fungi are progressing in natural and planted tree stands. In addition, given the increases in international trade and the effects of global climate change, forest pest problems are likely to become even more severe. It is thus important to understand how trees defend themselves against forest pests. Introduced forest pests can be particularly problematic as illustrated by the ravages of Dutch elm disease in eastern North America during the last century.

Host–pathogen interactions are important; in human health, the example of influenza is eloquent. Each year, there is a new strain of influenza virus against which humans, as hosts, produce antibodies. There is a host–pathogen interaction: humans try to fight against the influenza viruses and at the same time, viruses try to overcome our defence system. The same phenomenon happens in forests between trees and pathogenic fungi or insect pests. Trees use different mechanisms or strategies to defend themselves. They do not have an immune system to produce antibodies; rather, they produce complex chemicals or proteins. The production of these compounds can either be continuous, as in the case of waxes or lignin, or induced only when the tree is attacked. Pests usually adapt to the defence mechanisms developed by trees, for example by producing enzymes that detoxify the plant defensive compounds.

Although the regulation of genome expression has been widely documented in plants, the simultaneous study of two interacting genomes (those of the host and the parasite) is still largely unexplored territory. Poplar has become the model tree species for genomics research due to its extensive genetic resources, rapid early growth, and easy clonal propagation, as well as the existence of routine transformation protocols for it. Its genome sequence has recently been completed.

In addition, the genomes of a cortege of microorganisms associated with trees are being sequenced, and the LFC is participating in a proposal to completely sequence the genome of the poplar rust *Melampsora*, which would be the first tree pathogen to be sequenced. We have developed expertise in characterizing host–pathogen interactions and have already sequenced cDNA libraries of compatible and incompatible host–pathogen interactions in the poplar–*Melampsora* pathosystem.[2] We expect that such knowledge will lead to the discovery of novel resistance genes in the host and associated pathogenicity genes in the pathogen pest. These new gene targets could be used in molecular tree breeding programs. Identification of pathogen genetic profiles will make it possible to understand and direct the evolution of the pathogen as it responds to host resistance. This project should help Canada develop tools and resources for more sustainable forest stewardship in response to pests.

Biological control of pests using oligosaccharides to stimulate tree defensive mechanisms is also particularly promising. We have done some work with chitosan oligosaccharide derived from chitin, a constituent of the exoskeletons of arthropods such as crab, lobster, and shrimp. Industrial waste of this biological material can be recycled into a product for immunizing trees against pests. Potential applications of this technology include the control of pests in tree nurseries and plantations. We have shown that application of chitosan oligosaccharides induces the expression of various tree genes; the current challenge for us is thus to relate the expression of those genes specifically to tree defence responses.

Overall forest genomics is a knowledge-based approach. This means that the research performed and the models developed on poplar will be applicable to many other tree species and situations. For instance, the knowledge generated in studying poplar–*Melampsora* rust interactions could also be applicable to other pathosystems such as eastern white pine–white pine blister rust (*Pinus strobus*–*Cronartium ribicola*). This research could also generate economic benefits through the collection of intellectual property rights on discovered gene functions, molecular markers, and promoters.

I would like to thank the CFS Genomics Research Initiative, the Canadian Biotechnology Strategy, Université Laval, the Natural Sciences and Engineering Research Council of Canada, and Genome Canada–Génome Québec for financial support of our research, and the researchers and postgraduate students from Université Laval and Université de Sherbrooke for their commitment to it.

[2] A **compatible** host–pathogen interaction results in the successful invasion of the host by the disease; an **incompatible** one occurs when the disease induces a defence response in the resistant host, resulting in little or no disease invasion.

Genomics and Functions of the Insect-Induced Defences in Spruce and Poplar

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Brian Ellis has already introduced many aspects of the Treenomix project in his presentation [see page 17]. He and I, along with Carl Douglas and Kermit Ritland, are the four principal Treenomix researchers. In my presentation, I will give you an overview of Treenomix and of its gene discovery strategy. Then I will describe the forest health aspect of the project. To do so, I will address some of our targets and key resources in genome technology development, as well as some applications and selected discoveries. I will talk briefly about the functional discovery of conifer defence genes. Finally, I will propose some future directions for forest genomics.

Treenomix, the first forest genomics project funded in Canada, is supported by Genome British Columbia (BC) and the Province of British Columbia. As well as substantial funding, Treenomix has received constant management support from Genome BC and day-to-day research support from the province. Treenomix aims to integrate structural, functional, and comparative genomic approaches using the herbaceous plant *Arabidopsis* as a reference system. The project focuses on forest health, closely aligning its research with some key objectives of the tree improvement program of the BC Ministry of Forests and Range. Nationally, it has university, government, and industry partners; internationally, it has entered into formal partnerships with organizations in several countries, including the United States, Sweden, and Finland. Treenomix, together with other forestry projects funded by Genome Canada, has contributed to positioning Canada as an international leader in forest genomics.

Spruce and poplar are the study subjects of Treenomix's gene discovery efforts. Brian Ellis has already described Treenomix's contribution to the International *Populus* Genome Consortium. In addition, Treenomix researchers are studying spruce and poplar to identify and characterize the function of candidate genes relevant to the formation and quality of wood and fiber and to pest–host interactions.

Because of Genome Canada's major investment in forest genomics, researchers now have genomic tools specific to forestry. Treenomix is making DNA markers and public domain resources available to other researchers. It is also creating intellectual property such as patents.

Furthermore, Treenomix has produced highly qualified professionals, trained specifically in forest genomics. None of the project leaders had training in genomics. The team assembled to work on the project comprised postgraduates from diverse backgrounds, whose enthusiasm to work on forest genomics issues quickly produced a well-needed body of experts. Today, the Treenomix professionals also provide research support to academic, government, and industry forest stewardship programs. Treenomix aims to develop different capacities to address long-standing questions as well as new emerging ones. To achieve this, it integrates forest genomics research with traditional approaches in biotechnology and in tree breeding; this is affecting Canada's forest research in the academic, public, and private sectors.

Treenomix's gene discovery strategy consists of using different technology resources to investigate trees at the genomic, transcriptomic[1], and proteomic level. The focus on genomics must not obscure the importance of the proteome and the metabolome.[2] Consideration of proteomics by Treenomix researchers started relatively late in the project, when the power of combining gene expression profiles and annotations with protein expression profiles became apparent. This approach has proved to be helpful in discovering genes, especially those of spruce, as no conifer genome has yet been annotated. As for the metabolome, Treenomix is interested in it because it is very close to the tree's phenotype; the metabolome comprises the metabolites that make up the wood, the fiber, and the defence chemistry of a tree. By using these other approaches along with genomics, Treenomix researchers are much more efficient at finding the tree genes of interest.

The forest health aspect of the Treenomix project consists of addressing, at a genomic level, the interactions of spruce with insect pests. An example is the interactions of different species of spruce with western spruce budworm (*Choristoneura occidentalis*). During this symposium, we will hear more about spruce budworm: how it attacks a tree, how the tree defends itself, and so on. Spruce also interacts with a number of stem-boring insects; one such insect is white pine, or spruce, weevil (*Pissodes strobi*), which is detrimental to spruce stands and plantations in British Columbia.

White pine weevils attack spruce trees before or at bud break in May–June. The insects lay their eggs in feeding punctures made in the bark of the leader (the top part of the main stem). The larvae hatch and tunnel downwards as they feed on the living inner tissues of the bark, causing the top whorl shoot to wither. A few feeding larvae can in this way cut through two years of tree growth. In Sitka spruce (*Picea sitchensis*) plantations, weevil infestations cause deformations that greatly reduce the quality of trees and allow competing vegetation to overgrow. The plantations set by John King of the BC Ministry

[1] Relative to the full complement of activated genes, that is, messenger RNAs. Messenger RNAs serve as templates for protein synthesis.

[2] The proteome is the whole set of proteins expressed by an organism at a particular time and under specific conditions, and the metabolome is the whole set of metabolic entities and pathways of an organism.

of Forests and Range, for example, have been devastated by the white pine weevil. King and his team are currently evaluating spruce collected across the province and the country for insect resistance. Their tree breeding program includes crosses between resistant–resistant, resistant–susceptible, and susceptible–susceptible spruce lines. Treenomix can now use these trees to study tree defence and potential resistance mechanisms in collaboration with the ministry's Tree Improvement Branch.

For the first few years of Genome Canada funding, the mandate of Treenomix was to develop technologies. A major thrust of the project has indeed been to add new technologies to the existing biotechnology and tree breeding portfolio. In the next round (Genome Canada's Competition III), Treenomix will be able to address old as well as new questions by integrating the technologies developed with the existing approaches and resources. When the Ministry of Forests and Range established its tree breeding program, use of the trees for Genome Canada research projects was not considered. Now, however, the trees are available for forest genomics research, and this research in turn can be applied to public forest stewardship programs.

Complementary DNA (cDNA) sequences are a tool that can be used for gene discovery and marker development. The Treenomix expressed sequence tag (EST) sequencing program for spruce aims at sequencing 135 000 3′ reads and 22 500 5′ reads on selected cloned cDNAs; 100 000 3′ reads have already been done. Similarly, sequencing is well underway for poplar, which constitutes the Treenomix gene identification and annotation contribution to the International *Populus* Genome Consortium. This area of research is directed by Kermit Ritland, who is exploring all the sequences for marker development and undertaking microarray production and analysis as well as proteomic analysis. The results are recorded in databases hosted by the Genome Sciences Centre in Vancouver and can be accessed by Treenomix's collaborators. The databases provide an array of tools not normally available. In addition, Treenomix has developed microarrays for tree tissues at different development stages. The latest spruce microarray comprises close to 22 000 cDNA elements, which will make it a long-lasting research tool. Similarly, Treenomix is developing poplar microarray resources to help in the identification of genes for traits relevant to forest health and wood formation.

Treenomix applies its forest genomics resources to the development of a more comprehensive understanding of the mechanisms, genes, proteins, and metabolites involved in the defence and resistance of trees against insect pests and pathogens. Trees have a large range of constitutive and inducible defences, chemical and anatomical. Not much is known about the genetic regulation of these various defences and how they are interconnected; nor is it known if the induction of defences occurs at the cost of wood and fiber formation. Researchers are just beginning to dissect these mechanisms using biotechnology and molecular genetics approaches. The approach of one gene at a time that was previously used has been displaced since it did not allow the genetic regulatory elements to be clearly identified. Fortunately, research tools developed from a species can be applied to other Canadian forestry systems. For example, spruce microarrays can be used to obtain gene expression from lodgepole pine (*Pinus contorta* var. *latifolia*) infected with the mountain pine beetle–blue-stain fungi (*Dendroctonus ponderosae*–*Ophiostoma clavigerum*) complex.

Genome Canada's Competition III is an opportunity for researchers to propose future directions for forest genomics, and this symposium, a forum for them to build new alliances or strengthen existing alliances. In the future, I would like to better integrate my research with provincial and federal forest stewardship organizations to address problems of forest health and sustainable forest economy, always keeping in mind the particular forestry context. In forestry, tree resources have to be planned and developed for the long term. Indeed, trees remain at the same physical location for hundreds of years, and thus tree breeders cannot on short notice rotate to a different crop when it fails, Also, genomics research should be directed towards solving some of the problems faced by Canada in the management of its forests, such as the threat from indigenous or alien insect pests and pathogens and the effects of climate change. As well, genomics research can help in understanding the relationship between induced tree resistance and wood formation.

In closing I would like to acknowledge the fine work of my co-investigators on the Treenomix project: Brian Ellis, Carl Douglas, and Kermit Ritland as well as the team of technicians, research associates, and laboratory managers.

Bridging Structural and Functional Genomics in Spruce

Bob Rutledge | Laurentian Forestry Centre, Canadian Forest Service, Natural Resources Canada, Québec, QC

I am going to present some of the major aspects of a long-standing collaborative project that I have been conducting with my colleague Krystyna Klimaszewska, in addition to introducing research by Nathalie Isabel, who is our lead structural genomics researcher at the Laurentian Forestry Centre. These research projects are primarily targeted to commercial opportunities relating to enhancing forest productivity, through the identification and propagation of conifer trees with superior traits impacting, for example, fibre quality and enhanced growth rate. Furthermore, I will show how we are exploiting the unprecedented opportunities provided by functional genomics. I will focus on the genetic regulation of tissue development in conifers, as this is my area of specialty.

How then can the production of conifer seedlings with superior traits be accomplished? Essentially, three approaches can be used:

- conventional breeding — slow due to the long generation time of trees, but effective;
- genetic engineering — very rapid with unprecedented potential; and
- clonal propagation of elite trees — rapid and effective, but currently limited to juvenile trees.

Although genomics research can benefit all three approaches, it is the third one that has been the primary focus of our functional genomics research. Traditionally, the forest industry has extensively used rooted cuttings for clonal propagation of conifer trees. Unfortunately, this approach is limited to juvenile trees in that as conifers mature and become sexually active, they lose their ability to root. This in turn represents a significant limitation, due to the fact that many important traits can only be identified in mature trees.

An alternative propagation technology — which has been a major research area for both of my colleagues — is tissue culture-derived seedlings produced via "somatic" embryos, that is, embryos that develop from vegetative tissues. Tissue culture-based technologies overcome many of the limitations of rooted cuttings, providing, for example, the ability to produce unlimited numbers of seedlings and cryogenically preserve individual genotypes. These characteristics of tissue culture technologies have allowed their commercial exploitation for large-scale propagation of conifer seedlings. Importantly, clonal propagation using these techniques provides a commercial conduit for deployment of any new tree genotype by circumventing the long time frames associated with conventional propagation via seeds. The broad significance of somatic seedling production and its associated technologies is reflected by the many countries that have active research programs in this field; these include the United States, Sweden, New Zealand, Norway, and France.

Notwithstanding its scientific and commercial significance, a major limitation of somatic seedling production is the production of embryonic stem cells — through a process called somatic embryogenesis — which can only be accomplished using embryos extracted from seeds. Thus, similar to rooted cuttings of juvenile trees, this technology is currently limited to the propagation of unproven tree genotypes.

A major aspect of our research program centers on the exploitation of functional genomics to first better understand the genetic events that underlie somatic embryogenesis and then to develop protocols that would ultimately allow tissues other than seed to be used for initiating embryonic cultures. Ultimately, this could allow embryonic cultures to be initiated from the vegetative tissues taken from mature trees.

How could this be accomplished? Our approach has been to focus on a select group of genes coding for transcription factors, which are proteins that regulate the activity of other genes. An important advantage of working with this type of gene is the fact that each individual transcription factor controls the activity of thousands of genes. In fact, this is the underlying mechanism by which an elite subset of transcription factors called developmental regulators control the formation of stem cells, from which all tissues develop, including somatic embryos. Thus it is possible to modulate development processes by controlling the activity of just one developmental regulator.

How then can the developmental regulators underlying somatic embryogenesis be identified in conifers? The approach we have taken is to exploit the knowledge derived from the model plant *Arabidopsis*, under the premise that the genetic processes controlling cellular development in conifers are similar. Previous research conducted by my group investigated the function of developmental regulators controlling cone formation, which demonstrated a high level of functional similarity to genes that regulate flower development in *Arabidopsis*. Thus, it seemed likely that genes previously discovered to be involved in somatic embryogenesis in *Arabidopsis* would also be present in spruce — this is indeed what we have found.

Our research has subsequently led to the identification and isolation of several putative conifer gene homologues associated with the induction of somatic embryogenesis in *Arabidopsis*. We are now conducting functional testing of these gene homologues, with the main objective of determining how these genes might be manipulated to better understand conifer somatic embryogenesis in general, as well as whether

they could be used to induce somatic embryogenesis in tissues of mature spruce trees.

Another major research program involving transcription factors, led by Dr. Isabel, is based upon the likelihood that much of the natural variation in conifer growth and wood quality is linked to variation within the transcription factors that regulate these developmental processes. Dr. Isabel's primary approach is structural genomics, which consists of mapping the chromosomes of various conifer species. These genomic maps are now being utilized to study the natural genetic variation of a large number of conifer transcription factors, with the aim of linking specific types of variation to elite traits. Once identified, these transcription factors could be used as genetic markers to help accelerate conifer tree improvement via conventional breeding.

Proteomic Approach to Study Forest Tree–Pathogen Interaction

Abul K.M. Ekramoddoullah | Pacific Forestry Centre, Canadian Forest Service, Natural Resources Canada, Victoria, BC

In this presentation, I will show how my research team has used the proteomic approach to study tree–pathogen interaction. Except for alien pests introduced into the environment, tree diseases and insect pests are natural components of forest ecosystems. Nevertheless, the damage they cause to forests can have severe economic impacts. In British Columbia, this damage results in an estimated annual growth loss of 18%. Because of this, provincial and federal experts identified a number of research priorities in British Columbia, including root diseases such as armillaria (*Armillaria* sp.), tomentosus root rot (*Inonotus tomentosus*), and laminated root rot (*Phellinus weirii*); stem rusts such as white pine blister rust (*Cronartium ribicola*) and western gall rust (*Endocronartium harknessii*); and insects such as mountain pine beetle (*Dendroctonus ponderosae*), spruce budworm (*Choristoneura fumiferana* and *C. occidentalis*), and bark beetles (Scolytidae).

Damage to conifers by tree diseases and pests takes various forms. Douglas-fir (*Pseudotsuga menziesii*) decay due to laminated root rot spreads from tree to tree through root contact. Green conifer forests turn red due to attacks by the mountain pine beetle. White pine blister rust, introduced into North America in the early 20th century, causes stem cankers on western white pine (*Pinus monticola*); for this reason, western white pine has not been planted until just recently. Researchers are thus trying to find ways to improve pest resistance in conifers. To achieve this goal, my research team carried out a molecular analysis of tree–pathogen interactions with the primary aim of identifying genes that improve genetic resistance to pests. We chose proteomics as our primary approach.

Proteomics is the study of the total protein profile of a given organism or tissue. Even before the term "proteomics" was coined, I was extensively involved in studying the proteome of white pine blister rust. Proteomics researchers separate proteins of a given tissue using two-dimensional gel electrophoresis, which exploits the charge and size characteristics of the protein. Thousands of proteins can be separated this way. Then using software, the researchers analyze the proteins and differentiate between their profiles. In the study of forest pests, these profiles allow researchers to differentiate between pest-resistant and pest-susceptible trees. Proteins that are found to be unique in resistant trees are cut from the gels and digested with proteolytic enzymes such as trypsin to generate peptide fragments. Products from the proteolytic digestion are analyzed with MALDI (matrix-assisted laser desorption-ionization) tandem mass spectrometry. Databases of known proteins are then searched and the peptide fragments obtained are compared in order to find matches. If no hits are obtained, which is often the case with conifer proteins, the peptide fragments are further fragmented and the corresponding amino acid sequence is determined. From this information, one can design polymerase chain reaction (PCR) primers to clone the gene encoding the protein and then use the gene probe as a DNA marker to select trees for resistance. Another option is to use the amino acid sequence information to synthesize the peptide, produce an antibody, and observe the protein expression to determine its role in tree resistance.

In the white pine blister rust pathosystem model, proteomic analysis revealed both enhancement and suppression of protein biosynthesis in resistant trees after infection with the rust pathogen. In contrast, only suppression of protein biosynthesis occurred in infected susceptible trees. Among key findings are several pathogenesis-related proteins belonging to the PR-3, PR-5, and PR-10 groups in conifers. We found an anti-fungal peptide (PmAMP1) and the PR-3 protein to be associated with a resistance known as slow canker growth (see further on). We have also discovered two fungal proteins, one of which acts as an elicitor (triggers a defence response) and the other as a heat-shock protein (increases its activity when cells containing it are exposed to higher temperatures).

The proteins in the PR-3 family are also known as "chitinases." These enzymes cleave chitin, which is a structural component of fungi and insects. We have identified three chitinases, one in Douglas-fir and the other two in western white pine. In both pathosystems, they are upregulated[1] following infection. In Douglas-fir infected with root pathogens, we have observed chitinase accumulation in needles. We extrapolated these results to the stand and landscape level to predict productivity and tree growth loss. Interestingly, chitinase inhibits the growth of ice crystals during the freezing process and thus also acts as an antifreeze protein, protecting trees during winter months.

Cankers in western white pine sometimes heal with a receding canker margin; this indicates a form of resistance known as slow canker growth. My collaborator at the Canadian Forest Service's Pacific Forestry Centre, Rich Hunt, has selected for this type of resistance in western white pine, and slow-canker-growth resistant trees are now used in the breeding program in British Columbia. Such selection traditionally takes six to seven years. Now that two molecular markers for this type of resistance — PR-3 and PmAMP1 — are known, selection time could be reduced to a few months. When we analyzed tree tissues using Western immunoblot (a technique for detecting proteins by their response to labelled antibodies), we found that those from trees with slow-canker-growth resistance had two PR-3 chitinase bands, while those from susceptible

[1] That is, their expression is enhanced following pathogen infection.

trees had only one band. Bulk segregation analysis revealed that the gene encoding the PR-3 chitinase is only present in slow-canker-growth resistant trees. Resistant trees also have a significantly higher level of the anti-fungal peptide PmAMP1 than susceptible trees; we have developed a poster on this phenomenon.

We also observed a significantly higher accumulation of a PR-10 protein following fungal infection in a tree carrying a dominant resistance gene and used immunochemical techniques to study this protein's activity. We cross-sectioned tissue from needles of infected western white pine and probed it with gold particles tagged with PR-10 antibodies. Then we examined the tissue under the electron microscope. In white pine cells that had been infected — and thus that contained fungal cells — most gold particles were located on the cell wall. As this is the entry point of the fungus into the cell, it is indicative that this protein binds the fungus. To the best of my knowledge, this is the first demonstration that a PR-10 protein does bind a pathogen.

Interestingly, this PR-10 protein is upregulated during the winter months. Rich Hunt has shown that western white pine trees resistant to blister rust at high elevations become susceptible when moved to low elevations. Similarly, resistant trees from interior British Columbia become susceptible once moved to the coast. These results would suggest that low temperatures help trees to produce defence proteins. As a spin-off, we found that the protein can be used as a marker for frost hardiness and that trees that are the most frost hardy also have a higher level of this protein. We introduced the PR-10 gene into the model plants *Arabidopsis* and canola, an important agronomic crop, to see whether the gene confers frost tolerance to these plants. Both transgenic plants expressed the conifer PR-10 proteins, and PR-10 did provide frost tolerance to the transgenic *Arabidopsis*. Similar experiments are underway for canola.

PR-10 proteins are encoded by a multi-gene family; in western white pine alone, we have identified 19 members of this family. The expression of each gene member is under the control of its own promoter. This multi-gene family underwent diversification within conifers during evolution and was perhaps co-opted to assume various functions. We have characterized two PR-10 gene promoters: one is root specific, and the other is pathogen and wound inducible. The purpose of these promoters is to express the gene of interest in a tissue-specific manner and to express it only when there is a threat of invading insects or pathogens. We characterized some *cis*-acting DNA regulatory elements[2] in one PR-10 gene promoter: a G-box[3] involved in wound responsive expression, cold regulatory elements, a palindrome structure that has the potential binding site for transcription factors, elicitor responsive elements, a binding site for a well-known transcription factor for fungal elicitor response, a CGTCA (cytosine–guanine–thymine–cytosine–adenine) motif involved in MeJ (methyl epi-jasmonate) responsiveness, and an ethylene responsive element.

We have identified a dominant blister rust resistance (*R*) gene in western white pine from a State of Oregon breeding program. We tested the *R* gene on British Columbian (BC) isolates of the rust and confirmed that it is also effective against the BC form of the rust. As a result, we now select for the *R* gene in the trees used in the BC white pine breeding program. Trees carrying the *R* gene exhibit a hypersensitive reaction (HR) following blister rust infection: HR occurs when the host's cells surrounding the pathogen suddenly die, which eventually eliminates the fungus. The elicitor isolated from white pine blister rust that was mentioned previously can mimic this HR.

Using a biochemical model, we predicted that the nonvirulent gene product of the pathogen (presumably the elicitor) would directly or indirectly be recognized by the *R*-gene product of the host. This in turn would trigger a signal transduction pathway that would eventually unleash host defence-related proteins that in concert give rise to the HR. We have identified some promoter elements in western white pine that will allow us to characterize various transcription factors and thus to identify the regulatory genes. Our major effort now is to isolate the *R* gene and its product from western white pine. At present, 40 *R* genes have been cloned from a variety of plants. Deduced sequences for the proteins they encode reveal several common motifs, for example nucleotide binding sites, a leucine zipper, a leucine-rich repeat region, and a toll and interleukin receptor. We have used these common motifs to design PCR primers and to amplify resistance-gene analogues (RGAs) from western white pine. Thus far, we have isolated 120 RGAs and determined the complete genomic sequence for four of them and the cDNA (complementary DNA) sequence for two. We are producing an antibody to detect the corresponding *R* proteins.

Our strategy to isolate the *R* gene involves the following. Seeds carrying dominant *R* genes were germinated and the seedlings subjected to white pine blister rust. We are now evaluating, on a phenotypic basis, whether or not the seedlings are resistant or susceptible to the fungus. Concurrently, we are applying PCR to the DNA of megagametophytes from corresponding seeds using RGAs as primers. Whole PCR products will be used to construct the primary genetic map. The results from these two parallel studies will help us link RGAs to the *R* gene. If the distance between the RGAs and the *R* gene is relatively small, we will use positional cloning to evaluate them; otherwise, we will assess them by their interaction with blister-rust-derived elicitor in transgenic tobacco plants carrying the fungal elicitor gene.

[2] Regulatory elements whose action depends on their being physically linked to the regulatory target.

[3] A component of messenger RNA that binds purines to regulate purine metabolism and transport.

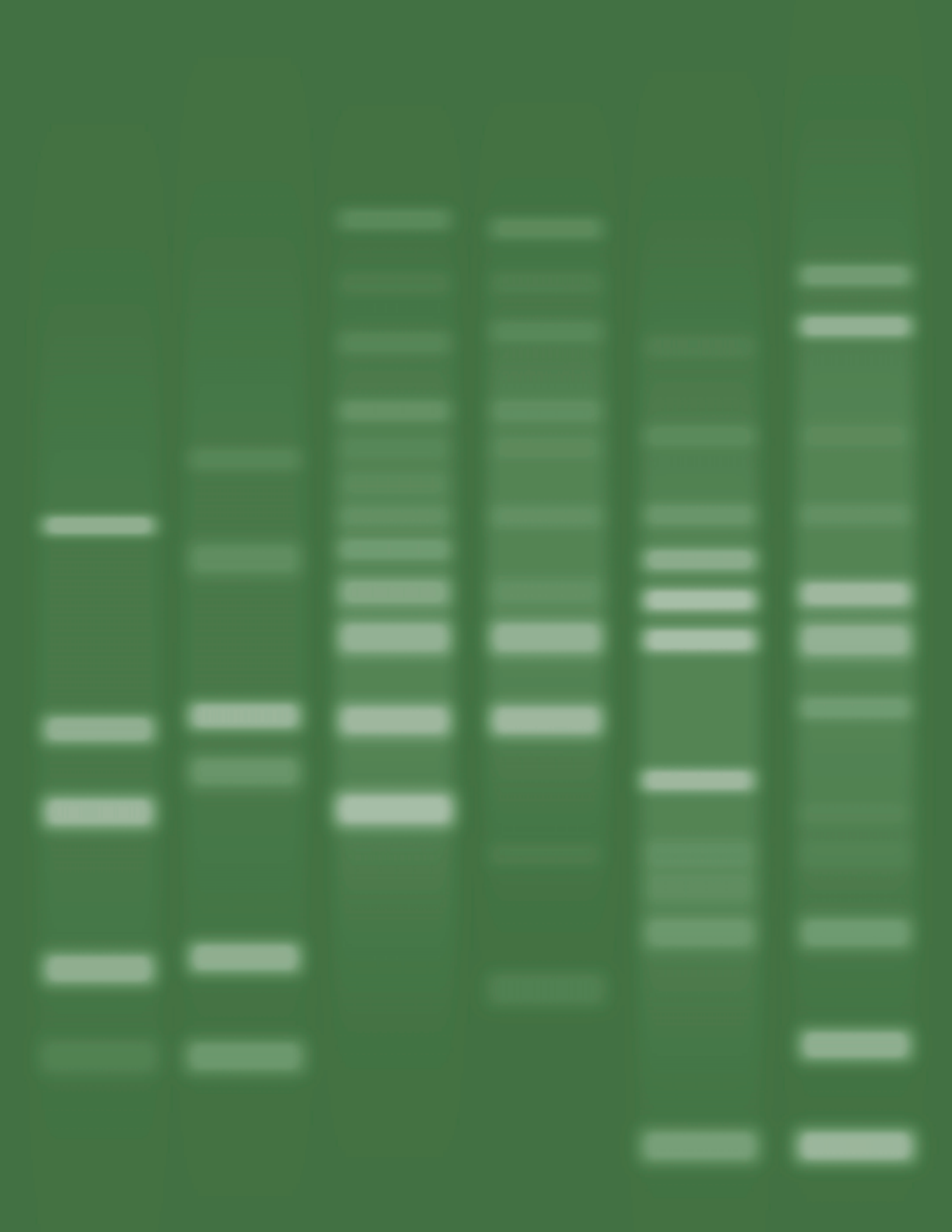
Finally, we have synthesized a monoclonal antibody to the fungal heat-shock protein we discovered. This involved engineering a 28-kDa (kilodalton)[4] single-chain antibody, which retains its protein-binding ability, from a 150-kDa antibody molecule. We plan to introduce the gene for this single-chain antibody into western white pine susceptible to blister rust infection. The antibody will deactivate the fungal protein, thereby slowing the growth of the fungus and allowing the trees' defence mechanisms to take over.

Our future research directions include looking for anti-fungal proteins in conifers; isolating and characterizing transcription factors useful for the regulation of genes upstream in the signal cascade pathway—our work on promoter characterization providing a head start; and ultimately, engineering trees with multilayer defences through the exploitation of conifer-derived gene promoters.

Among our partners, collaborators, and clients in this research, I would like to mention the British Columbia Ministry of Forests; Canfor; TimberWest; the Alberta Research Council, particularly for its work on transgenic canola; and Barbara Hawkins, University of Victoria, for her work on frost-hardiness testing.

[4] A dalton is a measure of molecular mass; one hydrogen atom has a mass of 1 Da.

GENOMICS OF INSECT PESTS AND THEIR VIRAL PATHOGENS



Canadian Research Involvement in International Insect Pest and Pathogen Projects

Keynote Address Peter Krell | Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON

It is a pleasure for me to highlight the impact that Canada is making on the international scene in the area of forest insect pests and pathogens. In the 1950s, an insect pathology research laboratory was built at the Great Lakes Forestry Centre (GLFC) [Canadian Forest Service (CFS), Natural Resources Canada (NRCan)] in Sault Ste. Marie, Ontario, to study microbial pest pathogens. Since then, Canada has been an international leader in forest pest research. Canadian forest pest researchers have had collaborations with many countries, and this presentation will cover only a sample of these. I will briefly describe the research of eight prominent Canadian scientists, including myself, working at university and government laboratories. I will conclude by giving you an overview of how, at an international level, Canadian scientists are involved in governance issues, participate on editorial boards, and have won recognition for their achievements in the area of forest pest and pathogen genomics.

Basil Arif of the GLFC has established international collaborations in China, Germany, Brazil, and the United States. His more recent research focuses on genomics, phylogeny, and evolution of three baculoviruses[1] of interest in the Canadian context. One of these, the *Neodiprion lecontei* nucleopolyhedrovirus (NeleNPV), is very effective against the red-headed pine sawfly (*Neodiprion lecontei*), which attacks and defoliates pine in plantations. Through a genomics approach, Dr. Arif and his colleagues have determined that NeleNPV represents an ancestral virus having close similarity to the *N. sertifer* nucleopolyhedrovirus (NeseNPV), which is effective against a different pest, the European pine sawfly (*N. sertifer*) (Lauzon et al. 2004).

Eric Carstens of Queen's University in Kingston, Ontario, collaborates with colleagues in Germany, China, and France. He is interested in the DNA replication complex of baculoviruses, particularly of the type species *Autographa californica* nucleopolyhedrovirus (AcMNPV). He has determined the temporal expression profiles of the synthesis of four viral proteins (IE-0, IE-1, P143, and LEF-3) thought to play a role in viral DNA replication, which takes place in the late phase of the viral infection of the host cell. He has demonstrated that these four proteins are available before DNA replication and therefore could theoretically be available for generating a DNA replicon[2] (Ito et al. 2004).

For my part, I have collaborators in Spain, the Netherlands, China, South Africa, Germany, and the United States. Like Drs. Arif and Carstens, I am interested in viral genomes, viral gene and genome phylogeny, and virus evolution. I have demonstrated that the phylogeny of the *lef-2* late expression factor (a gene involved in viral DNA replication) to some extent corresponds to its location on the respective genomes of different baculoviruses. For example, the *lef-2* sequence of the spruce budworm (*Choristoneura fumiferana*) baculovirus, CfMNPV, clusters with seven other group I nucleopolyhedroviruses, and the relative location of this gene on the genome is conserved among these viruses. In another phylogenetic cluster, however, the genomic location of the *lef-2* gene differs among viruses. This allowed me to conclude that the relative location of a baculovirus gene on the genome, as well as genome phylogeny, can be used to infer baculovirus evolution (Chen et al. 1999). By looking at wild samples of *Spodoptera exigua* nucleopolyhedroviruses (SeNPV) in the field, my Spanish colleagues and I have shown that they contain a mix of genotypes, suggesting that there is some synergy among the different genotypes (Muñoz et al. 1999).

I am also examining the function of some of the open reading frames that have been identified through genomics, including the *me-53* gene of the *Trichoplusia ni single* nucleopolyhedrovirus (TnSNPV) and the *Cf103* gene of the CfMNPV virus. Those two genes encode a protein containing a zinc-finger[3] motif, indicating they might act as a transcription factor. Since virus replication depends on host susceptibility, the viruses probably coevolved with their hosts. I have discussed this topic often with a German colleague, Johannes Jehle.

In addition to insect pathogens, I am interested in insect molecular biology, particularly with the aim of understanding insect development and the role of molting hormones in gene activation. In collaboration with Arthur Retnakaran and Qili Feng, colleagues from the GLFC, I have demonstrated for example that there is a peak in the level of the molting hormone ecdysone before insect pupation. The transcription factor hormone receptor 3 (HR3) is activated, and this presumably initiates a cascade of gene transcription leading to pupation. My research team, in close collaboration with scientists in Israel and at the Rohm and Haas Company in the United States, has found that an ecdysone analogue, RH-5992, induces transcription of the hormone receptor CHR3 in the spruce budworm, but not of the equivalent DHR3 in the fruit fly *Drosophila*. This ecdysone analogue thus acts specifically on lepidopterans (butterflies and moths) and has no effect on dipterans (flies) (Retnakaran et al. 2001).

[1] Viruses infecting specific arthropods, almost exclusively insects.

[2] A region of DNA that replicates as an individual unit, for instance, a virus chromosome.

[3] Zinc-binding loops or "fingers" that are part of certain proteins and that interact with DNA.

Michel Cusson of the Laurentian Forestry Centre (CFS, NRCan) in Quebec is working with colleagues in the United States, Belgium, and Burkina Faso. One of his projects focuses on an ichnovirus from a parasitic wasp. Ichnoviruses are symbiotic viruses specific to the wasp family Ichneumonidae; they encode proteins that abrogate the wasps' defences and block the metamorphosis of their larvae. Dr. Cusson aims to identify the viral genes related to the suppression of insect defences so that they can be introduced into baculovirus vectors to improve their utility as biological control agents (Béliveau et al. 2003). Another area of study is insect thermoregulation; he has discovered that grasshoppers can defend themselves from fungal attacks by increasing their own temperature through a thermoregulation process, hence reducing the ability of fungi to replicate and kill them as hosts. In addition, Dr. Cusson has used proteomics to determine the three-dimensional structure of a protein (farnesyl diphosphate synthase, or FPPS) involved in the production of juvenile hormone in lepidopterans. He has shown that structural components of the protein were conserved among a wide range of lepidopteran sources (Ouedraogo et al. 2003).

Along with his US collaborators, **David Theilmann** from the Pacific Agri-Food Research Centre (Agriculture and Agri-Food Canada) in Summerland, British Columbia, is interested in transcriptional regulation and baculovirus gene function. For example, he has developed a gene switch to induce gene expression based on an ecdysone receptor (EcR) response element (Dai et al. 2005). His initial experiments involving chloramphenicol acetyltransferase (CAT) gene expression demonstrate that gene induction is tightly controlled by the level of ecdysone added. Moreover, ecdysone analogues such as RG-102240 provide long-term induction of the CAT gene expression. CAT is a bacterial enzyme that inactivates the antibiotic chloramphenicol. These ecdysone analogues could thus be used in gene therapy to activate genes in mammalian expression systems. To study gene function, Dr. Theilmann has also used gene knockouts to demonstrate the essential role of the baculovirus *pe38* gene in DNA replication and virus production (Maynard et al. 2003).

Jean-Louis Schwartz and **Raynald Laprade** of the Université de Montréal, Quebec, do research on Cry1Ac, a (Cry) crystal-like toxin produced by the bacterium *Bacillus thuringiensis* that is effective against insect pests. They have been part of extensive national and international collaborations, including with laboratories in Austria, Italy, Thailand, France, the United States, and the United Kingdom. As part of their research, they used atomic force microscopy and other techniques to determine that Cry1Ac is tetrameric. They showed that when the toxin is inserted into an insect cell, its structure creates a pore in the cell membrane; ions leak out, leading to an electrolyte imbalance (Peyronnet et al. 2002).

Qili Feng of the GLFC has been involved in a major genome sequencing effort with colleagues in China and Japan. His research focuses on the genome of the major Canadian forest defoliator, the spruce budworm. Based on 35 000 expressed sequence tag (EST) reads, he was able to identify 9 574 unique spruce budworm genes, representing about 1.2% of its entire genome. The genomic database that resulted from his work now provides clues to developing effective species-specific insecticides. When combined with the genomic databases of other insects such as the fruit fly and the European corn borer (*Ostrinia nubilalis*), it can also be used to infer insect phylogeny. With other colleagues in the United States and Germany, Dr. Feng has dissected the juvenile hormone response element and built the phylogeny of insect ribosomal protein sequences (Kethidi et al. 2004).

I will now give an overview of Canada's advisory and administrative involvement on the international insect pest and pathogen issue. The Biocontrol Network is a Pan-Canadian consortium of 42 research scientists headed by Raynald Laprade and Jean-Louis Schwartz in Montréal and funded by the Natural Sciences and Engineering Research Council of Canada (NSERC). It is dedicated to reducing the reliance on chemical pesticides by providing for biologically based and environmentally benign alternatives. The network has formal affiliations with international research organizations such as CAB International (CABI); the International Organisation for Biological and Integrated Control of Noxious Animals and Plants (IOBC); the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia; the European Biological Control Laboratory (EBCL); the Institut National de la Recherche Agronomique (INRA) and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) in France; and the National Integrated Pest Management (IPM) Network and the US Department of Agriculture (USDA) in the United States. Those affiliations have facilitated the establishment of international research collaborations and the holding of workshops for network members.

Many Canadian scientists are officers or committee members of international governance organizations such as the International Committee on Taxonomy of Viruses (ICTV), the Society for Invertebrate Pathology (SIP), and the International Union of Microbiological Societies (IUMS). For example, three Canadians hold executive and committee chair positions at the ICTV, an organization responsible for taxonomic issues in the nomenclature of viruses, including vetting new proposals and revising the taxa as needed. SIP, an international organization dedicated to the study of pathogens of insects and other invertebrates, was originally founded by Canadians; in recent years, several Canadians have sat as executive members and as meetings and division committee chairs of SIP.

A number of Canadian scientists are editors or members of editorial boards of international scientific journals in the fields of invertebrate biology and pathology and hence are responsible for the vetting of material for publication.

For example, Canadians are in senior editorial positions of journals such as *Biocontrol Science and Technology, Biological Control*, and the *Journal of Invertebrate Pathology*.

Canadian scientists have made major international contributions to the study of insect viruses, particularly in the area of baculovirus genomics. Of the 24 complete baculovirus genome sequences reported in scientific publications and deposited in GenBank, an international DNA sequence database, 5 came from a research project led by Canadians and 6 additional ones from a research project with Canadian collaborators, accounting for almost half of the baculovirus sequences currently available.

Canadian scientists are well respected by their international peers and are often involved in intense discussions or debates at meetings. In an animated discussion, for example, David Theilmann argued his case for a revised baculovirus phylogeny with colleagues from Israel, the Netherlands, China, and Germany. In addition, at the SIP meeting in Helsinki, Finland, in August 2004, Canadian scientists clearly stood out as major players.

I conclude this presentation by reaffirming that Canada is indeed an international player in research on forest insect pests and pathogens and by thanking you for the opportunity to highlight the achievements of Canadian scientists in this area.

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Genomics of the Spruce Budworm and Its Viral Pathogens, and the Pursuit of Bioactive Molecules as Spin-Offs

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I will present an overview of the program funded by the Ontario Genomics Institute (OGI) on the genomics of spruce budworm and its viral pathogens and of the spin-off benefits from this research that have been realized and those that I hope to achieve.

Over the last few decades, the eastern spruce budworm (*Choristoneura fumiferana*) has been the most devastating forest insect pest in Canada, causing losses to harvestable timber in the billions of dollars. Insects and viruses have coevolved to accommodate the complicated larval physiology and the viral replicative needs. The tendency of virus evolution is towards a more attenuated parasite, resulting in an intricate insect–virus relationship that makes this particular environmental niche a most interesting study. With the advent of gene technology, it became possible to modify viruses and enhance their effectiveness against a particular insect pest in an environmentally safe manner. However, altering a viral genome can only be done after careful consideration is given to the efficacy of such a change and its effects on host range and specificity, nontarget organisms, and other aspects relating to environmental safety. As in many other fields, genomics and proteomics appear to hold the key that unravels the precise make-up of an organism, be it human, insect, or virus, and provide researchers with the tools needed to more effectively control insect infestations.

The OGI-funded structural and functional genomics program implemented at the Great Lakes Forestry Centre (GLFC) proceeds along two independent but closely interrelated studies on viral and insect genomics. The viral genomics studies involve sequencing a number of viral genomes, identifying all their genes and open reading frames (ORFs), categorizing genes according to temporal expression patterns and function, and developing microarrays to study gene expression in cell lines and in larvae. Similarly, for insect genomics, a number of complementary DNA (cDNA) libraries were generated for different larval tissues and at different developmental stages of the insects. From this library, expressed sequence tags (ESTs) were generated and genes annotated. Specific and global gene expression is then studied through a number of available technologies. The insect genes that are of particular interest are those involved in pathogenesis, host range and specificity, toxicity, molting, diapause, and resistance. These studies will lead to a number of applications such as the generation of environmentally safe biological control agents (transgenic viruses), transgenic plants, and spin-offs such as insect- and virus-based systems for the expression of exogenous proteins. Transgenic viruses have already been generated from these studies, and I will discuss them later in the presentation.

To generate spruce budworm ESTs, cDNA libraries were derived from whole larvae, epidermis, midgut, fat body, and midgut cell lines. These tissues are important sites that are critical for virus–insect interaction or virus replication or that support virus replication. ESTs are being generated based on the cDNA libraries, sequenced, blasted[1] against existing GenBank[2] libraries, and annotated. The libraries are being compared with similar ones of other insect species such as the silk moth, *Bombyx mori,* and differences among insects according to their habitat, evolution, and interactions in their respective environment determined. Our research team at the GLFC will look at specific and global gene expression, followed by functional genomics and gene knockouts. In summary, the application of ESTs will be in gene cloning, global gene expression, construction of physical maps, and annotation and profiling. To date, over 35 000 spruce budworm ESTs, generated from five cDNA libraries, have been sequenced. These ESTs contain almost 10 000 unigenes that match about 3 000 other genes in GenBank.

Qili Feng, part of the GLFC team, has developed an interactive Web site for other investigators to search our spruce budworm database, which can be accessed at http://pestgenomics.org/database.htm. Researchers can enter either their DNA sequence or a keyword and search the database to find matches. For example, by typing in a keyword and selecting the option "contigs," one can find information on the contigs, including their location and how they are identified. The SwissProt Database identifies a corresponding gene function. One can also link to the Web site of the National Center for Biotechnology Information, a resource for molecular biology information based in the United States, and search for further information there.

I shall give you a specific example of the research process that I just described. Larval molting in insects appears to be a simple process, proceeding from one stage to another during which the skin is shed. In reality, the process involves detailed regulation and coordination of hormonal and metabolic systems that result in up- or down-regulation of a number of genes, the products of which are critical for the rest of the process. Some genes are highly expressed; some are not expressed or are expressed at very low levels. From a single library, we have analyzed over 2 800 spruce budworm sequences. When blasted against other sequences from a variety of species

[1] BLAST is a computer program that identifies homologous genes in different organisms.

[2] A database of publicly available DNA sequences that is maintained by the National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD.

including human, we obtained 706 hits, the top hits being against sequences from other Lepidoptera. We have assigned functions to a large number of these genes in the intermolt and molting stages and, interestingly, there was a high proportion of genes encoding hydrolases, a group of enzymes that catalyze certain chemical bonds by adding or removing water. This is probably because a number of these enzymes — chitinases, proteases, cathepsins, and so forth — are needed to dissolve and degrade various tissues. One could thus insert genes encoding hydrolases into a baculovirus or a poxvirus to enhance their effectiveness against an insect pest, targeting a specific insect tissue to degrade.

We are in the process of generating microarrays to analyze gene expression in larvae going from the sixth larval to the pupal instar. We can identify which of the over 3 000 expressed genes have been upregulated and which have been downregulated. This work is proceeding well. We hope to further investigate the effect of virus infection on gene regulation within a specific larval tissue, as well as the effect of that particular insect tissue on viral gene regulation.

The virus replication cycle in susceptible cells is biphasic, meaning that two distinct phenotypes are generated at the end of this cycle. Both phenotypes have signature proteins. A budded virus is synthesized early in the infection and disseminates infection within larval tissues. Later in the infection, the virus becomes occluded in a protein matrix. Following the insect's death, the occluded virus is released into the environment and is responsible for spreading infection to other larvae. The genomes of both virus phenotypes (budded and occluded) are identical, but certain proteins are destined to be part of the structure of one phenotype but not the other.

We have sequenced a number of viral genomes. The size of the baculovirus genome ranges from 80 to 180 kilo base pairs (kbp). Looking at the genomes of all the lepidopteran baculoviruses that have been sequenced, one sees significant conservation of genes, their arrangements, and other structural features that are implicated in a variety of functions. One important feature of baculoviruses is the clear temporal division of classes of genes within the genome. Classes of genes are expressed at various times post-infection, depending on the need of the virus for a particular protein or function. One important feature of lepidopteran baculovirus genomes is the presence of genes that give the virus a certain selective advantage in nature but are not essential for virus replication, called auxiliary genes. They can thus be deleted from the genome and replaced with an exogenous gene encoding a protein of interest, for example one deleterious to the larva host. Gene expression in baculoviruses is certainly not random. It proceeds in a very orderly way, in a cascade fashion. Genes are regulated in time; they are associated with immediate early, early, late, or very late expression, corresponding to the time it takes for the DNA to begin replicating. This finding is critical: even though the baculovirus genome is small, it is expressed in an orderly fashion.

Peter Krell, a collaborator on our project at the University of Guelph, has used microarrays to analyze gene expression in insects at various times following viral infection. Microarrays measure concentrations of messenger RNA (mRNA), which encodes and carries information from DNA during transcription. In some cases, when Dr. Krell examined expression in a specific strand, he detected mRNA from the opposite strand, which was unexpected. This is possibly indicative of a previously unknown mechanism of gene regulation in baculoviruses that we may be able to apply to our work on virus replication. Although microarrays are valuable tools, they only give a rough measure of gene expression; researchers need to use other techniques to fully understand this complex process.

On the aspect of modification of baculoviruses, we have exploited genetic engineering to enhance their effectiveness against the spruce budworm. Naturally, environmental impact and safety to nontargets are paramount in this program. One of our approaches consisted of taking a budworm gene, splicing it in a host-specific virus, and expressing it back in the budworm. Thus, no exogenous genes or elements are introduced into the system. I will give you an example. We know that the hormone ecdysone initiates and regulates the larval molting process. When ecdysone (along with other factors) binds to DNA, it triggers the expression of a number of genes involved in molting — one is a transcription factor called CHR3. When the level of CHR3 drops to a certain value, we observe the expression of the enzyme dopadecarboxylase (DDC), which is needed for tanning and hardening of the new insect cuticle. If CHR3 is always expressed at a high level, the expression of DDC remains low. We engineered the gene encoding CHR3 into a baculovirus, and when budworm larvae were infected with this virus, they underwent molting but the cuticle did not tan properly. The larvae's mouthparts remained soft, so they could not forage. We have thus achieved feeding inhibition of the larvae, which is all one needs to control the spruce budworm. We achieved feeding inhibition by hyper-expressing a transcription factor that actually belongs to the insect, without the need to introduce something into this system and to alter the ecological niche.

Recently, we have become interested in viruses infecting orders of insects more ancient than Lepidoptera. Of particular interest are those infecting sawflies, belonging to the order Hymenoptera. Fossil records show that Hymenoptera are about 110 million years older than Lepidoptera. Viruses of sawflies (*Neodiprion* spp.) are the most efficacious of all viral control agents and rival chemicals in cost and efficacy. Before 1950, the European spruce sawfly (*Gilpinia hercyniae*) was a devastating forest insect pest, much like the spruce budworm is today. The introduction of a host-specific virus to this population — registered as a biological control agent in Canada by the Canadian Forest Service (CFS) — not only resulted in the collapse of infestations, but an epizootic[3] was established

[3] An epidemic outbreak of disease in an animal population.

that kept the insect under control until today. To find out why sawfly viruses are so effective, we decided to sequence the genome of the *Neodiprion lecontei* nucleopolyhedrovirus (NeleNPV), which infects the redheaded pine sawfly (*N. lecontei*). We found out that its approximately 81 kbp make it the smallest baculovirus genome sequenced to date. The NeleNPV genome is similar to the genomes of lepidopteran viruses in how it is organized, but differs markedly from these genomes in other respects. The NeleNPV genome contains far fewer genes and ORFs than the genome of the most studied lepidopteran baculovirus, *Autographa californica* nucleopolyhedrovirus (AcMNPV). NeleNPV also lacks proteins that are thought to be essential for the virus to function, yet it is a highly effective control agent.

Sawfly viruses replicate only in the larval midgut, while lepidopteran viruses replicate in a variety of tissues, including fat and epidermis. Obviously, as viruses evolved with their natural hosts, they managed to acquire new genes to allow them not only to invade other tissues, but also to survive the complicated physiology of the larva and its regular hormonal changes. I shall summarize some of these evolved mechanisms further on.

The NeleNPV genome contains all the essential genes required by baculoviruses to invade the midgut, except for a gene encoding an F-protein (or its functional homologue), which is an essential ingredient of the budded virus phenotype. As I emphasized previously, a typical insect virus produces a budded virus phenotype to infect larval tissues. We have thus concluded that this phenotype may not play a role in the biology of sawfly viruses. We are collaborating with colleagues at the University of Florida and the Institute of Biomedical Sciences in São Paulo, Brazil, to analyze more sawfly viruses. So far, our theory has been substantiated.

These data and other data generated from a number of baculoviruses point to an intricate relationship between insects and their viral pathogens — a coevolution. When I studied virology in Britain years ago, my fellow students and I were told in no uncertain terms that a virus infects a cell, multiplies, and kills the cell. Researchers now believe that as soon as a virus initiates infection, the host cell quickly tries to kill itself to protect the organism, and the virus works hard to keep the cell alive so that it can multiply. Baculovirus–insect coevolution could thus have taken place as follows. In the process of dealing with the apoptotic responses (programmed self-death) of the host cells, the virus acquired inhibitors of apoptosis proteins, some of which probably originated in the insect. As the virus acquired these genes, the insect in turn developed a cell-sloughing mechanism, clearly not to the advantage of the virus. The virus then had to acquire other mechanisms and factors to invade tissues beyond the larval midgut. These factors include the genes for IE-0, ORF Ac23, and a membrane fusion protein. I believe that it was at this stage that the viral genome evolved to synthesize a budded virus phenotype. Once the virus went past the midgut barrier, it had to contend with larval molting, a process that appears to be deleterious to virus replication. Then, the virus had to acquire a gene encoding an enzyme, steroid glucosyltransferase, that conjugates ecdysone with sugars to render it inactive. This arrests the molting process and allows the virus to replicate. It is essential to remember that all these evolutionary changes followed the general trend of viruses to become more attenuated and less effective against the natural host.

When we align two genomes of viruses infecting sawflies, belonging to an ancient order of insects, we see that while most genes are arranged in a collinear fashion, there is a nonsyntenic[4] region where the putative genes are more similar to host's than to other baculovirus genes. We believe that this region contained host genes that were lost as the virus evolved to the modern baculovirus. These studies will surely give a good insight into the functioning of the baculovirus genome. Along with insect genomics, they will also allow us to develop intelligent strategies for the control of forest and agricultural insect pests.

Our work has enabled us to transfer our technology to other sectors. We hold a worldwide patent on all transcription factors expressed in baculoviruses. We have ongoing collaborations with researchers in Britain, the United States, and China, where we transferred an entomopoxvirus fusolin gene to the Wuhan Institute of Virology, Chinese Academy of Sciences. We have entered into collaboration with OGI to engineer a virus against the velvetbean caterpillar (*Anticarsia gemmatalis*), a pest damaging soybean plantations in South America and other places. This will be of interest to the multibillion dollar soybean industry.

What are the expected outcomes from structural and functional genomics of insects and their viral pathogens? As the project develops, in harmony with similar projects in other parts of the world, we expect to

- identify key target areas for insect control (for example, the immune system);
- find bioactive molecules (antimicrobials, enzymes, antifreeze proteins);
- increase our knowledge on protein expression, transform germlines, and produce bioactive proteins;
- better understand plant–insect interactions to develop deterrents to insect feeding;
- improve biocontrol pathogens;
- construct a model for insect–parasite interactions;
- know more about the evolution of baculoviruses; and
- advance the field of comparative functional genomics.

[4] Located on different chromosomes in the two species.

As the list indicates, we are driven by our interest in biology to study genomics and not the other way round.

I would like to acknowledge others involved in this particular OGI program: Qili Feng and Arthur Retnakaran, CFS, GLFC; Chris Lucarotti, CFS, Atlantic Forestry Centre; Peter Krell, University of Guelph; David Theilmann, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre; Michel Cusson, CFS, Laurentian Forestry Centre; David Evans, University of Alberta; and Eric Carstens, Queen's University. We have collaborations in the United States with Richard Moyer, University of Florida; in England with Elisabeth Herniou, Imperial College London; in Brazil with Paolo Zanotto, University of São Paulo, and Flávio Moscardi, Brazilian Agricultural Research Corporation; and in China with Hu Zhihong, Wuhan Institute of Virology, Chinese Academy of Sciences, and the Southwestern Agricultural University.

Search for Target Sites for Insect Control

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Peter Krell and Basil Arif provided a good introduction to the research that I am going to present today. This research falls primarily under Program 4 of the Canadian Forest Service (CFS) Genomics R&D Initiative,[1] and specifically under the project entitled "Juvenile Hormone-Based Pest Management Tools for Forest Insects." Some of the research my colleagues and I have conducted also falls under Program 1 of this initiative,[2] within the project "Structural, Functional and Comparative Genomics of Insect Pests."

Two CFS laboratories are involved in this work: my laboratory at the Laurentian Forestry Centre in Québec, Quebec, and that of Qili Feng at the Great Lakes Forestry Centre (GLFC) in Sault Ste. Marie, Ontario. The research we perform is best described as a search for molecular target sites in the context of insect pest control.

What are molecular target sites, and why search for them? In the context of insect pest control, target sites are proteins — enzymes, receptors, or carrier proteins that are specific to insects or to a group of insects — typically involved in an insect-specific process such as molt, metamorphosis, or reproduction. Because the actions of these proteins are specific, it may be possible to interfere with them using inhibitors or antagonists, with minimal effects on nontarget organisms. Alternatively, when appropriate, the genes encoding these proteins can be used to engineer more effective microbial insecticides, as shown by Dr. Arif's work on the spruce budworm. The identification and characterization of such target sites can accelerate the development of novel, target-specific forest pest control products.

In the context of target-site research, we are interested in the following proteins: enzymes, hormone receptors, and carrier proteins, all of which have a ligand (a molecule that binds to a specific site on the protein). The ligand and the associated protein "recognize" each other in a manner similar to that of a key and a lock. In the case of enzymes, the ligand is a substrate that binds to the enzyme's catalytic cavity, hence triggering a catalytic process that transforms the substrate into new products. Hormones, as ligands, also have protein receptors to which they bind in a specific manner. This binding induces conformational changes in the receptor, which in turn allows the hormone–receptor complex to bind to a specific portion of DNA often referred to as a response element. This latter binding triggers DNA transcription and a cascade of molecular events of biological significance.

To interfere with a ligand–protein interaction, it is possible to find or design a competitive inhibitor or antagonist that has sufficient similarity to the natural ligand to bind tightly to the protein's active site, without triggering the biochemical reaction normally initiated by the natural ligand. This approach is commonly used in drug discovery.

What are the steps involved in this target-site strategy to develop pest control products? First, the researcher has to identify a new target site using one of several exploratory methods — for example, by constructing expressed sequence tag (EST) libraries or by choosing a known or suspected target site candidate based on what is known of the insect's biology. Second, the researcher has to clone the relevant complementary DNA (cDNA) — that is, the gene. With the gene in hand, various avenues may be considered. It may be appropriate to use the gene to engineer a microbial agent with the goal of enhancing its insecticidal activity; in this case, the modified microbial agents will need to be assayed to determine whether the genetic manipulation confers measurable enhancement of activity. Another avenue — and this is what we are aiming at with this research — is to produce the recombinant protein and develop an in vitro assay for the high-throughput screening of potential inhibitors or antagonists. With this type of approach, it will often be relevant to determine or model the three-dimensional structure of the protein so that we can examine the size and shape of the binding site and assess the likelihood of success of an inhibitor or antagonist strategy. Such structural knowledge allows us to use computer-assisted technology to do a first screen of potential inhibitor or antagonist molecules and then to test those leads in an in vitro assay. The molecules that show the most potential for pest control in these assays could then be tested in vivo.

The two main target-site research projects funded by the CFS Genomics R&D Initiative are aimed at blocking either the biosynthesis or the action of an insect hormone known as juvenile hormone (JH). However, some of our research involves other types of target sites not related to JH.

JH plays a role in insect development, particularly in that of lepidopteran insects such as our main target pest, the eastern spruce budworm (*Choristoneura fumiferana*). Shortly after hatching, the larva starts growing and goes through a number of larval molts, during which it sheds its old exoskeleton and acquires a new, larger one which permits further growth. After these larval molts, the larva resumes growth while retaining its juvenile characteristics; this is ensured by high levels of JH. When the larva reaches the final larval stadium, levels of JH fall to undetectable levels. Under these conditions,

[1] Program 4 of the CFS Genomics R&D Initiative is the Production of Environmentally Acceptable Forest Protection Methods.

[2] Program 1 of the CFS Genomics R&D Initiative is Forest Tree Production and Protection Systems.

secretion of the molting hormone ecdysone triggers metamorphosis and the pupal molt, which eventually gives rise to the moth.

It has long been known that if JH level can be artificially reduced early in larval life, this will cause precocious metamorphosis, early cessation of feeding, and a possible failure to complete development to the adult stage, as the pupa typically will not be viable. Our laboratories are examining two of the strategies for inducing precocious metamorphosis of the spruce budworm larvae: (1) blocking JH production using an inhibitor of a JH biosynthetic enzyme, and (2) blocking JH action by preventing binding to its receptor. Both strategies would have the same result.

There are six known forms of the sesquiterpenoid JH (Figure 1), the most common and simplest one being JH III, which is the only JH produced by most insects. Lepidoptera, however, produce four additional forms of this hormone, recognizable by the presence of one to three ethyl branches on the hormone's farnesyl backbone. We have hypothesized that one or more of the enzymes involved in the biosynthesis of these lepidopteran forms of JH have modifications in their catalytic cavity to accommodate these bulkier compounds.

We have recently shown that the Lepidoptera produce two very distinct farnesyl diphosphate synthase (FPPS) homologs. This enzyme is involved in the formation of the farnesyl carbon skeleton of JH and in other metabolic pathways. We have

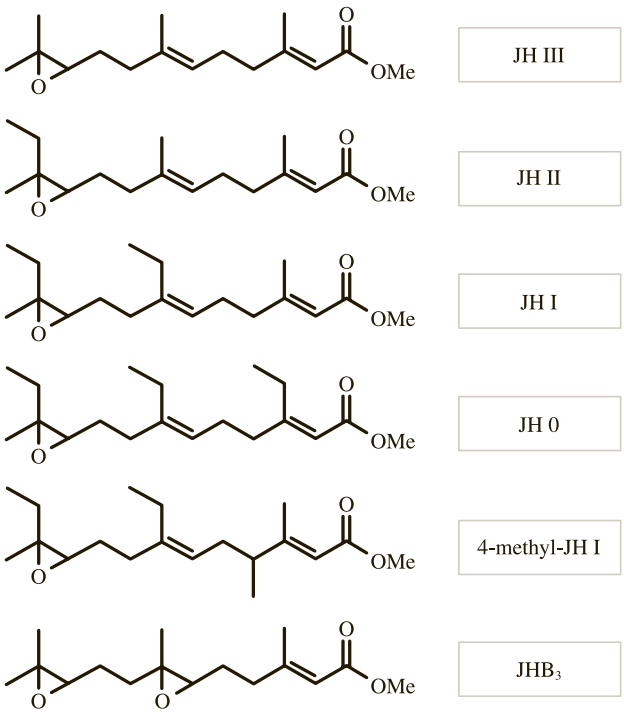

Figure 1. The six known forms of juvenile hormone. JH II, JH I, JH 0, and 4-methyl JH I have so far been found only in the Lepidoptera. (O=oxygen; Me=methyl.)

cloned FPPS cDNAs from lepidopteran representatives, including the spruce budworm, as well as from a number of non-lepidopteran insects. Through extensive amino acid sequence comparison, we showed that one of the two lepidopteran FPPSs (type I) displays several unique amino acid substitutions within its catalytic cavity. For example, we found four substitutions within a specific motif of this protein, which is well conserved among all living organisms, except the Lepidoptera. We used homology modeling and docking simulations to examine the potential impact of these substitutions on the volume of the catalytic cavity and found that there is actually an increase in the volume of the catalytic cavity of this enzyme relative to "conventional" FPPSs. In comparison, the second lepidopteran FPPS type has fewer substitutions within its active site and in this respect appears more "conventional" than the type-I protein, although unique in other respects. It is still not clear which of these two FPPS types is involved in JH biosynthesis, but we know that the expression of the type-II enzyme is essentially confined to the JH-producing glands, whereas that of the type-I enzyme is more ubiquitous (Cusson et al.). Whichever of these two FPPS homologs is involved in JH biosynthesis, the substitutions identified in each could facilitate the production of the ethyl-substituted precursor of JH. As a consequence, it would become possible to find an inhibitor that is specific to this particular catalytic cavity without affecting conventional FPPSs, making it a Lepidoptera-specific compound. We are now in the process of developing in vitro assays to test these hypotheses.

For the past few years, Qili Feng's group has been working on isolating a JH receptor. JH is an elusive molecule, and the challenge of cloning it has been taken up by several competitive laboratories. To achieve their goal, Dr. Feng and his team are using an original strategy based on the observation that JH can activate its own hydrolysis into an inactive form, JH acid. This hydrolysis is effected by a specific enzyme called juvenile hormone esterase (JHE), which is instrumental in preparing the insect for metamorphosis. JH can very rapidly activate transcription of the JHE gene; researchers believe that it does so by binding to its receptor, with the receptor–hormone complex subsequently binding to a response element within the promoter[3] of the JHE gene, which in turn activates JHE transcription. Qili Feng's group has identified the 30-base-pair response element within this promoter (Kethidi et al. 2004). They have made constructs of this promoter with luciferase, a reporter gene (or genetic marker), and transformed a culture of spruce budworm cells. When these cells are treated with JH, the reporter gene is induced in a measurable way, thereby providing a cell-based assay system. This system could be used to screen antagonists of JH, which would presumably prevent binding to the JH receptor and thus, JH activity. Using the response element DNA as a probe, they were able to identify a nuclear protein that binds

[3] A DNA site to which RNA polymerase binds and initiates transcription.

specifically to it and that is therefore a candidate JH receptor. Efforts are currently being made to purify this protein.

I now wish to turn your attention to the enzyme chitinase, which is involved in the breakdown of chitin, a homopolymer of *N*-acetylglucosamine and a major constituent of the insect cuticle secreted by the epidermis. Chitinase expression is induced by the secretion of the molting hormone ecdysone just before the molt. It helps break down the old cuticle when it is being shed. In this particular case, Qili Feng's group used a chitinase cDNA to modify *Autographa californica* nucleopolyhedrovirus, or AcMNPV (Zheng et al. 2002), so that it can be used as a viral insecticide against a number of insect pests. They have assayed this modified virus and compared it with the parent virus. The results indicate that the chitinase gene is an excellent candidate for the genetic improvement of baculoviruses.

What is the significance of this research for Canada? First, it helps put Canada on the leading edge of genomics-based pest control research and product development. Second, this research aims at broadening control options for forest pests by identifying effective and environmentally safe products. Indeed, there are currently very few options for the control of insect pests such as the spruce budworm. Also, some of the target-site proteins cloned at the GLFC are being used to engineer more effective viral insecticides aimed at the spruce budworm and other pests. As other speakers at this symposium have pointed out, some of the discoveries made through this research may affect other areas — for instance, the development of gene switches using an ecdysone receptor [see Krell, this publication]. It seems quite possible that researchers could also use JH receptors in similar gene switches, given that JH is not found in humans. Finally, the two laboratories involved in this research have significantly contributed to the training of highly qualified personnel in the area of insect biotechnology in the last five years, including eight postdoctoral fellows and numerous students and technicians.

Our research collaborators include Indiana University–Purdue University at Indianapolis; the University of Kentucky; the Gembloux Agricultural University in Belgium; the Highthroughput Factory at the RIKEN Harima Institute in Japan, where we have a collaborator helping out on homology modeling; the Southwest Agricultural University in China; and Peter Krell at the University of Guelph, Ontario.

The main sources of funding for our research have been the CFS Genomics R&D Initiative and Genome Canada, through the Ontario Genomics Institute. We have also received funds from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Biocontrol Network, the CFS, and the Ministère des Relations internationales du Québec.

In terms of future directions for our research, a number of possibilities can now be explored, but I think that efforts should be focused on the most promising target sites, while exploring new leads provided by ongoing microarray work. Indeed, the recently constructed spruce budworm EST libraries and the sequences of viral genomes will provide us with new tools for the development of DNA chips, or microarrays, that will help us identify genes that are potentially targeted by viral pathogens. We also need to bolster CFS expertise in structural bioinformatics and protein biochemistry to speed up the product development phase of this research. Lastly, industrial partnerships will have to be sought once in vitro and cell-based assays have reached the point where they can be used for screening compounds.

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Sawfly Nucleopolyhedrovirus Functional Genomics

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The balsam fir sawfly (*Neodiprion abietis*), indigenous to Newfoundland, was a forest pest of minor importance until 1990. Since then, however, it has caused moderate to severe defoliation in about 260 000 ha of the province's prime balsam fir (*Abies balsamea*) stands (Moreau 2006). The recent increase in sawfly damage seems to be connected to the silvicultural practice of precommercial thinning (Moreau et al. 2006).

Sawflies are primitive Hymenoptera in a suborder (Symphyta) originating about 250 million years ago, whereas Lepidoptera dates back about 150 million years ago. Sawfly nucleopolyhedroviruses (NPVs) are likely ancestral to the baculoviruses found in Lepidoptera and pre-date the separation of the NPVs and granuloviruses[1] in Lepidoptera (Garcia-Maruniak et al. 2004; Lauzon et al. 2004). Consequently, sawfly NPVs have smaller genomes and more simplified life cycles compared with lepidopteran NPVs.

Sawfly and lepidopteran NPVs differ in how they infect their hosts. The former only infect cells of the larval midgut; the latter first infect the larval midgut and then spread to internal tissues such as the body fat. Because sawflies are often gregarious and feed openly on the foliage and because the viral infection is restricted to their midgut much like a stomach flu, sawfly NPVs move through the population quickly. More than 80% of larval mortality in populations of balsam fir sawfly can be attributed to its species-specific NPV (NeabNPV) (Moreau 2004). The rate of spread of lepidopteran NPVs, however, depends on a number of factors; in larvae that are gregarious and tend to feed openly on foliage, the infection spreads more easily. Eastern spruce budworm (*Choristoneura fumiferana*), on the other hand, tends to be more resistant to infection by its baculoviruses due to reduced opportunities for exposure, and when exposure can occur, larvae are older, larger, and less susceptible. Early-instar budworms mine new needles and buds of balsam fir and white spruce (*Picea glauca*) and only emerge to feed openly on the foliage in the later instars or when population densities are high. As a result, the amount of spruce budworm mortality that can be accounted for by NPVs is less than 2% (Lucarotti et al. 2004). For NPV applications to be effective for forest insect pest control, a single application in the range of 1–10 billion occlusion bodies/ha is generally sufficient against sawflies; application rates a hundred to a thousand times higher are required to get any control of lepidopteran pests (Cunningham and Kaupp 1995).[2]

[1] The family Baculoviridae is subdivided into two genera, the nucleopolyhedroviruses and the granuloviruses.

[2] Occlusion bodies are protein structures containing the viruses. They are formed in the host cells and released into the environment when host cells die, thus ensuring virus survival outside the cells and subsequent infection of other host cells.

When it became apparent that NeabNPV was the principal cause of mortality in balsam fir sawfly populations (Moreau 2004), the team working on balsam fir sawfly–NeabNPV research at the Atlantic Forestry Centre, Canadian Forest Service (CFS), decided to focus its efforts on getting the nucleopolyhedrovirus registered as a biological control agent. Data from aerial efficacy trials conducted over three years were included in the registration package. The maximum test area for which a research permit in forestry is granted by the Pest Management Regulatory Agency (PMRA), Health Canada, is 5000 ha. We sprayed NeabNPV at a rate of 1–3 billion occlusion bodies/ha, applied in 2.5 L of 20% aqueous molasses solution from a Cessna 188 aircraft equipped with four under-wing Micronaire AU4000 atomizers. After the application, we observed an increase in NeabNPV prevalence in balsam fir sawfly populations, and in the following year, a rapid decline in populations that had previously been increasing or peaking (Moreau et al. 2005). A submission to the PMRA for registration of NeabNPV for use against balsam fir sawfly under the trade name Abietiv™ was sent in June 2004.

The balsam fir sawfly–NeabNPV project was supported with CFS salaries and facilities and through the CFS Genomics R&D Initiative and the Enhanced Pest Management Methods program. We initiated the project with a Natural Sciences and Engineering Research Council of Canada (NSERC)–CFS–Industry Research Partnership grant followed by NSERC support through the Biocontrol Network. Also, because we were able to apply NeabNPV on 5000 ha of forest per year during our efficacy trials, we received additional funds from the Province of Newfoundland and Labrador and our industrial partners Corner Brook Pulp and Paper Ltd. (a Kruger Inc. subsidiary) and Abitibi-Consolidated Inc. This allowed us to carry out the efficacy applications at a reduced cost of about $80/ha of forest treated.

The data collection to register NeabNPV as a biological control agent facilitated other related research on the balsam fir sawfly. We studied sawfly population dynamics, ecology, and impact on the forest to bridge knowledge gaps on the insect. We researched NeabNPV epidemiology, pathology, and prevalence in natural populations of its host. Finally, we ventured into the field of functional genomics. We published a genome sequence paper on the redheaded pine sawfly (*Neodiprion lecontei*) NPV (Lauzon et al. 2004), and another group centered at the University of Florida, Gainesville, simultaneously published a sequence paper on the European pine sawfly (*N. sertifer*) NPV (Garcia-Maruniak et al. 2004). This genome research, along with the sequence data that we had at the time for NeabNPV (S. Duffy et al., University of Victoria, British Columbia, personal communication), was included in our submission to the PMRA.

What is the relevance of functional genomics to our research? Baculoviruses can be viewed as living fossils in that they provide a record of the evolution of a group of DNA viruses. Through functional genomics and by comparing genomes, researchers can learn much about the evolution of baculoviruses and possibly about that of other DNA viruses. Functional genomics may also facilitate the registration of baculoviruses as biological control agents, allowing researchers to determine what the genome sequences of different baculoviruses have in common and whether or not there is something odd in a particular genome. Such information, one hopes, would speed up the baculovirus registration process. One might also develop environmentally safe pest control methods by genetically modifying baculoviruses; for example, a lepidopteran NPV could be made more effective by altering it so it behaves like a sawfly NPV.

I thank the Newfoundland and Labrador Department of Natural Resources, Abitibi-Consolidated Inc. and Corner Brook Pulp and Paper Ltd., the Biocontrol Network, the CFS, the Fundy Model Forest, the Québec Société de protection des forêts contre les insectes et les maladies (SOPFIM), and the Ontario Ministry of Natural Resources for their contribution to this research. I also gratefully acknowledge the work of Dr. Basil Arif (CFS, Great Lakes Forestry Centre) on the sequencing of the genome of the redheaded pine sawfly NPV and that of Dr. David Levin (University of Victoria) on the sequencing of the genome of the balsam fir sawfly NPV. Finally, for their work on the Abietiv™ field efficacy trials and balsam fir sawfly ecology and impact studies, I thank Dr. Eldon Eveleigh, Steve Holmes, Ed Kettela, Dr. Gaétan Moreau, Benoit Morin, Dr. Don Ostaff, Dr. Harald Piene, Dr. Graham Thurston, Charles Weaver, Grant White (CFS, Atlantic Forestry Centre), Dr. Dan Quiring (University of New Brunswick), and many graduate students and laboratory and/or field assistants.

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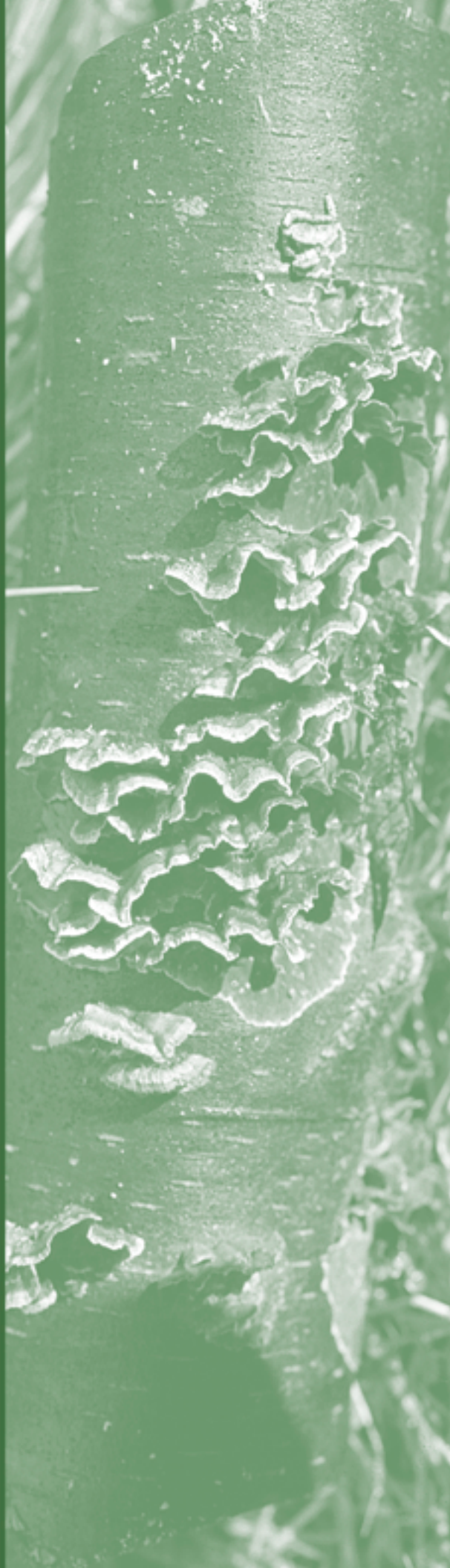
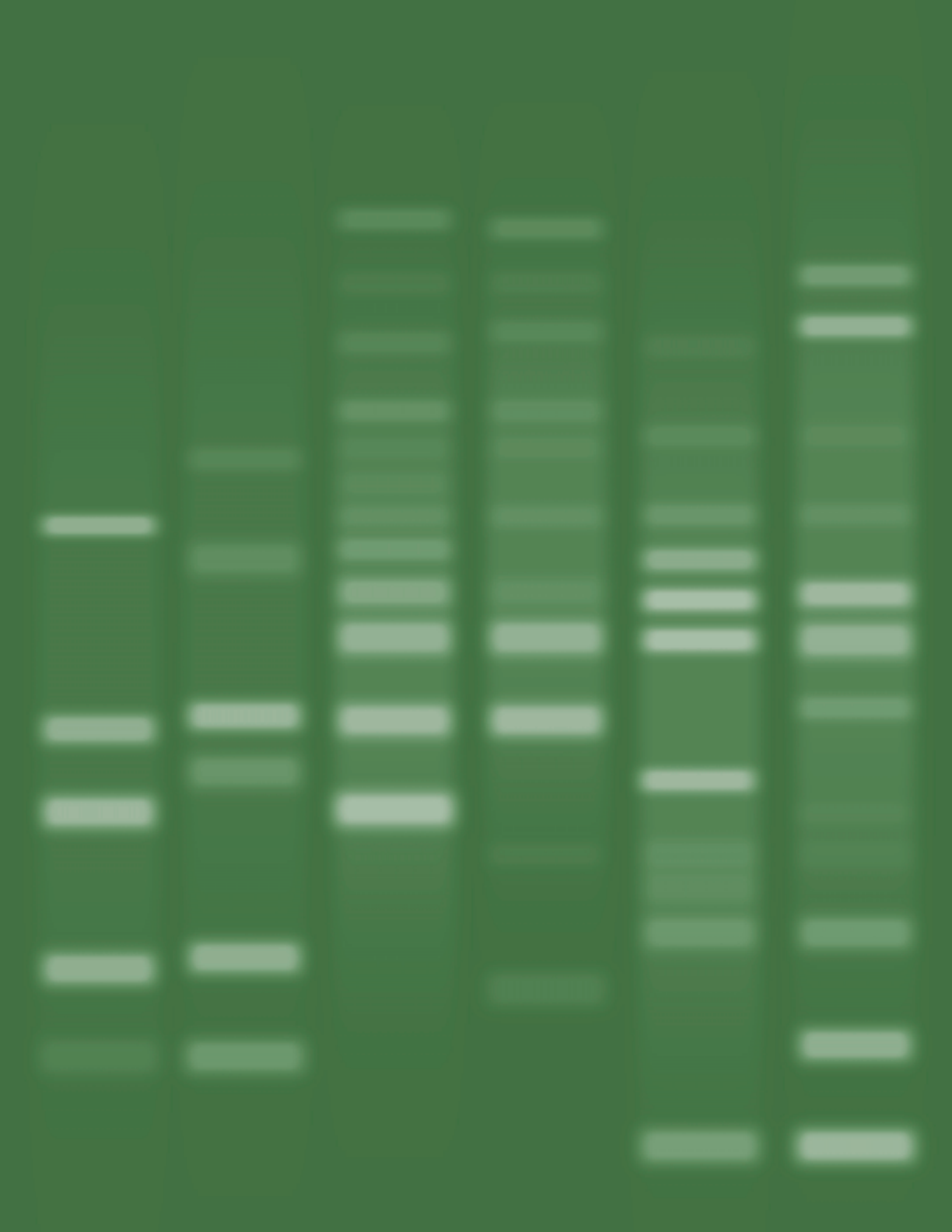
FUNGAL GENOMICS





Genomic Approaches to Identifying Fungal Enzymes for the Forest Industry

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Over the past century, economic growth in industrialized nations has depended on the use of fossil fuels and chemicals. However, this type of economy cannot be sustained forever; fossil fuels are nonrenewable resources, and the widespread use of chemicals is linked to the deterioration of the environment and human health. Many industrialized countries are thus now moving towards economies that are based on renewable resources and biological processes. For a country with abundant natural resources such as Canada, shifting to such an economy could lead to economic expansion, improved competitiveness, a cleaner environment, increased energy security, and more social cohesion of rural communities.

Up to about a hundred years ago, a bio-based economy — that is, an economy based on renewable biological resources — was the norm. In these pre-industrialized societies, most people struggled just to subsist; only a few could afford a high standard of living. Therefore, returning to a bio-based economy without associating it with technological advances is not likely to achieve the basic tenets of sustainable development — economic growth, a clean environment, and social benefits. In its strategic plan for sustainable development, the Government of Canada advocates the use of biocatalysis,[1] fermentation, and green technology to transform renewable bioresource feedstocks such as plants (crops, trees, and algae), animals, fish, microbes, and organic residues (municipal, industrial, agricultural, forestry, and aquaculture). These feedstocks can be used to manufacture industrial bioproducts such as biofuels, bioenergy, biochemicals, bioplastics, biosolvents, and biosurfactants.[2] Biotechnology and genomics are two of the enabling technologies needed to facilitate the development of bioprocesses.

The research described in this presentation was conducted by a team of researchers from four institutions in the Greater Montréal area: Concordia University, the Institut National de la Recherche Scientifique (INRS)–Institut Armand-Frappier, the Pulp and Paper Research Institute of Canada, and the Biotechnology Research Institute of the National Research Council of Canada. The research project, supported by Genome Canada and Génome Québec, uses biotechnological and genomic approaches to identify fungal enzymes for industrial and environmental applications. I will describe how enzymes can be used to process traditional forest products and to convert forest residues into new forest products. I will also review our efforts in finding enzymes that can potentially be used for these applications.

[1] The use of biological systems or their components, for example, enzymes, to carry out chemical reactions.

[2] Microbially produced compounds that lower surface tension between other compounds, hence functioning similarly to detergents.

Using Bioprocesses

Bioprocesses are generally believed to be less polluting but more expensive than chemical processes. However, in a life cycle assessment of over 20 case studies, the Organisation for Economic Co-operation and Development (OECD) showed that bioprocesses invariably produce less waste and consume less energy than chemical processes. Once bioprocesses are set up, their efficiency can be improved rapidly to make them less costly than chemical processes (OECD 2001). The case study involving the production of acrylamide illustrates this well and is discussed below.

Acrylamide is commonly used in life sciences laboratories for the separation of macromolecules through electrophoresis. Twenty years ago, acrylamide was synthesized by mixing acrylonitrile with heated sulphuric acid and copper. This chemical method was quickly replaced when researchers discovered that the enzyme nitrile hydratase could catalyze the conversion of acrylonitrile to acrylamide. The enzymatic method was superior to the chemical one in several ways (OECD 2001):

	Chemical process	**Enzymatic process**
Purity	70–80%	>99%
Process temperature	70°C	4°C to room temp.
Energy consumption	1.9 MJ/kg	0.4 MJ/kg
CO_2 emission	1.5 kg CO_2/kg	0.3 kg CO_2/kg

I was also able to determine the retail price of acrylamide over the years by checking prices for it in suppliers' catalogues. I found that in 1990 two grades of acrylamide were sold: a reagent grade (over 70% pure) at $90/kg and an electrophoresis grade (over 98% pure) at $620/kg. In 2004, only an electrophoresis (or molecular biology) grade (over 99% pure), was offered, retailing at $54/kg. By this time, only the enzymatic process was used to produce acrylamide. In addition, this example and others cited by the OECD show that bioprocesses are cleaner and more energy efficient than chemical processes.

Transforming Forest Products and Residues

The pulp and paper industry employs about 70 000 Canadians, and pulp mills are present across the country in all provinces. Canada is the world's fourth largest pulp and paper producer but its largest pulp and paper exporter. The pulp and paper industry contributes about $17 billion to Canada's trade balance.

Almost all pulp and paper products manufactured in Canada are made from wood. Of the dry weight of wood, 50% is composed of cellulose, the main fibre used for papermaking; about 20% consists of hemicellulose, mostly xylan; and the remaining 30% comprises mainly lignin with a low amount of resins and lipids. Mechanical and chemical methods are predominantly used in the manufacture of pulp and paper. Although many enzymes have been shown to be useful in pulping and papermaking (Figure 1), only a few are actually used (Bajpai 2004). The most extensive use of enzymes in the Canadian paper industry is the inclusion of xylanase in the pulp bleaching process; about 10% of the pulp is bleached this way. The mode of action of xylanase in the bleaching process is not fully understood; the proposed mechanism suggests that xylanase degrades surface xylan in the wood, thereby making the lignin more exposed to oxidizing chemicals (Paice et al. 1992) and reducing the amount of chemicals needed. There are other enzymes used during paper recycling, though to a lesser extent than xylanase; these consist of amylase, for de-inking, and cellulase, for re-pulping.

The Canadian transportation sector depends almost exclusively on petroleum. However, climate change and the projected shrinking supply of petroleum have prompted the search for alternative fuels. Substituting gasoline for ethanol is being strongly promoted in the United States, Brazil, and some Canadian provinces. Recent models of automobiles are designed to run on either E85 — 85% ethanol and 15% gasoline — or gasoline. Current production of fuel ethanol uses the enzymes amylase and glucoamylase to break down starch into glucose for fermentation into ethanol. The term "bioethanol" is used to describe ethanol produced from biomass such as wood, grasses, and organic wastes (Sheehan and Himmel 1999). In 2003, the Canadian section of Iogen Corporation built the world's first bioethanol demonstration plant.

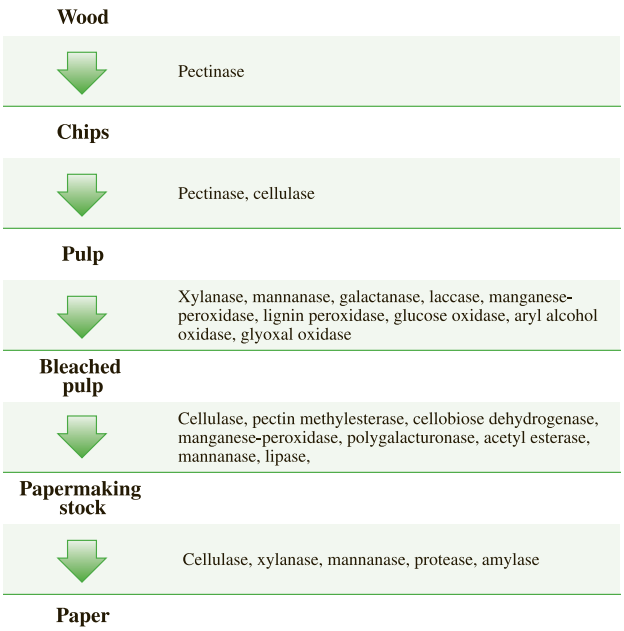

Figure 1. Enzymes used in papermaking.

Biomass feedstock for bioethanol is mainly composed of cellulose, followed by hemicellulose. As already mentioned, hemicellulose can be efficiently degraded by available enzymes, but breaking down cellulose into glucose is considerably more challenging. Although cellulase is often referred to as the enzyme that breaks down cellulose, the synergistic activity of at least three enzymes is required to degrade cellulose. Endoglucanase cleaves the cellulose polymers internally. Exoglucanase (also called cellobiose hydrolase) removes disaccharides from the ends of the cellulose polymers. Finally, beta-glucosidase cuts the cellobiose into glucose molecules, an excellent fermentation feedstock to produce ethanol (Tomme et al. 1995).

Canada harvests about 1 million ha of forest annually, generating about 38 million t of organic residues not used to manufacture products (CPPA 2001). If this amount of organic residues could be converted into fermentation feedstock, it would produce about 5 billion L of bioethanol, that is, roughly 10% of the country's gasoline consumption. The Canadian Forest Service, Natural Resources Canada, estimates that over 21 million ha of forest are damaged, and half of the harvested trees are infested. Altogether, organic residues produced by Canadian forestry activities provide ample potential feedstock for fermentation into ethanol.

Finding Fungal Enzymes

Enzymes are usually efficient in converting simple substrates into products and can function under mild conditions. However, the enzymes currently available are not efficient nor cost-effective in transforming complex substrates such as wood into new products. Moreover, researchers do not fully understand the combination of enzymes required to break down such a complex substrate.

Fungi are the major decomposers of the biosphere. Many fungi have evolved the ability to degrade wood by secreting extracellular enzymes that break down lignocellulose and then transporting the resulting sugars and nitrogen into their cells. Enzymes secreted by fungi are known to perform the most complex and diverse chemical reactions. The aim of our research project is to find such fungal enzymes and learn to use them to break down woody materials. We also want to improve our understanding of how fungi consume wood at the molecular level in order to gain insights into the enzyme combinations needed to decompose lignocellulose.

Figure 2 provides an overview of our research project. We begin by constructing cDNA (complementary DNA) libraries of fungi and using expressed sequence tags (ESTs) to identify a set of genes encoding secreted proteins. These target genes

are then cloned into expression vectors and inserted into organisms, the expression hosts, to produce recombinant proteins. The recombinant proteins are characterized biochemically and tested for their usefulness in industrial applications. The genes identified are also used to construct cDNA microarrays to study transcription profiling. In the hope of identifying enzymes with a broad range of activities, we have launched a gene discovery program on 15 evolutionarily diverse fungal species. These 15 fungi can be separated roughly into five groups:

(1) white-rot fungi: *Lentinula edodes, Phanerochaete chrysosporium,* and *Trametes versicolor;*
(2) pollutant-degrading fungi: *Aureobasidium pullulans, Amorphotheca resinae, Cunninghamella elegans,* and *Leucosporidium scottii;*
(3) lignin- and pitch-degrading fungi: *Aspergillus niger, Coprinus cinereus, Gloeophyllum trabeum,* and *Ophiostoma piliferum;*
(4) freeze-tolerant fungi: *Cryptococcus laurentii* and *Geomyces pannorum;* and
(5) thermophilic composters: *Thermomyces lanuginosus* and *Sporotrichum thermophile*.

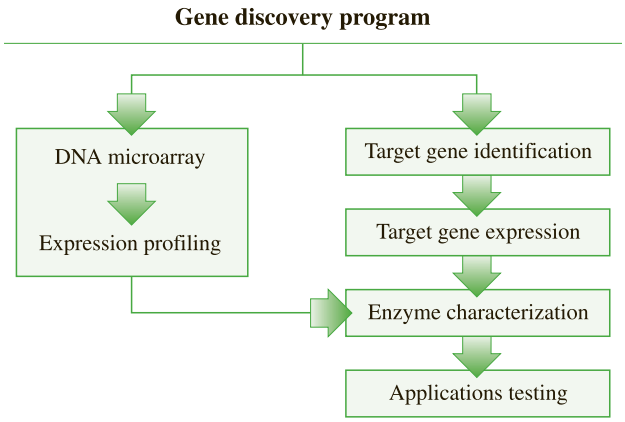

Figure 2. Overview of Génome Québec's fungal enzyme identification project.

For each species of fungus, we perform about 15 000 single-pass sequencing reactions of clones from normalized cDNA libraries, from the 5′ end, to generate ESTs. These 15 000 ESTs typically assemble into approximately 5 000 unigenes. From this random set of genes, we identify the potential gene targets.

All genes encoding secreted proteins are potential targets in our research project. Proteins secreted by eukaryote cells are normally translocated into the lumen of the endoplasmic reticulum as they are being synthesized. After a series of folding and processing, they are transported outside the cells via transport vesicles. The sorting of secreted proteins in the endoplasmic reticulum is based on the recognition of a signal peptide in the *N*-terminus of the protein. We use the program SignalP (Bendtsen et al. 2004) to identify the genes encoding secreted proteins. In addition, we use the BLAST family of programs and other bioinformatic tools to assign putative functions to the target genes. On average, about 4% of the genes examined are actually found to encode secreted proteins and of these genes, about 40% can have a putative function assigned. The function of the remaining genes can not currently be determined using bioinformatics tools. Table 1 summarizes the number of genes encoding the major classes of secreted enzymes in four of the fungal species examined.

The target genes compiled in Table 1 are sequenced completely from both ends using sequence-specific primers. They are further analyzed for features that may help sort the expressed proteins to cellular compartments other than the endoplasmic reticulum. The target genes deemed to encode secreted proteins following manual annotation are then processed for expression.

The protein-coding regions of the target genes are amplified and cloned into expression vectors. We use two fungal hosts, *Aspergillus niger* and *Pichia pastoris*, and one bacterial host, *Streptomyces lividans*, to express the secreted proteins. Of the genes encoding secreted proteins with predicted function, about 40% can be expressed functionally in *A. niger*. Most of these latter genes can also be expressed in the other fungus,

Table 1. Number of genes encoding four major classes of secreted enzymes and number of genes for which the function is not yet known for four fungal species.

	Enzymes				
Fungi	**Amylase**	**Cellulase***	**Lipase**	**Peroxidase**	**Unknown function**
Aspergillus niger	6	30	8	0	102
Cunninghamella elegans	0	5	19	0	112
Gloeophyllum trabeum	4	32	12	0	169
Phanerochaete chrysosporium	8	54	12	15	147

*Includes endoglucanase, cellobiose hydrolase, and beta-glucosidase.

P. pastoris. Similarly, most of the genes that cannot be expressed functionally in *A. niger* are not produced by *P. pastoris* either. However, about 20% of the genes that cannot be expressed in *A. niger* nor in *P. pastoris* are produced at detectable levels by the bacterium *S. lividans*.

The recombinant proteins expressed are harvested from the culture medium and concentrated using size-exclusion membranes. We perform basic biochemical characterizations such as temperature optimum, pH optimum, and temperature stability on the proteins using defined substrates. The recombinant proteins are also tested for their utility in various industrial applications. With colleagues at the Pulp and Paper Research Institute of Canada, we are testing these enzymes for their biobleaching and biopulping properties. We have also developed microplate assays to test the enzymes' ability to break down insoluble cellulose (Xiao et al. 2004). One of the challenges now is to establish high-throughput protocols for applications testing, as the same enzyme can find applications in many unrelated fields.

Genes that encode proteins involved in a specific biological process are often regulated in coordination. Hence, examining the transcription profiles of fungi exposed to different complex substrates is expected to yield information on the enzymes needed to break down those substrates. Moreover, the genes of unknown function that are upregulated in the presence of a defined substrate are likely to encode proteins that are involved in the catabolism of that substrate. The latter information could provide us with clues as to the physiological roles of genes whose function we cannot predict based on sequence analysis.

To date, we have constructed cDNA arrays for *Aspergillus niger*, *Cunninghamella elegans, Gloeophyllum trabeum*, and *Phanerochaete chrysosporium*. Our transcription profiling results show that the genes involved in the utilization of carbon sources are tightly regulated. For example, when cultured in xylose, *A. niger* upregulates the genes that encode xylose-utilizing and xylan-degrading enzymes by as much as 200-fold.

Conclusions

We have established the technology platforms and the analysis pipelines to identify and express fungal enzymes that are potentially useful in replacing chemical and mechanical processes in the manufacture of pulp and paper. Some of the enzymes identified may also find applications in the conversion of forest residues into fuels. A major challenge is to put in place application protocols to test a large number of enzymes. We plan to forge partnerships with the private and public sectors to develop new applications.

Acknowledgments

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Structural and Functional Genomics of Dutch Elm Disease Fungi

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Before I describe the genomics research performed by my team, I would like to take a brief historical detour. During the discussion session held on the first day of this symposium, someone asked me why Dutch elm disease was so named. Were the Dutch the culprits? "Quite the contrary," I replied. The name "Dutch elm disease" was coined as a tribute to the pioneering work by seven female researchers in the Netherlands, who, starting in1919, carried out the first scientific investigations of this disease of elms, then newly introduced into northwestern Europe. With the encouragement of Dr. Johanna Westerdijk, who headed the Willie Commelin Scholten Phytopathological Laboratory for more than 40 years, these scientists not only showed that the disease was caused by a fungus, but they also initiated a successful breeding program for improving the resistance of elms (Holmes and Heybroek 1990).

Components of Dutch Elm Disease

Dutch elm disease (DED) is a vascular wilt that can kill a mature elm within a few weeks. Since the end of World War I, two successive pandemics have decimated elm populations in Europe and North America. As a result, some rural and urban environments have been altered drastically in these parts of the world. For example, a century ago a typical eastern North American street would be lined with American elms (*Ulmus americana*). Indeed, the characteristic umbrella shape of elms and their high resilience to various abiotic stresses had made them a highly popular ornamental tree species. After the disease passed through, however, thousands of streets in hundreds of North American cities and towns were left without elms.

The need for efficient and sustainable control methods against DED has provided a strong incentive for studying the various components of the disease. Besides, the study of DED has aspects that make it quite interesting to plant pathologists. It is a nice model for wilt diseases in trees and provides a dramatic example of what alien pathogens can do to native tree populations. The causal agents of DED are easily manipulated in the laboratory and highly amenable to genetic manipulation, which is not always the case for pathogens of forest tree species. Moreover, relatively simple bioassays can be performed to understand the development of the disease. For example, pathogenicity assays can be carried out by injecting spores of the DED fungus directly into the xylem of elm saplings. After a few weeks, one can observe and quantify the typical external symptoms of the disease, that is, wilting of the leaves. Finally, there is abundant literature on this disease and its causal agents (for a recent review, see Gil et al. 2004).

The DED pathogens are ascomycete fungi; more specifically, they belong to a group collectively known as ophiostomatoid fungi. For many years, the disease was thought to be caused by a single fungal species, which was referred to as *Ceratocystis ulmi*. Nowadays, three different species have been reported to cause Dutch elm disease, and they have been reclassified in the genus *Ophiostoma*. Interestingly, the species that caused the first pandemic shortly after World War I, *Ophiostoma ulmi*, is now disappearing. It has been displaced by *O. novo-ulmi*, which is responsible for the current pandemic in both Europe and North America. Compared with *O. ulmi*, *O. novo-ulmi* is more aggressive and exhibits greater fitness (Brasier 1991). In the mid-1990s, Brasier and Mehrotra (1995) described a third species, *O. himal-ulmi*, endemic to the Himalayan region. Although *O. himal-ulmi* does not cause wilt on elms growing in this area, controlled inoculations of European elm species show that this fungus is highly aggressive. Therefore, if *O. himal-ulmi* was accidentally introduced into Europe or North America, it would likely make an already difficult situation worse.

Like most ophiostomatoid fungi, DED pathogens are vectored by insects, which in this case are elm bark beetles. The beetle itself is not a major problem. Native bark beetles have been living with elms for thousands of years and do not cause much damage. Unfortunately, the situation changed drastically upon the arrival of the DED pathogens, first in western Europe and later in North America. Spores of the pathogen are embedded in sticky mucilage, which facilitates their adhesion to the exoskeleton of beetles that come in contact with them. When the beetles feed into twig crotches in the crowns of healthy elms, they inject these spores into the elm vessels, where the fungus proliferates and eventually kills the tree (Lanier and Peacock 1981).

In another presentation at this symposium, Adrian Tsang described the different fungal species he was studying, including the sapstain fungus *Ophiostoma piliferum*. This fungus is closely related to the DED fungi. However, sapstain fungi are generally saprophytes; that is, they colonize the wood without degrading it or changing its structural properties. However, because they synthesize large amounts of melanin, they stain the wood, which is a problem to the forest products industry. Most sapstainers can be found in the genera *Ophiostoma*, *Ceratocystis*, and *Leptographium* (Seifert 1993).

Studies of a number of *Ophiostoma* species show that these are haploid organisms; their nuclear genome is believed to contain between 30 and 40 million base pairs (Dewar and Bernier 1995). A comparison with filamentous species such as *Neurospora crassa*, whose entire nuclear genome has been sequenced (Galagan et al. 2003), provides a rough estimate of 8 000 to 10 000 genes in the *Ophiostoma* nuclear genome. The chromosome number is variable, which is not unusual in fungi. It also comes as no surprise that pathogenicity has been shown

to be under polygenic control in *O. ulmi* and *O. novo-ulmi* (Brasier 1988). A few important genes have been mapped and/or cloned in the DED fungi; these include two pathogenicity genes, one gene involved in pigmentation, and one coding for cerato-ulmin. The latter belongs to a group of proteins known as hydrophobins and for many years was thought to be the main pathogenicity factor in the elm–*Ophiostoma* interaction. Studies of both null mutants[1] of *Ophiostoma* and mutants of *Ophiostoma* overexpressing cerato-ulmin suggest this hydrophobin is a fitness factor rather than a pathogenicity determinant (Bowden et al. 1996; Temple et al. 1997). A lot of research remains to be done, however, because the genes cloned so far amount to less than 0.2% of the nuclear genes one could expect to find in these fungi.

Genomic Analysis of the DED Pathogens

In 2001, researchers at four Canadian universities were awarded a grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) Genomics Projects Program to study the genomics of two closely related *Ophiostoma* species: the saprophyte *O. piceae* and the pathogen *O. novo-ulmi*. My group at Université Laval, Paul Horgen's group at the University of Toronto, and Will Hintz's group at the University of Victoria studied *O. novo-ulmi*, whereas Colette Breuil's group at the University of British Columbia carried out research on *O. piceae*. Genomic studies of *O. novo-ulmi* will be the focus of this presentation.

One aspect of the project involves structural genomics. The use of pulsed-field gel electrophoresis makes it possible to physically separate and distinguish individual chromosomes in *Ophiostoma* spp. (Dewar and Bernier 1995; Dewar et al. 1996, 1997). There is a high variability of the chromosome karyotypes in these species. We are also developing a linkage map for the Dutch elm disease fungi. Since the chromosomes can be separated, we can produce maps that include genetic markers physically located on the proper *Ophiostoma* chromosomes. Our current genetic map, based on the analysis of F_1 progeny from an *O. ulmi* × *O. novo-ulmi* cross, includes over 200 nuclear markers.

The bulk of our genomics project was to obtain expressed sequence tags (ESTs). The use of ESTs has its limitation in that they represent a very restricted part of the genome. However, given our limited funding, it provided a much less expensive alternative to the usual large-scale sequencing of total genomic DNA.

In Will Hintz's laboratory at the University of Victoria, we sequenced a reference library of complementary DNA (cDNA) clones from a yeast-like cell culture of *O. novo-ulmi* reference strain H327. Close to 6 000 ESTs were sequenced, 75% of which were readable. The latter included roughly 900 unique sequences for which we found homologs with known functions in public data banks — most of these sequences belonged to the metabolism and protein function classes according to the Munich Information Center for Protein Sequences (http://mips.gsf.de) — and an equal number of orphans, that is, coding sequences for which the search algorithms could not find homologs. A high number of the remaining *O. novo-ulmi* ESTs had strong homology to sequences with unknown functions.

EST sequencing is known to offer a diminishing return over time, a limitation we actually encountered in our project. Indeed, at first, because we knew so little about the *Ophiostoma* genome, virtually all of the sequences produced were novel. However, by the end of the sequencing effort, we were only getting about 30% new sequences per plate of 96 clones analyzed. One way to circumvent this problem is to construct cDNA libraries from the fungus exposed to different conditions. In this case, we were interested in both the temperature response of the pathogen and its different developmental stages. *O. novo-ulmi* is a dimorphic fungus; we thus constructed cDNA libraries from the yeast, mycelium, fruiting bodies, and parasitic state. We also used suppression subtractive hybridization (SSH), a method for enriching our samples for novel cDNAs (Diatchenko et al. 1999). More specifically, we obtained SSH-enriched libraries from *O. novo-ulmi* strain H327 growing at either high (31ºC) or low (15ºC) temperature — these are suboptimal temperatures compared with the optimal temperature of 25ºC (Brasier et al. 1981). We also made expression libraries enriched for sequences expressed during the formation of synnemata (asexual fruiting bodies) and perithecia (sexual fruiting bodies), as well as from elm calli previously inoculated with the fungus.

Enrichment by SSH is labor-intensive and we assessed whether it had been worth the effort. We compared the 4 500 readable ESTs from our *O. novo-ulmi* reference library with those from two small libraries obtained through SSH — from the fungus growing at high temperature, and during the formation of perithecia. The two subtracted libraries shared only 6% sequences, and using SSH allowed us to obtain, on average, 65% to 75% new sequences compared with the reference library.

I will now briefly describe our ongoing genomic study of pathogenicity. Before the start-up of this project, we had been analyzing genomic clones[2] for a number of putative *O. novo-ulmi* pathogenicity genes, such as the *Pat1* locus (Et-Touil et al. 1999). We also developed methods for insertional mutagenesis (mutagenesis of DNA by the insertion of one or more bases) in *O. novo-ulmi* and recovered mutants that displayed a lower aggressiveness towards elm saplings. In some cases, we were able to rescue the sequences we had mutated.

[1] Null mutants have a mutation that completely eliminates the function of a gene, in this case because it has been physically deleted.

[2] Clones made from a set of randomly generated overlapping DNA fragments representing the entire genome of an organism.

Functional genomic analysis allowed us to investigate the elm–*Ophiostoma* interaction from a broader perspective. We elected to use elm calli rather than saplings in our bioassays because calli do not contain the highly lignified tissues found in saplings, thus facilitating the recovery of cDNA. Obviously, the colonization of undifferentiated elm calli by *O. novo-ulmi* differs from the vascular wilt developing in elm saplings' highly differentiated xylem vessels. However, elm calli produce several reactions that are typically observed in mature trees when they are attacked by *O. novo-ulmi*, such as the production of mansonone phytoalexins (Yang et al. 1989), nonspecific toxic compounds. Moreover, we found that calli also produced phenolic compounds and suberin, which also have been associated with the response of mature elm trees to attack by *O. novo-ulmi*. It thus appears that calli are a useful system for investigating the elm–*Ophiostoma* interaction. An unsubtracted library was obtained from elm calli inoculated with *O. novo-ulmi*. It was found to contain a large number of ESTs showing significant homology with sequences coding for known proteins (mostly plant proteins and a few fungal proteins), the remaining being plant hypothetical proteins and orphans. Then, we subtracted this library against ESTs obtained from healthy, uninoculated calli, as well as from an axenic culture of the fungus, that is, a fungal culture free from other living organisms. This double subtraction resulted in an increase in the proportion of fungal sequences, including both known and putative sequences.

Annotation of the ESTs found in the *O. novo-ulmi* expression libraries is ongoing. So far, we have identified several sequences of interest. For example, the subtracted library obtained from synnemata included ESTs with homologs that encode conidiation-specific proteins whose exact functions are still unknown. As indicated earlier, present in the subtracted pathogenicity library were ESTs with high homology to fungal genes encoding hypothetical proteins. Although the latter library was enriched for fungal ESTs, it nevertheless included several homologs to known plant resistance genes. This is a significant achievement, given the fact that the molecular basis of resistance in elms remains poorly explored. Another interesting finding was the recovery of an EST coding for a transposase from the low temperature (15°C) library. Further analysis confirmed that this EST represents a Class II transposon[3] with an intriguing feature: it was highly methylated and displayed the typical signature of silencing by repeat-induced point mutations (RIP).[4] Detailed structural and physiological investigations of this and other transposons found in *Ophiostoma* spp. by my group are under way.

[3] A sequence of DNA that can move around to different positions within the genome of a single cell; in the case of a Class II transposon, it does so by moving directly from one position to another using a transposase to "cut and paste" itself within the genome.

[4] RIP is a genome defence mechanism unique to fungi by which duplicated DNA sequences are detected and modified by making guanine:cytosine to adenine:thymine mutations.

We used real-time polymerase chain reaction to study the expression of selected individual genes in the DED pathogens (Tadesse et al. 2003). More recently, we initiated a macroarray analysis of over 640 cDNAs recovered from elm calli infected with *O. novo-ulmi*, which so far indicates that at least one-third of the clones analyzed are differentially expressed. The construction of an *Ophiostoma* DNA microarray consisting of at least 2 000 different gene sequences is currently being planned.

Future Directions

As I stated earlier, the four laboratories that have taken part in this project had no prior experience in genomics and until recently were conducting studies on a few specific genes, one gene at a time. Therefore, EST analysis represented a definite shift in our research strategies, and it has been very instructive. So far, the benefits have been mostly in genome exploration: although 65% to 75% of the nuclear genome of *O. novo-ulmi* remains to be characterized, we have recovered over 2 000 unique ESTs, which is encouraging. Just a few years ago, only 20 genes or so had been identified in *O. novo-ulmi*. Another participant in this symposium [Peter Krell] indicated that only 20 genes or so are known in some viruses, which means we are more or less in the same boat. In those viruses, however, there are about 130 more genes to discover, whereas in the DED fungi, there are a few thousand more to characterize.

Our research is currently limited by the high number of *O. novo-ulmi* ESTs that do not yet have homologs in public data banks, even though the genomes of a few fungal species have been completely sequenced. In addition, several *O. novo-ulmi* ESTs have homologs with no known functions. However, more fungal sequences are being deposited in data banks every day and the situation is improving. An exciting outcome of our exploratory research is that we can now plan larger experiments to test hypotheses regarding the role of candidate genes in pathogenicity and in other aspects of *O. novo-ulmi*'s fitness, such as yeast–mycelium dimorphism and the transition between the saprophytic and pathogenic life styles. Although I have highlighted the fact that the DED fungi are pathogens, one should remember that these organisms must also survive as saprophytes to complete their life cycle.

As a follow-up to the research described above, we plan to use microarray technology for conducting large-scale functional analyses of *O. novo-ulmi*. We also intend to investigate population genomics of selected ophiostomatoid fungi. Both the *Ophiostoma* EST data and the recovered elm sequences in our pathogenicity library will be made public, with the hope that it will help us recruit new researchers interested in working on *Ophiostoma* species, elms, or both. We are conducting similar research on another ascomycete fungus, *Septoria musiva*, which is an important pathogen of hybrid poplars (for example, see Feau et al. 2005). The latter project benefits from the expertise developed while working on *O. novo-ulmi*,

as well as from the fact that poplar is a model species for tree genomics. In the long term, we hope results from our genomics research will help in developing successful control strategies against tree diseases such as DED.

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Genomics for Diagnostics and Gene Target Identification in Tree Pathogens

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There are two genomics projects on fungal pathogens funded at the Laurentian Forestry Centre [Canadian Forest Service (CFS), Natural Resources Canada]. The first project, which I lead, aims to develop a platform for molecular diagnosis of forest pests. The second one, led by Armand Séguin, focuses on how the genomes of trees and their pathogens interact. We hope to find the tools and knowledge to prevent, or if that is not possible, to mitigate the effects of forest pest epidemics and to improve our understanding of the interplay between the sets of genes involved in host–pathogen, host–nonhost, and compatible–incompatible interactions. One basic question we are trying to answer is, what are the unique sets of genes expressed by a fungus that define pathogenicity? Conversely, we are also asking, how do the gene expression profiles of pathogenic, saprophytic, and symbiotic fungi, such as mycorrhizae and endophytes, differ? Answering such questions could help us to develop better tools for identifying and monitoring fungal pathogens and to discover novel ways to prevent pathogen infections. Both projects fall within the realms of molecular diagnostics and genetic engineering, the two main platforms funded by the CFS in genomics.

Occurrences of alien pests are likely to increase, given the growth in international trade and the vulnerability of some ecosystems. The absence of coevolution between the native host and the alien pest or pathogen creates an ecological imbalance, and this, together with a lack of population surveillance after the introduction of the alien species in a novel ecosystem, can result in epidemics. Some of the worst disasters in forest ecosystems have been caused by alien pathogens. Chestnut blight (caused by the fungus *Endothia parasitica*) has completely changed an ecosystem once dominated by American chestnut (*Castanea dentata*); this once tall tree is now a rare understory species in northeastern North America. White pine blister rust (caused by the fungus *Cronartium ribicola*) is still spreading to new hosts and areas nearly a century after its introduction.

The last annual survey of forest diseases for Canada reported 52 million m^3 of timber losses to diseases. However, the impact of diseases on forests is not only measured in a loss in timber value or earnings. Entire fragile ecosystems in the Rocky Mountains are threatened by the spread of white pine blister rust and by the arrival of a novel hybrid between this exotic and a native rust. Key species such as limber pine (*Pinus flexilis*) and whitebark pine (*P. albicaulis*) are threatened by the disease, and in some stands, mortality can reach nearly 100%. The disappearance of these tree species could affect animals that depend heavily on pine nuts for food, such as Clark's nutcracker (*Nucifraga columbiana*), red squirrels (*Tamiasciurus hudsonicus*), and grizzly bears (*Ursus arctos*).

Sudden oak death (SOD), caused by the canker fungus *Phytophthora ramorum,* is the newest comer in the long list of infamous alien pathogens; it was detected in California in 1993 and described and named in 2000. SOD can infect a wide range of host species, including oak trees (*Quercus* spp.) and Douglas-firs (*Pseudotsuga menziesii*) but also several horticultural plants such as rhododendrons (*Rhododendron* spp.) and camellias (*Camellia* spp.).

Phytophthora ramorum belongs to a class of plant pathogens, the oomycetes, that has a deadly record. One of them, *P. infestans*, was the causative agent of the Irish potato famine, an event that saw the displacement of millions of Irish in the 1800s. Currently SOD is considered to be established in North America only in small forested areas in California and Oregon. In 2003, a single outbreak in rhododendrons at a British Columbia nursery was suppressed. However, in 2004, the disease was again introduced into British Columbia through infected nursery plants from California and is being controlled.

The SOD pathogen is well-equipped for dissemination and survival. It can produce different types of spores: floating spores that can be dispersed by water, airborne spores that are blown by the wind, and resting spores that can survive in the soil for up to 20 years. It can be transported on hikers' boots and car tires.

The CFS has been working closely with the Canadian Food Inspection Agency (CFIA) to develop detection and monitoring assays for the SOD pathogen. The Plant Health Division of the CFIA has the mandate to prevent the spread and introduction of foreign invasive regulated pests by enforcing quarantines, performing inspections, and applying eradication measures. Thus, the CFIA supports research on the pathogen and the development of survey and diagnostic tools. The CFS also collaborates with the US Department of Agriculture's Animal and Plant Health Inspection Service–Plant Protection and Quarantine, which like the CFIA is currently validating molecular diagnosis assays. Some Canadian provinces and American states have shown great interest in survey and mitigation tools, as have tree nurseries, private forest owners, and the public.

It is encouraging that the United States has been quick to recognize the serious implications of the SOD pathogen. An annual budget of $44 million was specifically dedicated to fight this pathogen, a large part of which was for mitigation, research and development, diagnostics, and monitoring. The US Department of Energy's Joint Genome Institute (JGI) received $4 million to sequence the SOD genome; the complete genome sequence was released at a meeting I attended in New Orleans in May 2004. The SOD pathogen turned out

to have a small genome with only 66 million base pairs (Mbp) and a predicted 16 000 genes, making its sequencing easy to achieve despite redundancy problems. The JGI claimed it was the fastest turnaround between the discovery of an organism (2000) and the release of its complete genome sequence (2004). Interestingly, even though *P. infestans* — the fungus that had caused the Irish potato famine — is closely related to SOD, its genome is much larger, having 256 Mbp.

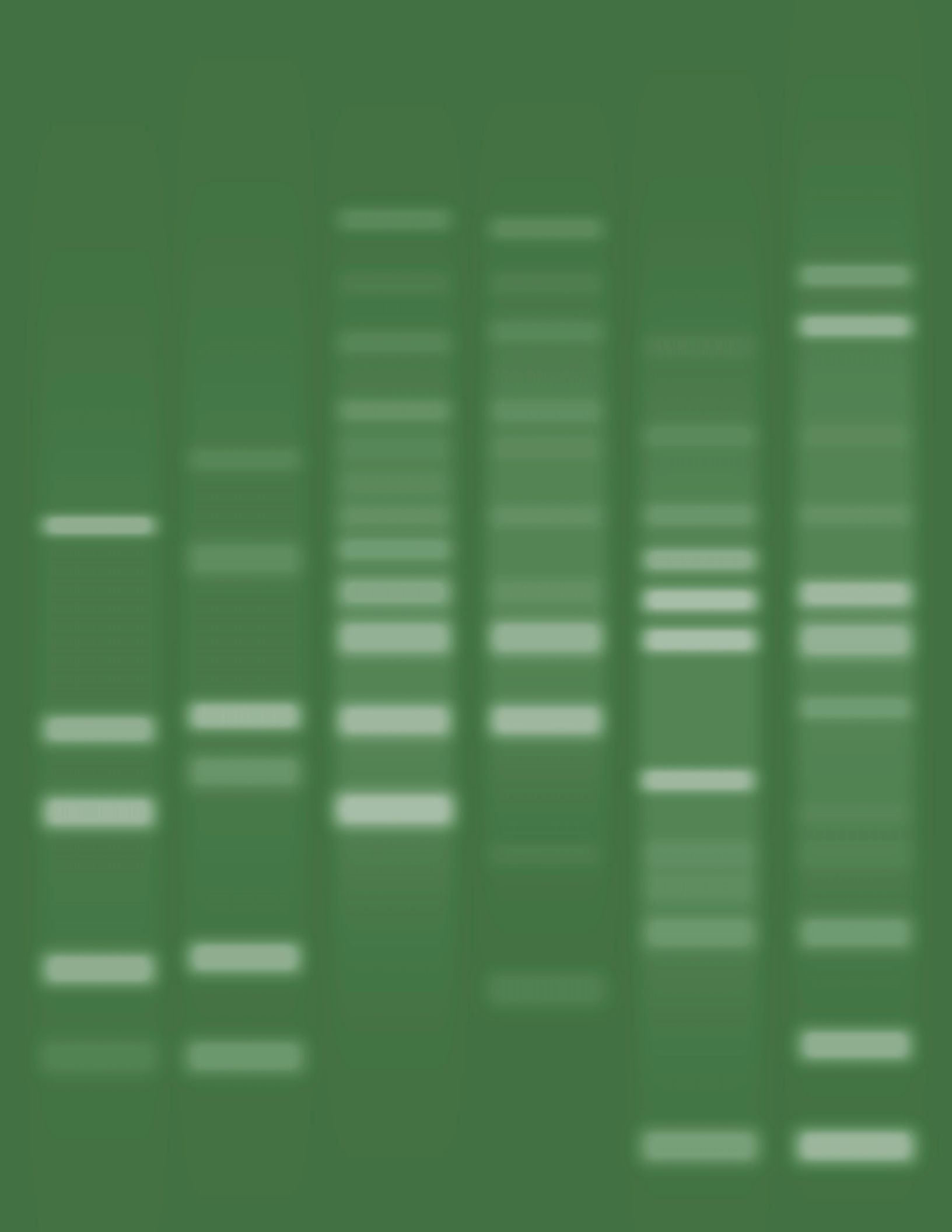
Interesting applications could be derived from the sequencing of the *P. ramorum* genome. Some of its 16 000 genes must be involved in pathogenicity, sporulation, host recognition, and other fungal functions. Two areas that interest me are gene and genome evolution. Genes can be classified as either rapidly or slowly evolving. Genes that evolve rapidly are probably those that are subject to either of two types of natural selection, balancing or positive. Balancing selection works to maintain polymorphisms, that is, multiple alleles per locus in a population, whereas positive (or directional) selection favors a single allele in the population. Rapidly evolving genes may be involved in, for example, adaptation to hosts, adaptation to new environments, host recognition, or response to host defence mechanisms. Scientists could target them to diagnose finer fungal taxonomic levels such as species, race, biotype, or mating type, or even to identify specific fungus individuals. They could also use them as targets for disease control by designing compounds or agents that interact with specific sets of genes or proteins involved in pathogenicity or host recognition.

Slowly evolving, highly conserved genes are subject to purifying (or stabilizing) natural selection, which works at eliminating extreme values of a character in the population, driving a population towards common traits. These genes are probably critical to pathogen survival and growth or involved in essential life processes such as respiration or the maintenance of basic metabolism. Scientists could target these genes for disease control through the disruption of essential life processes. By interfering with the functions of slowly evolving genes, they could develop efficient and broadly applicable disease control methods. As well, highly conserved genes are good subjects for diagnoses of higher taxonomic levels such as genus or family. Such work is especially important in dealing with alien pathogens. Indeed, with general probes that target an entire genus such as *Phytophthora* — whose members are all plant pathogens — we have a better chance of detecting and intercepting a potential alien pathogen *before* it can spread in its new environment.

With the entire *P. ramorum* genome sequence available, scientists can search the 16 000 genes and try to determine which ones are evolving rapidly (subjected to positive selection) and which ones are evolving slowly (subjected to purifying selection). This can be done by scanning the genome, looking at the distribution of codons, and measuring codon volatility, that is, the fraction of single nucleotide mutations in codons that result in amino acid changes. This measure can be computed across the genome and each gene can be classified according to its degree of volatility.

I hope that these genomic approaches will allow us to develop and refine novel detection, monitoring, and control methods to prevent the introduction of alien pathogens, or at least to mitigate their spread when, in spite of the precautions taken, they have succeeded in entering the country.

DISCUSSIONS



Forest Genomics: Future Priorities and Public Issues

In 2004, genomics research reached an important milestone in Canada. The federal research support program was concluding its initial phase, and the submissions for renewal were being prepared considering past performance and future priorities. To understand the dynamics and considerations at play and to guide the next steps for forest genomics, the organizing committee of Genomics for Future Forests felt that the symposium would be an ideal forum for the Canadian forest genomics community to collectively examine future priorities for forest genomics research, particularly as they apply to the Canadian Forest Service (CFS), Natural Resources Canada, and Genome Canada. In addition, a second topic was put forward for discussion. Participants were asked to consider how they would proactively respond to the environmental, ethical, economic, and social issues being raised by society about biotechnology.

Two discussion sessions took place; during each session, participants met first in small groups and then as one large group, exchanging ideas. To prepare for the get-togethers and to allow for individual consideration of issues, participants were provided with focus questions for stimulating exchange, as well as a brief outline of policy priorities at various federal government levels (Annex 1) and a description of the CFS intramural Genomics R&D Initiative for 2002–05 (Annex 2). Lyle Makowsky, President of InterQuest Consulting, facilitated the discussions.

The following notes are summarized from the flip chart records produced during the discussion sessions. These notes present key ideas as tracked by the discussion groups. They are not intended to be conclusive or comprehensive reviews of the topics discussed but should be seen as a profile of the discussions and key points raised.

Discussion Session 1: Advancing the Research Agenda

Step 1: Defining Stakeholders' Expectations

This step probed for stakeholders' expectations as voiced and interpreted by groups designated to represent a specific stakeholder group—the forest industry, the federal government, and the public (average Canadians). Each group responded to the following questions from the perspective of its assigned stakeholder.

What outcomes and impact do you [key stakeholder] expect from forest genomics research?

What are the greatest needs in the forest industry or sector that can be addressed by genomics research?

To what government policy priorities should the Canadian genomics research community respond?

What research applications from genomics could be seen as beneficial and acceptable to Canadians?

Forest industry expectations

- A better understanding of the role and benefits of genomics;
- Knowledge base for tool development to facilitate forest operations;
- Use of genomics to maximize tree growth, quality, and biomass utilization and to minimize loss to biotic and abiotic factors, e.g., to enhance traits for plantation management or characterization of trees for quality control;
- Value-added applications for short-term benefit to industry; and
- Use of genomics as tools to address ecological considerations and certification.

Federal government expectations

- **Short-term**
  - Research that increases knowledge;
  - Contribution to the innovation agenda, with increased intellectual property leading to business opportunities; and
  - Contribution to strategies linked to climate change, e.g., diagnostic and monitoring tools for forest health.
- **Medium-term**
  - Information for rational decisions to support biotechnology regulations.
- **Longer-term**
  - Industrial competitiveness and industrial uptake;
  - Level playing field with stronger research;
  - Bioenergy development;
  - A vision of what our forest nation should look like; and tools to evolve toward a new economy (new technologies, nontimber forest products, and green chemistry providing yet unknown benefits);
  - Global leadership in niche areas;
  - Contribution to sustainable forestry;
  - Assessment of safety on Canadians and of impacts on aboriginal communities; and
  - Assistance in meeting international commitments related to global change and global issues.

Expectations of the Canadian public

- Forest genomics research that is clear, transparent, understandable, with credible and defensible benefits; and
- Forest genomics research that will
  - Enhance fundamental knowledge;
  - Contribute to forest health;
  - Contribute to forest conservation;
  - Strengthen wood quality;
  - Extend development of bioenergy and renewable products;
  - Protect forests and climate; and
  - Support sustainable forestry.

Step 2: Selecting Overall Priorities

At this step, participants considered stakeholders' expectations as defined in the first discussion step and discussed potential priorities for genomics research to respond to these expectations. Participants were divided into four groups and tasked with outlining their top research priorities by exploring the following questions:

Where should we [research community] focus forest genomics research over the next five years?

What research priorities would best meet stakeholders' expectations?

Priority set 1

- Forest health (host–pest interactions);
- Understanding the structures of conifer genomes;
- Wood quality;
- Socioeconomic research;
- Tools for gene expression;
- Research on genetic resources; and
- Others
  - Sequencing of the spruce genome;
  - Establishing links between conifer and poplar genomes;
  - Using knowledge derived from the poplar genome to address sustainability questions; and
  - Devising protocols for marker development and validation.

Priority set 2

- Holistic approach to understanding forest ecosystem interactions;
- Species conservation;
- Increased use of genetic improvements in forest management;
- Tools for management of invasive species, e.g., detection and control;
- Protection from pests and diseases; and
- Value-added products and biomass conversion.

Priority set 3

- Understanding tree responses to diseases and pests;
- Development of release strategies and government regulations that enable innovation;
- Basic gene discovery: wood quality, pest resistance, commercial products, pharmaceuticals, and nutraceuticals;
- Communications strategies; and
- Comparison of poplar and conifer genomes.

Priority set 4

- Quality of forest products and efficient use of these products;
- Forest protection;
- Forest health; and
- Comparison of tree and pest genomes.

Priorities common to all sets

- Forest health (host–pest interaction, both native species and exotics);
- Genomic tools for conifers; understanding of the structure of conifer genomes;
- Response to and protection from diseases and pests;
- Tools for management of invasive species, e.g., detection and control;
- Basic gene discovery:
  - Specific traits;
  - Tools for gene expression;
  - Research on genetic resources; and
  - Sequence of the white spruce genome;
- Comparative genomics for:
  - Poplars and conifers; and
  - Trees and pests;
- Socioeconomic research;
- Government regulations that enable innovation; and
- Efficient use of forest products for:
  - Competitiveness;
  - Wood quality;
  - Value-added products; and
  - Health benefits, e.g., pharmaceuticals and nutraceuticals.

Step 3: Exploring Ways to Advance the Agenda

This step involved an open exchange of ideas from the whole group in response to the following question:

How can we coordinate our efforts to advance the Canadian research agenda for forest genomics?

Suggestions to coordinate efforts

- Pursue co-location of CFS and university scientists where feasible;
- Explore communication tools for university and government scientists;
- Reconcile different financial grants for a better distribution to government–academic–industry consortia;
- Recognize the dollar scale difference between projects funded through Genome Canada (average $10 million) and through the CFS (average $300 000, including salaries of principal investigators and technicians);
- Convene the community more often with face-to-face meetings and via a network;
- Explore funding sources to support smaller-scale projects;
- Consider how large research platforms could provide a service to smaller projects (some Genome Canada projects already play that role);
- Provide training and resources for smaller-scale projects;
- Provide a clear list of existing resources and their location in relation to the research agenda;
- Look at issues related to intellectual property;
- Draw upon resources and influence from the National Research Council, Agriculture and Agri-Food Canada, and Environment Canada, and ensure links with the provinces;
- Explore the key question of how to get the broader community engaged; and
- Explore issues related to the requirement for matching funds, e.g., the provinces cannot always match federal dollars.

Discussion Session 2: Responding to Research Challenges

Step 1: Defining the Challenges or Issues

At this discussion step, participants were asked to describe challenges faced in the forest genomics research field in four domains: society and public acceptance, industry uptake, government research policy, and the research community. Participants were divided into four discussion groups; each group explored one of the domains using the following questions:

What are the challenges or issues we face in pursuing forest genomics research?

What are the three to five challenges that most limit our ability to advance research and have it accepted?

Societal/public challenges

- Need for a communications strategy, e.g., risk reduction around invasive species (insects and diseases) and fundamental knowledge leading to increased credibility;
- Need for public acceptance and support of the industry;
- Need to address environmental concerns, e.g., conservation of biodiversity and habitats; and
- Capacity and availability of highly qualified people and transferability to related industries.

Industry uptake challenges

- Economic viability and "industry fit" as per intellectual property issues, time to market from research to development, and regulatory constraints;
- Public and consumer acceptance;
- Capacity and willingness to adopt and market technology challenge "start-ups";
- Competitiveness issues; and
- Lack of, confusing, or overlapping policies.

Government policy challenges

- Coordination, governance, and communication, including among related government departments;
- Funding;
- Commercialization and intellectual property;
- Regulations for release strategies; and
- Erosion of core expertise both within and outside government.

Research community challenges

- Long-term core funding for technology platforms;
- Development of a long-term strategy to curate genetic resources;
- Open access to core facilities, genomic resources, and tools;
- Intellectual property guidelines;
- Others:
  - Bridging the gap between genomics research and forestry;
  - Training and recruitment of highly qualified personnel; and
  - Communication among stakeholders.

Step 2: Responding to the Challenges

Participants explored how the genomics research community could engage key parties to address the identified challenges through the following questions:

How do we engage stakeholders and the public to address these challenges?

How can we build capacity and will in our community to address these challenges?

Canadian public

Engaging the public

- Develop communications strategies for specific target groups;
- Utilize an annual forest genomics symposium as a promotion vehicle;

- Facilitate public debate; and
- Engage senior officials to discuss forest genomics in a public context.

Building capacity and will in our forest genomics research community

- Convey a sense of urgency, e.g., pathogens in urban forestry;
- Engage the teaching community to develop greater public awareness of forest resources and their benefits;
- Invest in human resource capacity, e.g., training;
- Demonstrate benefits related to quality of life; and
- Get involved in international cooperation.

Industry

Engaging industry

- Have discussions with industry at executive and management levels;
- Develop a primer on forest genomics for industry and the public; and
- Develop educational outreach, exchanges, and visits of establishments.

Building capacity and will in our forest genomics research community

- Provide more funding to act as a catalyst for "community effort"; and
- Promote more efficient resource use and sharing for engaging industry.

Government

Engaging government

- Explore how to facilitate communication among different stakeholders (government, academia, industry, nongovernmental organizations, and the public), commencing with developing a strategy and designating leaders;
- Address regulations related to release strategies for transgenic trees (must be science-based, transparent, with risks –benefits weighed).

Building capacity and will in our forest genomics research community

- Improve coordination and governance to link genomics, forest industry, and communities; and
- Build awareness and develop a common language.

Research community

Engaging the research community

- Focus on funding and the need to increase the R&D budget;
- Launch a campaign to demonstrate the benefits of genomics research in forestry; and
- Demonstrate a return on investment.

Building capacity and will in our forest genomics research community

- Train scientists in public relations;
- Create a Canadian Forest Genomics Network (start with CFS and Genome Canada); and build a common electronic platform (e.g., common Web site).

Conclusion

The dialogue sessions concluded with a brief summary of the scope and findings of the two sessions conducted over the two days. The major recurring theme was the need for better coordination and communication in the research community and a network to facilitate this. Participants recommended proceeding with the creation of such a network and developing a forward-looking approach to bring together the research community.

Annex 1: Outline of 2004 Policy Priorities of the Federal Government

Speech from the Throne to Open the Third Session of the 37th Parliament, February 2004

- Strengthen social foundations (health, children, people with disabilities, aboriginal Canadians, and communities);
- Build a strong economy for the 21st century (e.g., developing and applying biotechnology, environmental technology, information and communications technologies, health technologies, and nanotechnology);
- Position Canada at the forefront of the next big technological revolution (e.g., bridging commercialization gap for S&T, fostering regional and rural development, sustainable development); and
- Affirm Canada's pride and influence in the world.

Natural Resources Canada

- Provide information to make balanced decisions regarding natural resources;
- Ensure sustainable economic, social, and environmental benefits for present and future generations;
- Develop strategies that reduce environmental impacts in the natural resources sector; and
- Secure the natural resources sector.

Canadian Forest Service

- Mitigate the effects of climate change on Canada's forests;
- Provide science-based knowledge;
- Encourage sustainable development of Canadian forests;
- Focus on urban forests and economic development of rural communities;
- Foster industrial competitiveness; and
- Position Canada as a global forest nation.

Canadian Biotechnology Strategy

- Develop new stewardship framework supported by innovative programs (regulatory foresight, long-term impacts, dialogue with Canadians, and international cooperation);
- Coordinate R&D system (focused on clusters and skills);
- Accelerate commercialization (financing and intellectual property);
- Improve access to global markets;
- Build alliances for international development; and
- Enhance global security.

Biotechnology Strategic Plan of the Canadian Forest Service

- Provide the knowledge base and technologies to contribute to enhanced tree productivity on selected lands:
  - Develop fundamental understanding of production and protection systems;
  - Develop technologies to produce genetically improved, superior, and fast-growing trees; and
  - Develop environmentally acceptable forest protection methods.
- Develop safe and effective deployment strategies for biotechnology-derived products:
  - Develop scientific expertise and provide advice to ensure environmental safety;
  - Promote frameworks for science-based regulations and protection of intellectual property; and
  - Promote public awareness of the benefits and costs of biotechnology.
- Foster the use of forest biotechnology:
  - Develop innovative client partnerships and technology transfer mechanisms;
  - Assertively market spin-off technologies; and
  - Foster responsible use of biotechnology in forestry applications.

Annex 2: 2002–05 Canadian Forest Service Genomics R&D Initiative

Program 1: Forest Tree Production and Protection Systems

- Structural, functional, and comparative genomics of insect pests and their viruses.
- Molecular and genetic characterization of conifer host–root rot fungi.

Program 2: Molecular Markers for Diagnosis, Monitoring, and Early Selection

- Structural genomics of conifer species.
- Novel platform for molecular diagnosis of forest pests.

Program 3: Production of Genetically Improved Trees

- Resistance of white pine to blister rust fungus.
- Study of transcription factors controlling embryogenesis in spruce.
- Creation of a National Tree Functional Genomics Platform for collaborators.

Program 4: Production of Environmentally Acceptable Forest Protection Methods

- Interacting genomes in tree defence response.
- Juvenile hormone-based pest management tools for forest insects.
- Selective functional genomics of insect pests and their key parasite complexes.
- Genetic engineering of viruses and insects for pest management and protein expression.

GLOSSARY

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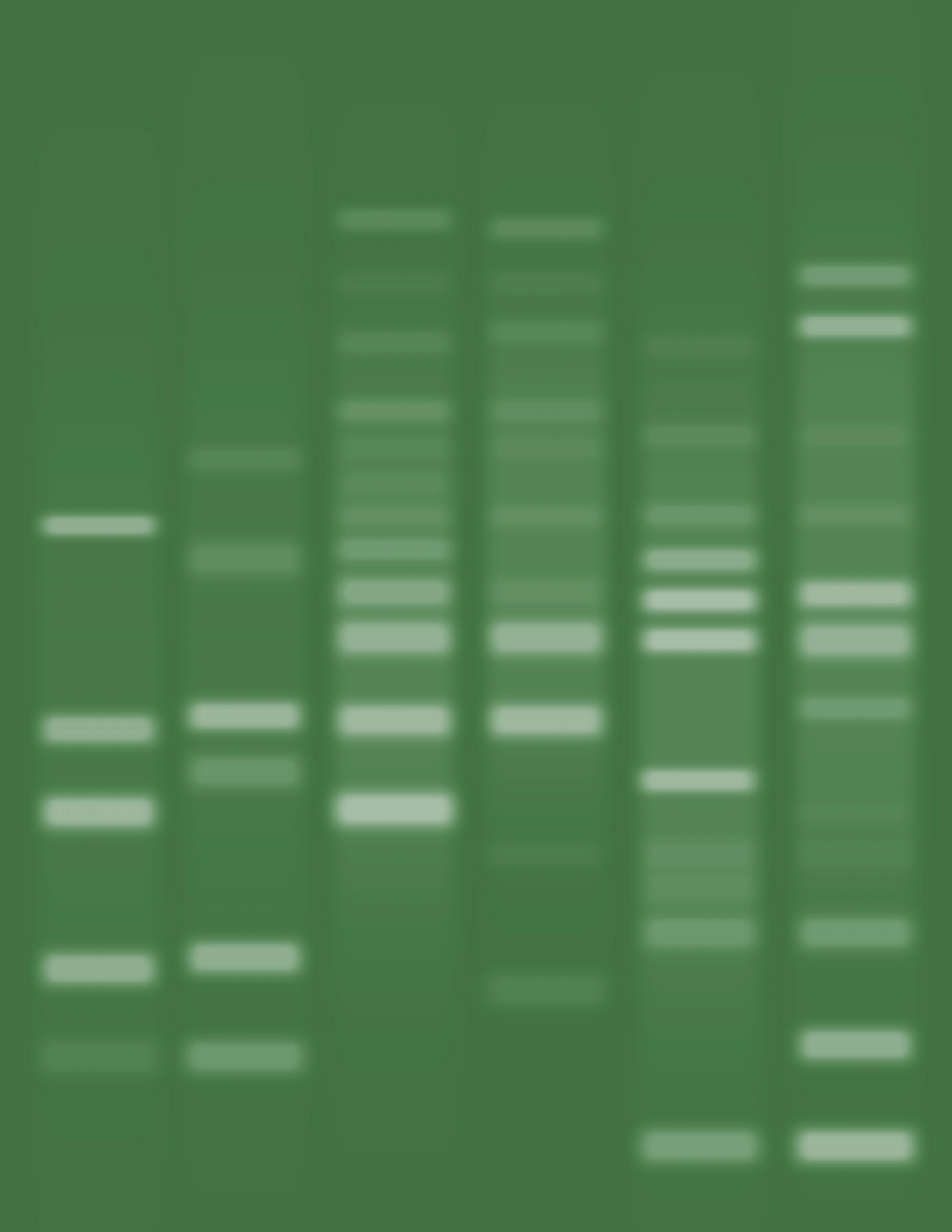

Glossary of Selected Terms

billion: In this publication, refers to a thousand million (10^9), as per North American usage (in the United Kingdom, it usually means one million million (10^{12})).

cDNA: See *complementary DNA*.

codon: A unit of gene sequence composed of three nucleotides and coding for a single amino acid; a codon is thus the basis of protein synthesis.

complementary DNA (cDNA): DNA synthesized from messenger RNA templates.

contig: Cloned stretches of overlapping regions of DNA for a particular chromosome; short for contiguous sequence.

DNA: Deoxyribonucleic acid; a molecule that forms the basis for encoding the genetic information of most organisms; composed of two complementary strands of nucleotides organized in a double helix and capable of self-replication. In cells of higher life forms, DNA is organized into chromosomes in the nucleus and is found in a circular form in organelles such as mitochondria and chloroplasts. The sequence of nucleotides provides the information that is passed on to offspring.

downstream genes: Genes located "downstream" from a particular DNA sequence relative to the direction of transcription and translation, that is, located to the 3′ side of that particular sequence.

EST: See *expressed sequence tag*.

expressed sequence tag (EST): A short (200–500 base pairs) cDNA sequence from mRNA derived from a specific cell population and used for cloning a large number of genes that are expressed in that population.

five prime (5′) and three prime (3′) ends: Either end of a strand of nucleic acid; the 5′ end bears a phosphate and the 3′ end a hydroxyl group; in DNA, the two strands of nucleic acid are arranged so that the 3′ end of one strand is aligned with the 5′ end of the other.

gene knockouts: A strategy to determine the function of a specific gene by inactivating (knocking out) that gene in an organism and studying the consequences of this modification.

gene switch: Chemicals that inhibit or induce gene expression; for instance, a gene off-switch would be a regulator protein that inhibits the action of any gene under its control, while the on-switch would be an inducer chemical that turns the gene-inhibiting regulator protein into a gene-activating protein.

genomic clone: Clones made from a set of randomly generated overlapping DNA fragments representing the entire genome of an organism.

macroarray: See *microarray*.

messenger RNA (mRNA): RNA that serves as a template for protein synthesis.

microarray: An array of microscopic DNA spots on a glass slide, silicon chip or other such surface that allows high-throughput analysis of gene expression profiling; *macroarray* may be the preferred term for arrays with larger and fewer spots.

mRNA: See *messenger RNA*.

open reading frame (ORF): An RNA or a DNA sequence that is uninterrupted by stop codons (i.e., has no signal to stop reading) and thus may encode part or all of a protein.

quantitative trait locus (QTL): A polymorphic locus or genetic marker that contains alleles that differentially affect the expression of a continuously distributed phenotypic trait.

read: Each sequencing operation in the process of determining the order of the millions of nucleotides in a particular DNA.

response element: A sequence of nucleotides in a gene that can be bound by a protein called a transcription factor. Binding of a transcription factor to a response element regulates the production of specific proteins by inhibiting or enhancing the transcription of genes that encode those proteins.

RNA: Ribonucleic acid; found in the cells of organisms, where it plays an important role in protein synthesis; in some organisms, such as certain viruses, it is the carrier of genetic information.

single nucleotide polymorphisms (SNPs): Variations that occur when a single nucleotide in the DNA sequence of a genome is altered.

somatic embryogenesis: The process of forming organized embryonic structures morphologically similar to zygotic embryos, but initiated from somatic (non-zygotic) cells.

transcription: The synthesis of an RNA copy from a sequence of DNA (a gene); the first step in gene expression.

transcription factors: Proteins that regulate the activity of other genes.

unigene: Derived from UniGene, a system for partitioning database gene sequences into a nonredundant set of gene-oriented clusters, each of which contains sequences that represent a unique gene, or "unigene." UniGene is managed by the National Center for Biotechnology Information, National Institutes of Health, Bethesda, MD.

unigene set: An EST database containing only one copy of each unique sequence (see above).

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Bob Rutledge is a research scientist at the Laurentian Forestry Centre (LFC), Canadian Forest Service (CFS). In 1986, he received a doctorate in molecular genetics from Carleton University, Ottawa. He followed this with a postdoctoral fellowship at Agriculture Canada's Plant Research Centre, where he participated in a project to genetically engineer herbicide resistance in canola. Dr. Rutledge joined the Petawawa National Forestry Institute (CFS) in 1990 as a research scientist within the conifer biotechnology group and initiated research into conifer gene function and developmental genetics. The group moved to the LFC in 1996, and Dr. Rutledge subsequently initiated a collaborative project to extend propagation technologies to mature conifers, with the objective of cloning elite trees via somatic embryogenesis. Most recently his work has expanded into a broader program involving the application of functional and structural genomics to conifers.

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Armand Séguin has been a research scientist at the Canadian Forest Service's Laurentian Forestry Centre (LFC) since 1995. He obtained his doctorate in 1990 from Université Laval, Québec, and did postdoctoral studies at the Salk Institute for Biological Studies in California. Since then, he has become a recognized researcher in tree biotechnology and molecular genetics. Currently, he is working with a team at the LFC to find ways to improve tree resistance to pests through genetic engineering and to better understand tree response to stress and pest attack. Dr. Séguin is also an adjunct professor at Université Laval and at Université de Sherbrooke, where he endeavors to inspire his students to apply their fundamental knowledge to finding ways to genetically improve trees.

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Adrian Tsang has been a professor in the Biology Department of Concordia University, Montréal, since 1991. He received his doctorate in biochemistry from York University, Toronto, in 1978 and then did postdoctoral studies on the molecular mechanisms of development at the Imperial Cancer Research Fund, London, England, as a North Atlantic Treaty Organisation Science fellow. Before joining Concordia, Dr. Tsang was a faculty member at York University and McGill University. Over the years, he switched his research focus to protein production in fungi and then to fungal genomics, with the aim of identifying fungal enzymes for industrial and environmental applications. In 1999, he became the founding director of the Centre for Structural and Functional Genomics, a state-of-the-art core genomics facility. Dr. Tsang led the Genome Canada project "A Genomic Approach to Identify Fungal Enzymes for Industrial and Environmental Applications" from 2002 to 2004, which brought together researchers from Concordia University, the National Research Council of Canada's Biotechnology Research Institute, the Pulp and Paper Research Institute of Canada, and the INRS–Institut Armand-Frappier.