#### STEPHEN P. DIFAZIO

Department of Biology West Virginia University Email: spdifazio@mail.wvu.edu

#### RESEARCH INTERESTS

Plant genomics; molecular ecology; ecosystem genomics; forest biotechnology; gene flow and establishment in plant populations; plant reproductive biology; landscape ecology; biotechnology risk assessment

#### PROFESSIONAL EXPERIENCE

# Assistant Professor, Department of Biology, West Virginia University.

August 2005 to present.

# Research Scientist, Environmental Sciences Division, Oak Ridge National Lab January 2002 to October 2005

Associate Research Professor, Plant Sciences Department, University of Tennessee June 2004-present

# Graduate Research Assistant, Dept. of Forest Science, Oregon State University

October 1995 to December 2001

Led a project to assess genetic impacts of hybrid poplar plantations on wild populations.

Developed molecular markers and performed paternity analyses for populations.

Designed and implemented spatial simulation model for transgene flow.

# Faculty Research Assistant, Department of Forest Science, Oregon State University

March 1995 to October 1995

Sequencing of genes controlling flower development in Douglas-fir and black cottonwood.

Bulked segregant for molecular markers associated with stem forking in Douglas-fir.

# General Biology Teaching Assistant, Oregon State University, Corvallis

January 1994 to March 1994

Taught laboratory classes for 80 students

# Graduate Research Assistant, Pacific Northwest Research Station, U.S. Forest Service/Oregon State University, Corvallis

July 1992 to March 1995

Studied factors influencing the reproductive biology of Pacific yew populations

# Forestry Extension Worker, Peace Corps, Totonicapan, Guatemala

July 1989 to March 1992

Promoted forestry, agroforestry and soil conservation in Mayan villages

# Research Technician, Repligen Corporation, Cambridge, Massachusetts

January 1989 to June 1989

Tested anti-microbial polymer surfaces with yeast

# Research Technician, Children's Hospital Medical Center, Boston, Massachusetts

June 1987 to January 1989

Performed genetic analysis of neurological mutations in mice

# Research Assistant, Harvard School of Public Health, Boston, Massachusetts

March 1986 to May 1987

Tested artificial blood emulsions in rats

#### **EDUCATION**

Ph.D. 2002 Oregon State University (Corvallis), Forest Genetics, GPA, 4.0 M.S. 1995 Oregon State University (Corvallis), Ecology, GPA, 3.84 B.S. 1989 Northeastern University (Boston, Massachusetts), Biology, GPA, 3.95

# **HONORS**

Outstanding PhD Student, 1999; Fowells Fellowship, 1998; EPA STAR Fellowship, 1997-1999; Moldke Fellowship, 1996; Ell Presidential Scholarship, 1984-1989; Phi Kappa Phi, 1988-1989; Alumni Award for Most Professional Promise, 1989; Junior Ring Award, 1988

**FOREIGN LANGUAGE:** Fluent in Spanish

**COMPUTER LANGUAGES: PERL, C, SAS, HTML** 

**OPERATING SYSTEMS:** Windows, Unix, Linux, DOS

# **GRANTS FUNDED**

DOE/Program for Ecosystem Research. 2/05-2/08. \$5,042,000. HERMES: Hierarchical Experimental Responses from Macromolecular to Ecosystem Scales. /S.P. DiFazio, S.D. Wullshcleger, T.J. Tschaplinski, C.W. Schadt, A. Rogers, and C.R. Kuske.

NSF/FIBR. 10/04-10/09. \$5,000,000. Community Genetics, Heritability & Evolution: Consequences of Extended Phenotypes. T.G. Whitham, R. Lindroth, S.P. DiFazio, B. Potts, S. Shuster, C. Gehring, G. Allan, J. Marks, S. Hart, and P. Keim.

NSF Plant Genome. 10/04-10/06. \$1,385,384. VCA: Populus Genome Curation. G.A. Tuskan, S.P. DiFazio, Z.M. Cheng, E. Retzel, and D. Rokhsar.

DOE/Basic Energy Science. 10/04-10/06. \$602,000. Chromosome-Scale Assembly of the Poplar Genome. S.P. DiFazio, N. Islam-Faridi, L.E. Gunter, and G.A. Tuskan.

DOE/Terrestrial Carbon Sequestration. 3/04-2/07. \$1,201,324. New genetic tools for modifying tree properties to enhance carbon sequestration: Microarray expression analysis of poplar regulatory gene families. A. Brunner, S, Givan, S. Strauss, B. Goldfarb, and S. DiFazio.

Oak Ridge National Laboratory Directed Research and Development Program, 5/02-10/03, \$140,000, "Ecosystem Genomics: An Emerging Opportunity for Environmental Research. S.P. DiFazio.

Oak Ridge National Laboratory Directed Research and Development Program, 9/02-10/04, \$670,000, "Genomic Characterization of Belowground Ecosystem Responses to Climate Change. S.P. DiFazio, M.W. Fields, S. M. Tiquia, C.C. Brandt, R.J. Norby, J. Zhou, J. C. Schryver, and J.F. Weltzin.

DOE/Terrestrial Carbon Sequestration, 10/02-9/05, \$5,142,024 "Genome-Enabled Discovery of Carbon Sequestration Genes in Poplar" G.A. Tuskan, J. Davis, S. Strauss, S.P. DiFazio, and 14 co-PI's.

USDA Initiative for Future Agriculture and Food Systems, 9/00-9/04, \$539,000, "Flowering control in Transgenic Trees: Stability and RNAi Gene Suppression." S. H. Strauss, A. Brunner, S.P. DiFazio, J. Skinner, and R. Meilan.

National Science Foundation, 9/99-8/04, \$350,000, "Industry/University Cooperative Research Center: Tree Genetic Engineering Research Cooperative." S. H. Strauss, R. Meilan, A. Brunner, J. Skinner, and S.P. DiFazio.

USDA Biotechnology Risk Assessment, 10/97-9/00, \$166,000, "Gene flow and simulation modeling of transgene spread in poplar." S. H. Strauss, S. P. DiFazio, S. Leonardi, S.Garman, W. T. Adams, and D. Hibbs. (Lead author and project leader)

EPA STAR Graduate Fellowship, 10/97-9/00, \$90,000, "Potential impacts of transgenic poplar cultivation." S.H. Strauss and S.P. DiFazio. (Lead author and project leader)

#### **PUBLICATIONS**

#### **Peer Reviewed**

\*Co-First author

- Tuskan, G.A., S.P. DiFazio, U. Hellsten, S. Jansson, et al. The genome of black cottonwood, Populus trichocarpa (Torr. & Gray ex Brayshaw). Submitted to *Science*.
- Groover, A.T., S.D. Mansfield, S.P. DiFazio, G. Dupper, J.R. Fontana, R. Millar, and Y. Wang. *In Press*. The *Populus* homeobox gene *ARBORKNOX1* reveals overlapping mechanisms regulating the shoot apical meristem and the vascular cambium. *Plant Molecular Biology*.
- Wullschleger, S.D., T.M. Yin, S.P. DiFazio, T.J. Tschaplinski, L.E. Gunter, M.F. Davis, and G.A. Tuskan. 2005. Genotypic variation in growth and biomass distribution for two advanced-generation (F<sub>2</sub>) pedigrees of hybrid poplar (*Populus* spp.). Canadian Journal of Forest Research 35: 1779-1789.
- \*Yin, T.-M., \*S.P. DiFazio, L.E. Gunter, S.S. Jawdy, W. Boerjan, and G.A. Tuskan. 2004. Genetic and Physical Mapping of *Melampsora* Rust Resistance Genes in *Populus* and Characterization of Linkage Disequilibrium and Flanking Genomic Sequence. *New Phytologist* 165(1):95-105.
- Yin, T.M., S.P. DiFazio, L.E. Gunter, D. Riemenschneider, and G.A. Tuskan. 2004. Large-scale <u>Heterospecific Segregation Distortion in Populus Revealed by a Dense Genetic Map</u>. *Theoretical and Applied Genetics*. 109(3):451-463.
- Tuskan, G.A., L.E. Gunter, Z.K. Yang, T.M. Yin, M.M. Sewell, and S.P. DiFazio. 2004. <u>Characterization of Microsatellites Revealed by Genomic Sequencing of Populus</u> <u>trichocarpa</u>. Canadian Journal of Forest Research. 34(1):85-93.
- Burczyk, J., S.P. DiFazio, and W.T. Adams. 2004. Gene flow in forest trees: How far do genes really travel? Forest Genetics, in press.
- DiFazio, S.P., G. Slavov, J. Burczyk, S. Leonardi, and S.H. Strauss. 2004. <u>Gene Flow From Tree Plantations and Implications for Transgenic Risk Assessment</u>. *In* C. Walter and M. Carson (eds.), Plantation Forest Biotechnology for the 21<sup>st</sup> Century. Research Signpost, Kerala, India. pp. 405-422.
- Slavov, G., S.P. DiFazio, and S.H. Strauss. 2004. <u>Gene flow in forest trees: Gene migration patterns and landscape modelling of transgene dispersion in hybrid poplar</u>. *In* H.C.M. den Nijs, D. Bartsch, and J. Sweet (eds.), Introgression from Genetically Modified Plants into Wild Relatives. CABI Publishing, Wallingford, Oxfordshire.
- Wullschleger, S.D., and S.P. DiFazio. 2003. <u>Emerging use of gene expression microarrays in plant physiology</u>. *Comparative and Functional Genomics* 4: 216-224.
- Wullschleger, S.D., G.A.Tuskan, , and S.P.DiFazio. 2002. Genomics and the Tree Physiologist. Tree Physiology 22: 1273-1276.
- Meilan, R., K.-H. Han, C. Ma, S.P. DiFazio, J.A.Eaton, E. Hoien, B.J. Stanton, R.P. Crockett, M.L. Taylor, R.R. James, J.S. Skinner, L. Jouanin, G. Pilate, and S.H. Strauss. 2002. <u>Growth and glyphosate tolerance of transgenic poplars I. Two-year field performance</u>. Canadian Journal of Forest Research 32: 967-976.

- Meilan, R., D.J. Auerbach, C. Ma,, S.P. DiFazio, and S.H. Strauss. 2002. <u>Stability of herbicide</u> resistance and GUS expression in transgenic hybrid populars (Populus spp.) during several years of field trials and vegetative propagation. HortScience 37(2): 277-280.
- Strauss, S.H. S.P. DiFazio, , and R. Meilan. 2001. <u>Genetically modified poplars in context</u>. Forestry Chronicle, 77(2):1-9.
- Brunner, A.M., W.H. Rottmann, L.A. Sheppard, K. Krutovskii, S.P. DiFazio, S. Leonardi and S.H. Strauss. 2000. <u>Structure and expression of duplicate AGAMOUS orthologs in poplar</u>. Plant Molecular Biology 44 (5):619-634.
- James, R., S.P. DiFazio, A. Brunner and S.H. Strauss. 1998. <u>Environmental effects of genetic engineering of woody biomass crops</u>. Biomass & Bioenergy 14(4): 403-414.
- DiFazio, S.P., M.V. Wilson, and N.C. Vance. 1998. <u>Factors limiting seed production of Taxus</u> brevifolia (Taxaceae) in western Oregon. American Journal of Botany 85(7): 910-918.
- DiFazio, S.P., N.C. Vance, and M.V. Wilson. 1997. Strobilus Production and growth of Pacific yew under a range of overstory conditions in western Oregon. Canadian Journal of Forest Research 27: 986-993.
- DiFazio, S.P., N.C. Vance and M.V. Wilson. 1996. Variation in sex expression of Pacific yew in western Oregon. Canadian Journal of Botany 74: 1943-1946.

# **Other Publications**

- DiFazio, S. 2005. <u>A pioneer perspective on adaptation</u>. Functional Genomics of Environmental Adaptation in *Populus* The 12<sup>th</sup> New Phytologist Symposium, Gatlinburg Tennessee, USA, October 2004. *New Phytologist*.165: 661-664.
- DiFazio, S.P., L. E. Gunter, G. Wickham, J. Zhou, C. C. Brandt, J. C. Schryver, and R. J. Norby. 2004. <u>Genomic Characterization of Belowground Ecosystem Responses to Climate Change.</u> *Laboratory Directed Research And Development Program Fy 2004 Annual Report.* Oak Ridge National Laboratory, Oak Ridge, TN.
- DiFazio, S., S. Jawdy, L. Gunter, B. Wilson, and A. Brunner. 2004. <u>Ecosystem Genomics—An Emerging Opportunity for Environmental Research</u>. *Laboratory Directed Research And Development Program Fy 2003 Annual Report*. Oak Ridge National Laboratory, Oak Ridge, TN. pp. 342-351.
- Martin, F., G.A. Tuskan, S.P. DiFazio, P. Lammers, G. Newcombe, and G.K. Podila. 2004. Symbiotic sequencing for the *Populus* mesocosm. *New Phytologist* 161: 330-335.
- Lammers, P., Tuskan, G.A., DiFazio, S.P., Podila, G.K., and Martin, F. 2004. <u>Mycorrhizal</u> symbionts of Populus to be sequenced by the United States Department of Energy's Joint Genome Institute. *Mycorrhiza* 14: 63-64.
- Tuskan, G.A., S.P. DiFazio, and T. Teichmann. 2004. <u>Poplar genomics is getting popular: The impact of the poplar genome project on tree research</u>. *Plant Biology*. 6: 2-4.
- Strauss, S.H., and S.P. DiFazio. 2004. <u>Hybrids Abounding (Book Review)</u>. *Nature Biotechnology* 22: 29-30.
- DiFazio, S.P. 2002. Biotechnology in tree improvement: Controversy versus capability. *Southeast Biology* 49(3): 288-289.
- Slavov,G.T., S.P. DiFazio, and S.H. Strauss. 2002. Gene flow in forest trees: From empirical estimates to transgenic risk assessment. *In* Ecological and Agronomic Consequences of Gene Flow from Transgenic Crops to Wild Relatives. Ohio State University, Columbus, pp. 106-119.
- DiFazio, S.P. 2002. <u>Potential Impacts of Hybrid Poplar Cultivation on Black Cottonwood Populations: Gene Flow and Simulation Modeling.</u> PhD Dissertation. Oregon State University, Corvallis. 244 pp.
- Strauss, S.H., R. Meilan, S.P. DiFazio, A.M. Brunner, J.S. Skinner, R. Mohamed and J. Carson. 2000. Tree Genetic Engineering Research Cooperative Annual Report: 1999-2000. Forest Research Laboratory, Oregon State University.

- Butler, B.J., S.P. DiFazio, M. Duane, M. Stoddard, and T. Neal. 2000. Sustainability and biodiversity of tropical ecosystems symposium. Meeting Report. *Environmental Conservation* 27(1), 82-83. 2000.
- Meilan, R., C. Ma, S. Cheng, J.A. Eaton, L.K. Miller, R.P. Crockett, S.P. DiFazio, and S.H. Strauss. 2000. <u>High levels of Roundup® and leaf-beetle resistance in genetically engineered hybrid cottonwoods</u>. *In*: K.A. Blatner, J.D. Johnson, and D.M. Baumgartner, eds., Hybrid Poplars in the Pacific Northwest: Culture, Commerce and Capability. Washington State University Cooperative Extension Bulletin MISC0272, Pullman, WA. pp. 29-38.
- DiFazio. S.P., S. Leonardi, S. Cheng, and S.H. Strauss. 1999. Assessing potential risks of transgene escape from fiber plantations. In P.W. Lutman (ed.) Gene flow and agriculture: relevance for transgenic crops. Symposium Proceedings No. 72. British Crop Protection Concil, Farnham, UK. pp. 171-176.
- Strauss, S.H., R. Meilan, S.P. DiFazio, A. Brunner, S. Leonardi, J. Skinner, K. Krutovskii, and R. Mohamed. 1999. Tree Genetic Engineering Research Cooperative (TGERC) Annual Report: 1998-1999. Forest Research Laboratory, Oregon State University, Corvallis.
- Strauss, S.H., R. Meilan, S.P. DiFazio, R. Mohamed, A. Brunner, S. Leonardi, J. Skinner, and K. Krutovskii. 1998. Tree Genetic Engineering Research Cooperative (TGERC) Annual Report: 1997-1998. Forest Research Laboratory, Oregon State University, Corvallis.
- DiFazio, S.P. 1995. The Reproductive Ecology of Pacific Yew (Taxus brevifolia Nutt.) Under a Range of Overstory Conditions in Western Oregon. M.S. Thesis, Oregon State University, Corvallis, OR. 178 pp.

# SYMPOSIA ORGANIZED

<u>Functional Genomics of Environmental Adaptation in *Populus*</u>. with M. Campbell, F. Martin, R. Norby, H. Slater, and J. Tuskan. October 10-13, 2004. Gatlinburg, Tennessee <u>Sustainability and Biodiversity of Tropical Ecosystems</u>, with B.J. Butler, M. Duane, M. Stoddard, and T. Neal. **May 27** 2000. Corvallis, Oregon.

# **INVITED PRESENTATIONS**

The *Populus* Genome Sequence: An Invaluable Resource for Industrial Biotechnology. <u>The World Congress on Industrial Biotechnology and Bioprocessing</u> (Session Chair). Orlando, FL. April 22, 2005.

From Ecosystem Genomics to Genome Ecology: Opportunities at the Interface of Complex Systems. <u>Landscapes, Genomics, and Transgenic Conifer Forests Environmental Leadership Forum.</u> Duke University, Durham, NC. November 17-19, 2004.

Map-Based Assembly and Structural Characterization of the *Populus* Genome. <u>IUFRO Forest</u> <u>Genetics and Tree Breeding in the Age of Genomics</u>. Charleston, SC, November 1-5, 2004

A Structural Overview of the *Populus* Genome. <u>Functional Genomics of Environmental Adaptation in *Populus*</u>. Gatlinburg, TN, October 10-13, 2004.

The International Populus Genome Sequencing Project: Unveiling the Secrets of a Pioneer Tree. <u>II Meeting of the Spanish Forest Functional Genomics Network</u>. Pontevedra, Spain, September 29-30, 2004.

The International Populus Genome Sequencing Project: Unveiling the Secrets of a Pioneer Tree (Keynote Address). <u>2004 Poplar Council of Canada Annual Meeting</u>. University of British Columbia, Vancouver, BC. August 8-11, 2004.

Outcrossing Risks for Transgenic Hybrid Poplars. IUFRO Forest Biotechnology 1999. University of Oxford, Oxford, UK. July 14, 1999.

#### **PRESENTATIONS**

S.P. DiFazio, T.M. Yin, N. Putnam, and G.A. Tuskan. Tools and Strategies for Identifying Genes for Complex Traits in *Populus* (Keynote). IUFRO Tree Biotechnology 2005. Pretoria, South Africa. (Prepared by S. DiFazio, presented by G. Tuskan).

Structural Characterization of the *Populus* Genome. <u>Plant and Animal Genome Conference XIII</u>. San Diego, CA. January 15-19, 2005.

The Poplar Genome. University of Tennessee Symposium on the Functional Genomics of Woody Plants. October 17, 2003.

Poplar Genomics: Opportunities for Accelerated Domestication and Insights on Adaptation. ORNL Genome Science and Technology Series. July 31, 2003.

<u>Ecological Implications of the Biotechnology Revolution in Forestry</u>. University of Tennessee, February 17, 2003.

Gene Flow from Hybrid Poplar Plantations and Implications for Transgenic Risk Assessment. International Poplar Symposium III, Uppsala, Sweden. August 28, 2002.

Biotechnology in Tree Improvement: Controversy versus Capability. S.P. DiFazio and S. Strauss. Meeting of the Association of Southeast Biologists, Boone, North Carolina, April 11, 2002.

A Landscape Modeling Approach to Assessing Gene Flow from Transgenic Poplar Plantations. S.P. DiFazio, S. Leonardi, S. Cheng, B. Straub, S. Garman, T. Adams, and S. Strauss. Tree Biotechnology in the New Millennium. Skamania, Washington, July 24, 2001.

Potential Impacts of Hybrid Poplar Plantations on Black Cottonwood Populations. S.P. DiFazio, S. Leonardi, S. Garman, T. Adams, and S. Strauss. Meeting of the International Poplar Council. Vancouver, Washington. September 26, 2000.

Use of Biotechnology in Improvement of Woody Biomass Crops. S.P. DiFazio and S.H. Strauss. Society of American Foresters National Convention. Portland, Oregon. September 14, 1999.

Potential Impacts of Hybrid Poplar Plantations on Black Cottonwood Populations. S.P. DiFazio, S. Leonardi, S. Garman, S. Cheng, T. Adams, and S. Strauss. Western Forest Genetics Association 1999 Annual Meeting. Flagstaff, Arizona. July 27, 1999.

Assessing potential risks of transgene escape from fiber plantations. S.DiFazio, S.Leonardi, S. Cheng, and S.H. Strauss. British Crop Protection Council Symposium: Gene Flow and Agriculture, Relevance for Transgenic Crops. University of Keele, Staffordshire, UK. April 13, 1999.

#### POSTER PRESENTATIONS

HERMES. Hierarchical Experimental Responses at Macromolecular to Ecosystem Scales. Genome Science and Technology Program Retreat. University of Tennessee. February 25, 2005.

Ecosystem Genomics: An Emerging Opportunity for Environmental Research. Complex Biology Review, ORNL, August 1, 2003.

Potential Risks of Transgenic Poplar Cultivation. S.P. DiFazio, S. Leonardi, S. Garman, B. Straub, and S. Strauss. EPA STAR Fellows Conference, Washington, DC. July 9-12, 2000.

Genetic engineering of hybrid poplar: High levels of Roundup and leaf-beetle resistance in genetically engineered hybrid cottonwoods. Society of American Foresters National Convention. Portland, Oregon. September 11-13, 1999.

Potential Impacts of Transgenic Poplar Cultivation. S.P. DiFazio, S. Leonardi, S. Garman, S. Cheng, T. Adams, and S. Strauss. EPA STAR Fellows Conference, Washington, DC. June 12-14, 1998.

#### **CLASSES TAUGHT**

FS699 <u>Applications of the Polymerase Chain Reaction in Natural Resource Research</u>. (Co-taught with A. Brunner). Fall 1999. 8 students.

FS507. Sustainability and Biodiversity of Tropical Ecosystems. (Co-taught with L. Norris, B.

Butler, M. Duane, T. Neal, and M. Stoddard). Spring 1999. 17 students.

FS505. R/C Molecular Biology and Forestry Research: Applications and Principles (Co-taught with A. Brunner). Winter 1999. 8 students.

#### **CLASS LECTURES**

PS605 Plant Genomics. Chromosomal Mapping and Assembly in poplar. Spring 2004.

FS548 Weed Ecology. Risk Assessment for Genetic Engineering. Fall 1999

FOR441 Silvicultural Principles. Genetic Improvement and Clonal Forestry. Spring 1999.

ENT691 Biological Invasions. Reaction-diffusion models of invasions. Winter 1999.

RE571 Biosystems Modeling Techniques. Cellular automaton models. Winter 1998.

FS548 Weed Ecology and Management. Seed dispersal and gene flow. Fall 1997.

FS548 Weed Ecology and Management. Evolution of plant breeding systems. Fall 1997.

Z565 Molecular Ecology. Gene flow in poplar. Winter 1997.

FS544 Forest Genetics. Strategies for breeding resistance to Melampsora rust. Spring 1997.

FS545 Advanced Community Ecology. Interactions between plants and animals. Spring 1996.

#### **GRADUATE COMMITTEES**

Sara Jawdy, M.S. Ongoing. Department of Plant Sciences, University of Tennessee. Matt Kaproth, M.S. Ongoing. Department of Biology, West Virginia University.

#### **APPRENTICES**

Jessica Adams. Summer 2005. Molecular methods for estimating belowground biomass.

Ryan Cunningham. June 2004 – August 2005. Bioinformatics for the *Populus* Genome.

Harvey T. Jones. Summer 2002. Nucleotide polymorphisms in poplar floral homeotic genes.

David Auerbach. Summer 1999. Somaclonal variation in genetically engineered hybrid poplar.

Jennifer Ross. Summer 1998. Using DNA markers to study modes of reproduction in trees. Nathaniel Ford. Summer 1997. Detecting hybrid poplar seedlings using molecular markers. Laure Muir. Summer 1995. Bulked segregant analysis of forking in Douglas-fir.

# **PROFESSIONAL ACTIVITIES**

Host for Professor Barbara Wilson, Jackson State University, in the Historically Black Colleges and Universities Program, Summer 2003.

Proposal Review Panel, DOE Program for Ecosystem Research, May 2005.

Editorial Review Board, Tree Physiology, 2004.

Reviewed manuscripts for American Journal of Botany (2), Canadian Journal of Forest Research, Comparative and Functional Genomics, Conservation Genetics, Molecular Breeding, New Phytologist (3), Plant Physiology, Plant Science, Theoretical and Applied Genetics, Tree Physiology (5), and Trees, Structure and Function.