

IN VIVO

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HAROLD VARMUS AND CARL LEOPOLD

TO ADDRESS THE 37th ANNUAL MACUB CONFERENCE AT LONG ISLAND UNIVERSITY

Long Island University will host the 37th Annual Fall MACUB Conference on Saturday, November 6, 2004. The conference theme is *"Cancer and the Environment: Their Impact on the Future"* and will feature addresses by Drs. Harold Varmus and Carl Leopold



Harold Varmus, former Director of the National Institutes of Health and co-recipient of a Nobel Prize for studies of the genetic basis of cancer, has served as the President and Chief Executive Officer of Memorial Sloan-Kettering Cancer Center in New York City since January 2000.

Much of Dr. Varmus' scientific work was conducted during 23 years as a faculty member at the University of California, San Francisco, where he, Dr. J. Michael Bishop and their co-workers demonstrated the cellular origins of the oncogene of a chicken retrovirus. This discovery led to the isolation of many cellular genes that normally control growth and development and are frequently mutated in human cancer. For this work, Drs. Bishop and Varmus received many awards, including the 1989 Nobel Prize for Physiology or Medicine. Dr. Varmus is also widely recognized for his studies of the replication cycles of retroviruses and hepatitis B viruses, the functions of genes implicated in cancer, and the development of mouse models for human cancer, the focus of much of the current work in his laboratory at Memorial Sloan-Kettering Cancer Center.

In 1993, Dr. Varmus was named by President Clinton to serve as the Director of the National Institutes of Health, a position he held until the end of 1999. He initiated many changes in the conduct of intramural and extramural research programs, recruited new leaders for most of the important positions at the NIH, planned three major buildings on the NIH campus, and helped to increase the NIH budget from under \$11 billion to nearly \$18 billion.

In addition to authoring over 300 scientific papers and four books, including an introduction to the genetic basis of cancer for a general audience, Dr. Varmus has been an advisor to the federal government, pharmaceutical and biotechnology firms, and many academic institutions. He served on the World Health Organization's Commission on Macroeconomics and Health, chairs the Board of Directors of Public Library of Science and the Scientific Board of the Grand Challenges in Global Health, and is involved in initiatives to promote science in other countries. He has been a member of the U.S. National Academy of Sciences since 1984 and of the Institute of Medicine since 1991.



Carl Leopold

Upstate New York and the mountains of Costa Rica may have little in common geographically, but they share a determined advocate for the protection of biodiversity who is not afraid to get his nails dirty to accomplish it. Dr. Leopold will share highlights from his life in conservation. One focus of his talk will be the he and his wife have undertaken in Central America, which began with several trips as tourists in the late 1980s. Particularly troubling to him was the rate the country's tropical forests were being converted to pasture. While the Costa Rican government encourages reforestation, such efforts are required to be monocultures and often include exotic species. In 1993, Carl and his wife Lynn pooled resources with three other families to purchase 350 acres near the city of Dominical and initiate their own forest restoration project. Employing a well-known Leopold strategy, they planted trees on the depauperate farm. The trees grew very rapidly, but trees aren't the only things growing quickly. The Tropical Forestry Initiative, as the project is known, now boasts an annual bird census, biodiversity monitoring, medicinal plant research, wetland restoration, forest policy analysis, workshops, and a full fledged internship program. Thousands of seedlings of up to 43 native species are produced yearly by the Tropical Forest Initiative nursery for planting on the farm or distribution to nearby landowners. Dr. Leopold characterized the project as great fun. "It's very rewarding going down and doing something constructive rather than lying on the beach getting a sunburn," he said. Dr. Leopold, author of five books and approximately 300 scientific papers, majored in botany at UW-Madison and earned an M.A. and Ph.D. at Harvard. As a professor at Purdue, University of Nebraska, and Cornell, he studied the physiology of seeds, unlocking some secrets which confer their remarkable ability to survive and remain viable even in a dry state. The key, he explained, is the production of soluble sugars which form a glass-like matrix when water is removed, protecting a cell's contents until moisture returns. Now, a California company is building on his work to develop an inhalable system of insulin delivery for diabetics. Treatments involving the dry preservation of other hormones may follow. Dr. Leopold, now 81, retired from teaching in 1990, but has hardly slowed down. In addition to his Costa Rica project, he has served on the Aldo Leopold Foundation Board of Directors since its inception in 1982 and continues to play a key role in it's growth and evolution. He maintains an active involvement in environmental issues closer to his Ithaca home, as Founding President of the Finger Lakes Land Trust. Through a combination of nature preserves and conservation easements, the Trust protects roughly 4,000 acres amidst seven deep glacial lakes in western New York.

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Articles can be submitted electronically to invivo@mec.cuny.edu or mailed as a printed copy (preferably with a diskette that contains the file) to the Editorial Board at Medgar Evers College. All submissions should be formatted double spaced with 1 inch margins. The title of the article, the full names of each author, their academic affiliations and addresses, and the name of the person to whom correspondence should be sent must be given. As a rule, full length articles should include a brief abstract and be divided into the following sections: introduction, materials and methods, results, discussion, acknowledgments and references. Reviews and short communications can be arranged differently. References should be identified in the text by using numerical superscripts in consecutive order. In the reference section, references should be arranged in the order that they appeared in the text using the following format: last name, initials., year of publication. title of article, journal volume number: page numbers. (eg. - ¹Hassan, M. and V. Herbert, 2000. Colon Cancer. *In Vivo* 32: 3 - 8). For books the order should be last name, initial, year of publication, title of book in italics, publisher and city, and page number referred to. (eg. - Prosser, C.L., 1973. *Comparative Animal Physiology*, Saunders Co., Philadelphia, p 59.). Abbreviations and technical jargon should be avoided. Tables and figures should be submitted on separate pages with the desired locations in the text indicated in the margins.

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A Comparison of Species Diversity at Twelve Study Sites, Jamaica Bay, New York

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ABSTRACT

The objective of the present study was to identify the vascular plant species at twelve study sites in Jamaica Bay, New York: (1) Dead Horse Bay; (2) Gerristen Creek; (3) Paerdegat Basin; (4) Fresh Creek; (5) Spring Creek; (6) Hawtree/Bergen Basin; (7) Bayswater State Park; (8) Dubos Point; (9) Brant Point; (10) Broad Channel, New York; (11) JFK Runway By-pass, and one island, (12) Ruffle Bar. Collecting trips to the study sites were made approximately once a month during the growing season from March, 2000 through November 2000, and at site 6 from July 2000 to October 2000, and March 2001 through June 2001. Objectives for each trip included the collection of voucher specimens and accumulation of information on abundance and apparent habitat preference for each species. More than 500 specimens form the basis for this study. Taxonomically problematic specimens were sent to various experts for identification. Bayswater State Park, was the richest site; 185 species of vascular plants were identified at this site. Site 11, JFK Airport, was the least diverse site floristically; 25 species were identified there. Ten New York State endangered species were identified. These rare species were mapped with GIS coordinates at each of the 12 study sites.

INTRODUCTION

The objective of the present study was to identify the vascular plant species at twelve study sites Jamaica Bay: (1) Dead Horse Bay; (2) Gerristen Creek; (3) Paerdegat Basin; (4) Fresh Creek; (5) Spring Creek; (6) Hawtree/Bergen Basin; (7) Bayswater State Park; (8) Dubos Point; (9) Brant Point; (10) Broad Channel, New York (Table 1); (11) JFK Runway By-pass, and an island (12) Ruffle Bar. A second objective was to identify rare and/or endangered vascular plants at the above sites. The aforementioned study sites encompassing 1,210.4 hectares were located at 40°-35°, Latitude 73°-52° longitude, New York. Within the twelve study sites were a variety of habitats including salt marsh, successional fields, shrublands, woodlands, freshwater wetlands dominated by *Phragmites australis*, fresh and brackish-water impoundments, and several landfill sites. This study was part of a large comprehensive study of the Jamaica Bay Ecosystem Restoration Project (JABERP).

The Jamaica Bay Ecosystem Restoration Project (JABERP) was co-sponsored by the U.S. Army Corps of Engineers (USACE); the New York City Department of Environmental Protection (DEP); the New York City Department of Parks and Recreation;

the New York State Department of Environmental Conservation (NYSDEC); U.S. EPA's Harbor Estuary Program (HEP); the National Marine and Fisheries Service (NMFS); Gateway National Recreation Area; National Park Service (NPS); the U.S. Environmental Protection Agency (EPA); and the U.S. Fish and Wildlife Service (USFWS).

Gateway National Recreation Area (GNRA) was created by an act of Congress in 1972, to preserve the science, beauty, flora and fauna, and recreational opportunities of the estuaries and beaches in the New York City metropolitan area¹. Gateway was the nation's first urban national park. Urban and industrial developments have modified the natural environment by dredging and filling, construction, pollution from biological and chemical contaminants and over harvesting or eradication of native plant species. Despite these varied numerous deleterious impacts, the natural environment and many native plants have been remarkably preserved. Stalter and Lamont² recorded 476 species of vascular plants at Jamaica Bay Wildlife Refuge (GNRA) and 482 vascular plant species at Sandy Hook, New Jersey³. Venezia and Cook⁴ reported over 650 vascular plant species for all units of Gateway National Recreation Area, though many species on their list were not vouchered.

There have been numerous ecological and floristic inventories of Gateway National Recreation Area. Most notable, were those by Cook⁵, Greller⁶, Johnson⁷, Lent⁸, Rogers et al.⁹, Stalter and Schmidt¹⁰, Stalter and Lechthaler¹¹, Stalter and Cook¹², Stalter and Greller¹³, and Stalter et al.¹⁴. Many "in house" descriptions of vegetation and/or the ecology of various units in Gateway National Recreational Area have been prepared; these references were also included in the Literature Cited section of Stalter's¹⁵ JABERP report.

METHODS

Collecting trips to the twelve Jamaica Bay study sites were made approximately once a month during the growing season from March 2000 through November 2000, at site 6 from July 2000 to October 2000 and March 2001 through June 2001. Objectives for each trip included the collection of voucher specimens and accumulation of information on abundance and apparent habitat preference for each species.

More than 500 specimens form the basis for this study. Taxonomically problematic specimens were sent to various experts for annotation; experts consulted include Steven Clemants (Juncaceae and the genus *Suaeda*), Ihsan Al-Shebaz (Brassicaceae), Bruce Hansen (various families), Eric Lamont (Asteraceae), Robert Meyer (Poaceae), Richard Mitchell (Polygonaceae), Charles Sheviak (*Spiranthes*) and Gordon Tucker (Cyperaceae). A complete set of voucher specimens has been deposited at the herbarium at Gateway National Recreation Area, New York.

The annotated checklist contains an inventory of the vascular plants that reproduce spontaneously and persist for more than one year without cultivation, including native taxa, naturalized and adventive weeds, and escapes from cultivation. Plants are arranged in the checklist by division and then alphabetically by family, genus, and species. Each entry includes the following information sequence: scientific name; pertinent synonym, enclosed in brackets; and site location. Indication of species rarity across all of New York is based upon the New York Division of Natural Heritage (Indicated in the checklist as NYHP) list of rare plant species¹⁶.

Nomenclature follows Gleason and Cronquist¹ but recent monographs and revisions of Kartesz¹⁸ were also consulted. In most cases nomenclature presented in this flora agrees with Kartesz¹⁸ but when differences occur, the name as presented in Kartesz¹⁸ is listed as a synonym enclosed in brackets.

RESULTS AND DISCUSSION

The vascular flora of twelve study sites

consisted of 252 species within 169 genera and 61 families. One hundred sixty-nine species, 54% of the flora, were native to the area. A summary is given in Table 1 and a list of species is included in the appendix.

The largest families in the flora were the Asteraceae, 49 species and the Poaceae 35 species. Together they comprised 33% of the flora. Other large families were the Fabaceae and Polygonaceae each with 15 species, and the Rosaceae (12 species). *Polygonum* was the largest genus, 13 species. *Trifolium* (6 species) and *Cyperus* (5 species) ranked second and third in species composition. The Poaceae contained the greatest number of non-native species, 18. Fifteen species of Asteraceae and 12 species of Rosaceae were non-native. The genus *Polygonum* contained the largest number of non-native species, 7.

A comparison of vascular plant species at twelve study sites is presented in Table 2. Non-native species, 50.6% of the total species at all sites, slightly outnumbered native species. Spring Creek had the highest percentage of non-native species, 58.2% while, 48.2% of the species were non-native at Brant Point. The percentage of the non-native species at Jamaica Bay Wildlife Refuge, 48.7%, was similar to the percentage of non-native species at the twelve study sites, 50.6%. The twelve study sites and Jamaica Bay Wildlife Refuge have a higher percentage of non-native vascular plant species than New York State where 35% of the vascular plant species were not native to the region (Table 3).

Ten New York State rare vascular plant species were identified at the twelve sites: *Aster subulatus*, *A. tenuifolius*, *Cuscuta pentagona*, *Cyperus schweinitzii*, *Echinacea purpurea*, *Eupatorium hyssopifolium*, var. *laciniatum*, *Quercus phellos*, *Solidago rigida*, *Solidago sempervirens* var. *mexicana* and *Tradescantia ohiensis*. Site 2, Gerristen Creek, had the greatest number of rare species, seven while site 1, JFK Airport, had no rare species.

A comparison of species richness among 12 study sites is presented in Table 4. Bayswater State Park, contained the largest number of vascular plant species, 185, while site 11, JFK Runway By-pass had the fewest number of species, 25 (Table 3). Bayswater State Park and Brant Point had the highest species area quotient, 6.25 while the JFK site had the lowest species area quotient, 0.24 (Table 2). The species area quotient is only valid when sites of approximately equal size are compared. Ruffle Bar, the largest site, 416.4 ha, contained 109 species; the species area quotient for the site was 0.26. Brant Point, the smallest site, 8 ha, contained 50 species; the species area quotient of Brant Point was 6.25. A brief description of the species diversity native/non-native status and New York State rare plant species for each study site follows.

Table 1. Summary of the vascular flora at 12 Study Sites, Jamaica Bay, New York.

	Fern Allies	Ferns	Gymnosperms	Dicots	Monocots	Total
Families	1	1	2	49	8	61
Genera	1	1	2	127	38	169
Species	1	1	2	190	58	252
Native Species	1	1	1	94	34	131
Introduced Species	0	0	1	96	24	121

Site 1. Dead Horse Bay, New York.

The vascular flora of Dead Horse Bay, New York consisted of 153 species including 121 dicots and 31 monocots. Seventy-eight species were non-native, 51.6% of the flora. One New York State rare plant, *Cuscuta pentagona*, was identified at the site.

Site 2. Gerritsen Creek, New York.

The vascular flora of Gerritsen Creek, New York consisted of 136 species. One hundred twelve dicots and 24 monocots existed at the site. Seventy-two species were non-native, 52.9% of the flora. Rare plants at this site were *Solidago rigida*, *Solidago sempervirens* var. *mexicana*, *Eupatorium hyssopifolium* var. *laciniatum*, *Cuscuta pentagona*, *Echinacea purpurea*, *Quercus phellos* and *Cyperus schweinitzii*. Gerritsen Creek contained the greatest number of New York rare plants⁷. Five percent of the species at Gerritsen Creek were New York State rare plants.

Site 3. Paerdegat Basin, New York.

The vascular flora of Paerdegat Basin consisted of 116 species, 97 dicots and 19 monocots. Sixty non-native species comprised 51.7% of the flora. Rare plants included *Aster tenuifolius* and *Cuscuta pentagona*.

Site 4. Fresh Creek, New York.

One hundred sixty two species of vascular plants were identified at Fresh Creek, New York. One hundred thirty one dicots, 30 monocots, and one fern ally,

Equisetum arvense, were identified at this site. Eighty-eight species, 54.3% of the flora, were non-native. Rare plants identified were *Aster subulatus* and *Solidago sempervirens* var. *mexicana*.

Site 5. Spring Creek, New York.

The vascular flora at Spring Creek, New York consisted of 143 species including 119 dicots, 23 monocots and one Pinophyta, *Pinus thunbergii*. Eighty-three species were non-native, 58% of the flora. Rare plants identified at the site included *Cuscuta pentagona* and *Tradescantia ohiensis*.

Site 6. Hawtree/Bergen Basin, New York.

The vascular flora at Hawtree/Bergen Basin consists of 170 species, 138 dicots, 31 monocots and one native Pinophyta, *Juniperus virginiana*. Eighty-five species, 50% of the flora were non-native. Rare plants identified at the site included *Aster tenuifolius*, *Cuscuta pentagona* and *Tradescantia ohiensis*.

Site 7. Bayswater State Park, New York.

The vascular flora at Bayswater State Park consisted of 185 species, the richest site in terms of species diversity. One hundred fifty two dicots, 33 monocots and *Pinus thunbergii* made up the vascular flora. Eighty-nine species, 48.1% of the flora were non-native. The rarest plant identified at the site was *Aster tenuifolius*. Additional species of trees and shrubs have been planted at Bayswater State Park, e.g. *Pinus rigida*; planted species were not included in the species list.

Table 2. Vascular plant species at twelve study sites, Jamaica Bay, New York.					
Study Site	Total Number of Species	Native Species	Non-native Species	% Non-native Species	
1. Dead Horse Bay	153	75	78	51.6	
2. Gerristen Creek	136	64	72	52.9	
3. Paerdegat Basin	116	56	60	51.7	
4. Fresh Creek	162	74	88	54.3	
5. Spring Creek	143	60	83	58.0	
6. Hawtree/Bergen Basin	170	85	85	50.0	
7. Bayswater State Park	185	96	89	48.1	
8. Dubos Point	71	39	32	45.1	
9. Brant Point	50	29	21	42.0	
10. Broad Channel	68	31	37	54.4	
11. JFK Runway By-pass	25	14	11	44.0	
12. Ruffle Bar	109	62	47	43.1	
Total	1,338	685	703	50.6	

Table 3. Frequencies of native versus non-native plants at New York State, Jamaica Bay Wildlife Refuge, New York, Liberty Island, New York, Ellis Island, New York and twelve study sites, Jamaica Bay, New York.					
	Locality				
	New York State	Jamaica Bay, NY	Liberty Island, NY	Ellis Island, NY	Twelve Study Sites, Jamaica Bay, NY
Native Species	2078	234	34	98	685
Non-native Species	1117	222	63	149	703
% Non-native Species	35.0	48.7	65	60.3	50.6
Total Species	3195	456	97	247	1388

Table 4. Comparison of species richness among 12 study sites

Site	Area (ha)	Species Richness	Species Area Quotient
1. Dead Horse Bay	191.2	153	0.80
2. Gerristen Creek	33.7	136	4.04
3. Paerdegat Basin	83.7	116	1.39
4. Fresh Creek	65.3	162	2.48
5. Spring Creek	197.8	143	0.72
6. Hawtree/Bergen Basin	46.0	170	4.00
7. Bayswater State Park	29.6	185	6.25
8. Dubos Point	24.1	71	2.95
9. Brant Point	8.0	50	6.25
10. Broad Channel	11.6	68	5.86
11. JFK Runway By-pass	103.0	25	0.24
2. Ruffle Bar	415.4	109	0.26
Total	1210.4	252	0.21

Site 8. Dubos Point, New York.

The vascular flora at Dubos Point consisted of 71 species including 47 dicots, 22 monocots, one fern ally, and one pine. Thirty-two species, 45.1% of the flora were non-native to the region. The rarest plants encountered were *Aster subulatus* and *Aster tenuifolius*.

Site 9. Brant Point, New York.

The vascular flora at Brant Point, New York, consisted of 50 species, 38 dicots and 12 monocots. Twenty-one species, 42% of the flora were non-native. The rarest plants identified were *Aster tenuifolius* and *Tradescantia ohiensis*.

Site 10. Broad Channel, New York.

The vascular flora of Broad Channel consisted of 68 species. Fifty eight dicots and 10 monocots were identified at this site. Thirty-seven species, 54.4% of the flora were not native to this region. The rarest plants encountered were *Aster tenuifolius* and *Solidago sempervirens* var. *mexicana*.

Site 11. JFK Airport, New York.

The JFK site was the least floristically rich site; 25 species of vascular plants were identified, including seventeen dicots and 8 monocots. Eleven species, 44% of the flora, were not native to this region. No New York State rare or threatened plants were observed here.

Site 12. Ruffle Bar, New York.

The vascular flora of Ruffle Bar consisted of 106 species including 88 dicots, 16 monocots, one fern, *Thelypteris palustris* and 2 Pinophyta, *Juniperus virginiana* and *Pinus thunbergii*. This inaccessible site is infrequently visited by humans because Ruffle Bar is an island in Jamaica Bay. This site had the lowest percentage (42.4%) of non-native plant species. Forty-five species, were not native to this region. The rarest plants encountered were *Aster tenuifolius* and *Cuscuta pentagona*.

ACKNOWLEDGMENTS

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Appendix: Species Distribution at 12 study sites, Jamaica Bay, New York. Site 1 (DH) Dead Horse Bay, Site 2 (G) Gerristen Creek, Site 3 (P) Paerdegat, Site 4 (FC) Fresh Creek, Site 5 (S) Spring Creek, Site 6 (H/B) Hawtree/Bergen, Site 7 (BS) Bayswater State Park, Site 8 (DP) Dubos Point, Site 9 (BP) Brant Point, Site 10 (BC) Broad Channel, Site 11 (AP) JFK, and Site 12 (RB) Ruffle Bar.

Species	Study Sites											
	1 DH	2 G	3 P	4 FC	5 S	6 H/B	7 BS	8 DP	9 BP	10 BC	11 AP	12 RB
LYCOPODIOPHYTA												
Equisetaceae												
<i>Equisetum arvense</i> L.					x				x			
POLYPODIOPHYTA												
Thelypteridaceae												
<i>Thelypteris palustris</i> Schott.												x
PINOPHYTA												
Cupressaceae												
<i>Juniperus virginiana</i> L.	x		x								x	
Pinaceae												
* <i>Pinus thunbergii</i> Parl.					x		x	x				x
MAGNOLIOPHYTA-MAGNOLIOPSIDA												
Aceraceae												
* <i>Acer negundo</i> L.	x	x			x	x			x	x		
* <i>Acer platanooides</i> L.	x	x	x	x	x	x	x			x		x
<i>Acer rubrum</i> L.							x					
* <i>Acer saccharinum</i> L.		x	x	x	x	x	x					
Amaranthaceae												
<i>Froelichia gracilis</i> (Hook.) Moq.	x	x										
<i>Amaranthus hybridus</i> L.						x						
* <i>Amaranthus retroflexus</i> L.						x						
Anacardiaceae												
<i>Rhus copallinum</i> L.	x	x	x	x	x	x	x		x			x
<i>Rhus glabra</i> L.	x				x	x	x	x		x	x	
<i>Rhus hirta</i> (L.) Sudworth[<i>Rhus typhina</i> L.]			x		x	x						
<i>Toxicodendron radicans</i> (L.) Kuntze	x	x	x	x	x	x	x	x	x			x
Apiaceae												
* <i>Daucus carota</i> L.	x	x	x	x	x	x	x		x			x
Apocynaceae												
<i>Apocynum cannabinum</i> L.	x	x	x	x	x	x	x	x				x

Species	Study Sites											
	1 DH	2 G	3 P	4 FC	5 S	6 H/B	7 BS	8 DP	9 BP	10 BC	11 AP	12 RB
Asclepiadaceae												
* <i>Asclepias syriaca</i> L.	x	x		x	x	x	x	x				x
<i>Asclepias tuberosa</i> L.							x					
Asteraceae												
* <i>Achillea millefolium</i> L. subsp. <i>lanulosa</i> (Nutt.) Piper.	x	x		x	x	x	x					
<i>Ambrosia artemisiifolia</i> L.	x	x	x	x	x	x	x		x	x		x
* <i>Arctium minus</i> Schk.		x					x					
* <i>Artemisia ludoviciana</i> Nutt.				x	x	x		x				x
* <i>Artemisia vulgaris</i> L.	x	x	x	x	x	x	x		x			x
<i>Aster dumosus</i> L. [A. <i>cordifolius</i> Michx.]												x
<i>Aster subulatus</i> Michx.				x		x		x				
<i>Aster tenuifolius</i> L.			x			x	x		x	x		x
<i>Aster viminifolius</i> Lam.	x	x	x	x								x
<i>Baccharis halimifolia</i> L.	x	x	x	x	x	x	x	x	x	x	x	x
<i>Bidens bipinnata</i> L.						x	x					
* <i>Centaurea maculosa</i> Lam.	x	x	x			x	x					x
* <i>Chrysanthemum leucanthemum</i> L. [Leucanthemum <i>vulgare</i> Lam.]		x	x									
<i>Chrysopsis graminifolia</i> (Michx.) Elliott			x			x						
* <i>Cichorium intybus</i> L.	x	x	x	x	x	x	x	x				
<i>Cirsium arvense</i> (L.) Scop.												x
<i>Conyza canadensis</i> (L.) Cronq. var. <i>canadensis</i>	x	x	x	x	x	x	x		x	x		x
<i>Coreopsis lanceolata</i> L.		x		x								
<i>Erechtites hieracifolia</i> (L.) Raf.						x						
<i>Erigeron strigosus</i> Muhl.	x	x	x	x	x		x			x		
<i>Eupatorium hyssopifolium</i> L.	x			x		x	x					x
<i>Eupatorium pilosum</i> Walt.	x											
[<i>Eupatorium rotundifolium</i> L. var. <i>ovatum</i> (Bigel.) Torr.]												x
<i>Eupatorium rugosum</i> Houtt.	x	x				x						
<i>Euthamia graminifolia</i> (L.) Nutt.	x	x	x	x	x		x					x
<i>Euthamia tenuifolia</i> (Pursh) Nutt.	x	x	x	x								
<i>Gaillardia pulchella</i> Foug.												
<i>Galinsoga quadriradiata</i> Ruiz & Pavon	x						x					
<i>Gnaphalium obtusifolium</i> L.	x	x	x	x	x	x	x		x			
* <i>Helianthus petiolaris</i> Nutt.	x	x		x	x	x						
<i>Helianthus giganteus</i> L.							x					
<i>Heterotheca subaxillaris</i> (Lam.) Britt. & Rusby	x	x	x	x	x	x						x
* <i>Hieracium caespitosum</i> Dumort.					x	x						
<i>Hypochoeris radicata</i> L.			x	x	x		x					
<i>Iva frutescens</i> L.	x	x	x	x	x	x	x		x	x	x	x
<i>Krigia virginica</i> (L.) Willd.			x									
<i>Lactuca canadensis</i> L.	x											
* <i>Matricaria discoidea</i> DC. [<i>Matricaria matricarioides</i> auct. Non (Less.) Porter]	x	x	x	x	x	x	x			x		
<i>Rudbeckia hirta</i> L. var. <i>pulcherrima</i> Farw.		x										
* <i>Senecio vulgaris</i> L.	x		x	x	x	x	x			x		
<i>Solidago canadensis</i> L. var. <i>scabra</i> Torr. & Gray [S. <i>altissima</i> L.]			x	x	x	x				x		x
<i>Solidago rugosa</i> Miller	x	x	x	x	x	x	x					
<i>Solidago rigida</i> L.		x										
<i>Solidago sempervirens</i> L.	x	x	x	x	x	x	x		x	x		x
* <i>Sonchus asper</i> (L.) Hill	x	x	x	x	x	x	x			x		x
* <i>Sonchus oleraceus</i> L.	x											
<i>Taraxacum officinale</i> Weber	x	x	x	x	x	x	x					
<i>Tragopogon dubius</i> Scop.	x			x	x	x	x					
<i>Xanthium strumarium</i> L. var. <i>canadense</i> (Miller) T. & G.	x			x	x	x						
Berberidaceae												
* <i>Berberis thunbergii</i> DC.							x					
Betulaceae												
<i>Betula populifolia</i> Marsh.	x	x		x	x		x					x
Bignoniaceae												
<i>Campsis radicans</i> (L.) Seemann		x										
<i>Catalpa bignonioides</i> Walter	x			x		x	x					

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Boraginaceae												
* <i>Echium vulgare</i> L.						x						
Brassicaceae												
* <i>Alliaria petiolata</i> (Bieb.) Cavara & Grande				x								
* <i>Arabidopsis thaliana</i> (L.) Henyl.				x	x							
* <i>Barbarea vulgaris</i> R. Br.		x										
<i>Bertonia incana</i> (L.) DC.		x										
<i>Cakile edentula</i> (Bigel.) Hook.	x	x		x	x	x	x			x		x
* <i>Capsella bursa-pastoris</i> (L.) Medik.						x						
* <i>Lepidium virginicum</i> L.	x	x	x	x	x		x		x	x		x
* <i>Lunaria annua</i> L.				x								
* <i>Raphanus raphanistrum</i> L.						x						
<i>Sisymbrium altissimum</i> L.					x							
<i>Thlaspi arvense</i> L.												
Campanulaceae												
<i>Triodanis perfoliata</i> (L.) Nieuwl. [<i>Specularia perfoliata</i>] (L.) A. DC.]	x						x					
Cannabaceae												
<i>Humulus japonicus</i> Siebold & Zucc.					x	x						
Caprifoliaceae												
* <i>Lonicera japonica</i> Thunb.	x	x	x	x	x	x	x	x	x			x
* <i>Lonicera tartarica</i> L.							x	x	x			x
<i>Sambucus canadensis</i> L.	x					x	x					x
Caryophyllaceae												
* <i>Arenaria serpyllifolia</i> L.	x	x	x	x	x	x	x					x
* <i>Cerastium fontanum</i> Baumg. subsp. <i>vulgare</i> (Hartman) Grueter & Burdet [<i>C. holosteoides</i> Fries var. <i>vulgare</i> (Hartman) Hylander, <i>C. vulgatum</i> L.]												x
* <i>Dianthus armeria</i> L.	x			x	x	x	x				x	
<i>Honkenya peploides</i> (L.) Ehrh. [<i>Arenaria peploides</i> L.]	x											
* <i>Saponaria officinalis</i> L.	x	x	x	x	x	x	x	x	x	x	x	
* <i>Scleranthus annuus</i> L.	x	x	x	x	x	x	x					x
* <i>Silene latifolia</i> Poiret subsp. <i>alba</i> (P. Mill). Greuter & Burdet [<i>Silene alba</i> (P. Mill) Krause; <i>Lychis alba</i> P. Miller]	x	x	x	x		x	x		x			x
* <i>Silene vulgaris</i> (Moench) Garcke. [<i>Silene cucubalis</i> Wibel]	x		x	x	x	x			x		x	
<i>Spergula arvensis</i> L.		x										
<i>Spergularia marina</i> (L.) Griseb.			x	x		x	x					
Celastraceae												
* <i>Celastrus orbiculata</i> Thunb.	x	x		x	x	x	x			x		x
Chenopodiaceae												
* <i>Atriplex patula</i> L.	x	x	x	x	x	x	x		x	x		x
* <i>Atriplex prostrata</i> Boucher [<i>A. hastata</i> sensu Allen non L.; <i>A. patula</i> L. var. <i>hastata</i> (L.) A. Gray]										x		x
* <i>Chenopodium album</i> L.	x	x	x	x	x	x	x		x			x
* <i>Chenopodium ambrosioides</i> L.	x			x			x					x
<i>Cycloloma atriplicifolium</i> (Spreng.) Coult.	x	x		x	x	x						x
<i>Salicornia europaea</i> L.	x	x	x	x		x	x	x	x	x		x
<i>Salicornia virginica</i> L.								x				
<i>Salsola kali</i> L.	x					x	x	x		x		x
<i>Suaeda linearis</i> (Elliot) Moq.				x			x	x	x		x	x
Cistaceae												
<i>Lechia maritima</i> Leggett.	x			x	x	x	x	x				x
Clusiaceae												
<i>Hypericum punctatum</i> Lam.	x	x	x	x	x	x	x					x
Convolvulaceae												
* <i>Convolvulus arvensis</i> L.	x	x	x	x	x	x	x		x	x		x
Cornaceae												
<i>Nyssa sylvatica</i> Marshall							x					
Cucurbitaceae												
<i>Sicyos angulatus</i> L.					x							
Cuscutaceae (Formerly Convolvulaceae)												
<i>Cuscuta campestris</i> Yunker [<i>C. pentagona</i> Engelm.]	x	x	x			x						x

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	1 DH	2 G	3 P	4 FC	5 S	6 H/B	7 BS	8 DP	9 BP	10 BC	11 AP	12 RB
Elaeagnaceae												
* <i>Elaeagnus angustifolia</i> L.	x	x	x	x	x		x			x		
* <i>Elaeagnus umbellata</i> Thunb.	x											
Euphorbiaceae												
<i>Acalypha virginica</i> var. <i>rhomboidea</i> (Raf.) Cooperider [<i>Acalypha rhomboidea</i> Raf.]	x	x		x								
<i>Chamaesyce maculata</i> (L.) Small [<i>Euphorbia maculata</i> L.; <i>E. supina</i> Raf.]	x	x	x	x	x	x	x	x				x
* <i>Euphorbia cyparissias</i> L.	x		x	x	x	x						
Fabaceae												
<i>Gledisia triacanthos</i> L.	x	x	x	x	x							x
* <i>Lotus corniculatus</i> L.		x	x									
* <i>Medicago lupulina</i> L.	x	x	x	x	x	x	x				x	
<i>Medicago sativa</i> L.						x						
* <i>Melilotus alba</i> Medikus	x	x	x	x	x	x	x				x	x
* <i>Melilotus officinalis</i> (L.) Lam.	x	x	x	x	x	x	x			x	x	
<i>Robinia pseudoacacia</i> L.	x	x	x	x	x	x					x	
<i>Strophostyles helvola</i> (L.) Ell.	x	x	x	x	x	x	x			x		x
* <i>Trifolium arvense</i> L.	x	x	x	x	x	x	x				x	
* <i>Trifolium aureum</i> Pollich	x	x	x	x	x		x			x		
* <i>Trifolium campestre</i> Schreber [<i>T. procumbens</i> L.]		x		x	x							
* <i>Trifolium hybridum</i> L.		x	x	x	x	x	x			x		
* <i>Trifolium pratense</i> L.	x	x	x	x	x	x	x			x		
* <i>Trifolium repens</i> L.	x	x	x	x	x	x	x			x		
* <i>Wisteria sinensis</i> (Sims.) Sweet							x					
Fagaceae												
<i>Quercus ilicifolia</i> Wangenh.			x									
<i>Quercus palustris</i> Muenchh.	x			x	x	x	x					
* <i>Quercus phellos</i> L.		x										
<i>Quercus velutina</i> Lam.			x				x					
Gentianaceae												
<i>Centaureum pulchellum</i> (Swartz) Druce.							x					
Geraniaceae												
* <i>Geranium dissectum</i> L.	x	x	x	x	x	x	x					x
Lamiaceae												
* <i>Lamium amplexicaule</i> L.	x	x	x	x	x		x			x		x
Lauraceae												
<i>Sassafras albidum</i> (Nutt.) Nees.						x	x					
Lythraceae												
* <i>Lythrum salicaria</i> L.				x		x			x			
Malvaceae												
* <i>Albutilon theophrasti</i> Medik.				x					x			
* <i>Hibiscus syriacus</i> L.						x						
* <i>Malva neglecta</i> Wallr.		x		x								
Mimosaceae												
* <i>Albizzia julibrissin</i> Durazz.						x						
Molluginaceae												
* <i>Mollugo verticillata</i> L.	x	x	x	x	x	x	x	x				
Moraceae												
* <i>Morus alba</i> L.	x	x	x	x	x	x	x	x	x	x		x
Myricaceae												
<i>Myrica pensylvanica</i> Mirbel.	x	x	x	x	x	x	x	x	x	x		x
Nyctaginaceae												
* <i>Mirabilis nyctaginea</i> (Michx.) MacM.	x						x					x
Oleaceae												
<i>Fraxinus americana</i> L.									x			
* <i>Ligustrum amurense</i> Carr.		x	x	x		x	x	x				
Onagraceae												
<i>Epilobium coloratum</i> Biehler			x									
<i>Oenothera biennis</i> L.	x	x	x	x	x	x	x	x		x		x
<i>Oenothera laciniata</i> Hill.	x		x	x	x	x		x	x			x
Oxalidaceae												
<i>Oxalis dillenii</i> Jacq.	x	x	x	x	x	x	x	x				
Phytolaccaceae												
<i>Phytolacca americana</i> L.	x	x	x	x	x	x	x	x	x	x		x
Plantaginaceae												
* <i>Plantago aristata</i> Michx.	x											
* <i>Plantago lanceolata</i> L.	x	x		x	x	x	x	x	x			x

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	1 DH	2 G	3 P	4 FC	5 S	6 H/B	7 BS	8 DP	9 BP	10 BC	11 AP	12 RB
<i>*Plantago major</i> L.		X	X	X	X	X	X	X		X		
<i>*Plantago rugelii</i> Decne.	X			X	X							
Plantanaceae												
<i>*Platanus acerifolia</i> (Ait.) Willd.	X						X					
Plumbaginaceae												
<i>Limonium carolinianum</i> (Walt.) Britt.	X	X	X	X	X	X	X	X	X	X	X	X
Polygonaceae												
<i>Polygonella articulata</i> (L.) Meissner.	X		X	X	X							X
<i>*Polygonum arenastrum</i> Jord. ex Bor.	X	X				X	X	X				
<i>Polygonum aviculare</i> L.	X	X	X	X	X	X				X		
<i>*Polygonum bellardii</i> All.						X						
<i>*Polygonum cespitosum</i> Blume var. <i>longisetum</i> (DeBruyn) Stewart							X					
<i>*Polygonum cuspidatum</i> Sieb. & Zucc.	X	X	X	X		X	X	X	X			X
<i>*Polygonum lapathifolium</i> L.						X	X					
<i>*Polygonum orientale</i> L.						X						
<i>Polygonum persicaria</i> L.						X						
<i>Polygonum punctatum</i> Elliott		X										
<i>*Polygonum pensylvanicum</i> L.		X	X	X	X	X				X		
<i>*Rumex acetosella</i> L.	X	X	X	X	X	X	X	X				X
<i>*Rumex crispus</i> L.	X	X	X	X	X	X	X	X	X	X		X
Portulacaceae												
<i>*Portulaca oleracea</i> L.	X	X			X	X	X	X	X		X	
Pyrolaceae												
<i>Chimaphila maculata</i> (L.) Pursh							X					
Ranunculaceae												
<i>Ranunculus scleratus</i> L.							X					
Rhamnaceae												
<i>*Rhamnus frangula</i> L.	X	X	X	X	X	X	X					X
Rosaceae												
<i>Amelanchier canadensis</i> (L.) Medikus						X						
<i>Geum canadense</i> Jacq.							X	X				
<i>*Malus baccata</i> (L.) Borkh.				X	X							
<i>*Malus pumila</i> P. Mill.				X	X		X					X
<i>*Malus sylvestris</i> P. Mill.							X		X			
<i>*Potentilla argentea</i> L.	X	X		X	X		X					X
<i>*Potentilla norvegica</i> L.				X	X	X						
<i>*Potentilla recta</i> L.	X	X	X			X	X					
<i>*Potentilla simplex</i> Michx.	X			X	X	X	X	X		X		X
<i>Prunus maritima</i> Marsh.	X						X					X
<i>*Prunus persica</i> (L.) Batsch						X		X				X
<i>Prunus serotina</i> Ehrh.	X	X	X	X	X	X	X	X		X		X
<i>*Pyrus communis</i> L.				X	X		X					
<i>*Rosa multiflora</i> Thunb. Ex Murr.	X	X	X	X	X	X	X	X		X		X
<i>*Rosa palustris</i> Marsh.		X		X	X				X			X
<i>*Rosa rugosa</i> Thunb.	X						X	X				
<i>Rubus flagellaris</i> Willd.	X	X	X	X	X	X	X		X		X	
Rubiaceae												
<i>*Galium friflorum</i> Michx.												
<i>*Galium mollugo</i> L.						X	X					
Salicaceae												
<i>*Populus deltoides</i> Marshall	X	X		X	X	X		X	X	X		
<i>xPopulus grandidentata</i> Michx.				X	X	X	X			X		X
<i>*Populus x canescens</i> (Ait.) Sm. [alba x tremula]		X		X		X						
<i>*Salix babylonica</i> L.		X	X		X		X					
<i>Salix bebbiana</i> Sarg.				X								
<i>Salix discolor</i> Muhl.				X	X	X	X	X				
<i>Salix exigua</i> Nutt.					X							
<i>Salix nigra</i> Marshall				X								
Scrophulariaceae												
<i>Linaria canadensis</i> (L.) Dum-Cours.	X	X	X	X	X	X	X	X		X		X
<i>*Linaria dalmatica</i> (L.) Miller				X								
<i>*Linaria vulgaris</i> P. Miller	X	X	X	X	X	X	X					X
<i>*Verbascum blattaria</i> L.	X		X	X	X	X	X				X	
<i>*Verbascum thapsus</i> L.	X	X	X	X	X	X	X			X		X
<i>*Veronica arvensis</i> L.	X	X	X	X	X	X				X		
<i>Veronica serpyllifolia</i> L.	X		X			X						

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Simaroubaceae												
* <i>Ailanthus altissima</i> (P. Mill.) Swingle	x	x	x	x	x	x	x	x				x
Solanaceae												
<i>Datura stramonium</i> L.	x			x		x		x				x
* <i>Lycium barbarum</i> L.						x						x
* <i>Lypersicon esculentum</i> L.	x											
* <i>Petunia violacea</i> Lindl.												
* <i>Solanum dulcamara</i> L.	x	x	x	x	x	x		x	x	x		x
<i>Solanum carolinense</i> L.	x						x					
* <i>Solanum nigrum</i> L. var. <i>virginicum</i> L. [<i>S. americanum</i> Miller]			x	x		x						
Ulmaceae												
<i>Celtis occidentalis</i> L.				x	x	x						
* <i>Ulmus pumila</i> L.				X			X					
Tiliaceae												
<i>Tilia americana</i> L.							X					
Violaceae												
<i>Viola sororia</i> Wild. [<i>V. papilionacea</i> Pursh. <i>V. priceana</i>]												X
Vitaceae												
* <i>Ampelopsis brevipedunculata</i> (Maxim.) Trautv.					X		X			X		X
<i>Parthenocissus quinquefolia</i> (L.) Planch.	x	x		X	x	x	x	x	x	x		X
* <i>Parthenocissus tricuspidata</i> (Sieb. & Zucc.) Planch.							X					
<i>Vitis aestivalis</i> Michx.						X						
MAGNOLOPHYTA-LILIOPSIDA												
Agavaceae												
* <i>Yucca filamentosa</i> L. [<i>Y. flaccida</i> Haw.]								x				
Commelinaceae												
* <i>Commelina communis</i> L.		X		X	x		X	x				
<i>Tradescantia ohiensis</i> Raf.												
Cyperaceae												
<i>Carex pellita</i> Muhl.	x							x	x			
<i>Carex vulpinoidea</i> Michx. [<i>Carex annectans</i> (Bickn.) Bickn.]				x	x	x					x	
<i>Cyperus grayi</i> Torr.											x	
Cyperaceae												
<i>Cyperus lupulinus</i> (Sprengel) Marcks. ssp. <i>macilentus</i> Fern.												x
<i>Cyperus retrosus</i> Chapm.			X									X
<i>Cyperus schweinitzii</i> Torr. orr.	X	x										
<i>Cyperus strigosus</i> L.							X					
<i>Eleocharis parvula</i> (R. & S.) Link.	X			X			X	x				
[<i>Scirpus americanus</i> Pers.] <i>Scirpus pungens</i> Vahl				X								
<i>Scirpus robustus</i> Pursh												x
Iridaceae												
<i>Sisyrinchium augustifolium</i> P. Mill.							x					
Juncaceae												
<i>Juncus bufonius</i> L.		X	x	x			X	x				
<i>Juncus gerardi</i> Loisel		X	x	x		X	x	x	x			
<i>Juncus tenuis</i> Willd. var. <i>tenuis</i>	X	x		X	x	x	x	x	x			X
Liliaceae												
* <i>Allium vineale</i> L.	X	x		X	x	x	x	x	x	x		
* <i>Asparagus officinalis</i> L.	X	x	x	x		X		X				X
<i>Hemerocallis fulva</i> (L.) L.				X	x		X	x				X
Monocots												
* <i>Ornithogalum umbellatum</i> L.												
Poaceae												
* <i>Agrostis gigantea</i> Roth.	x							x				
<i>Agrostis perennans</i> (Walt.) Tucheran	x							x				
* <i>Aira caryophyllea</i> L.	x	x	x	x	x	x					x	

Species	Study Sites											
	1 DH	2 G	3 P	4 FC	5 S	6 H/B	7 BS	8 DP	9 BP	10 BC	11 AP	12 RB
<i>Aristida dichotoma</i> Chapman								x				
<i>Ammophila breviligulata</i> Fern.	x			x	x	x	x	x				x
<i>Andropogon gerardii</i> Vittman							x					
* <i>Bromus hordeaceus</i> L.	x											
* <i>Bromus inermis</i> Leysser.	x			x			x					
* <i>Bromus secalinus</i> L.			x	x		x				x		x
* <i>Bromus tectorum</i> L.	x		x	x	x	x				x		x
* <i>Calamagrostis epigejos</i> (L.) Pers.						x						
<i>Cenchrus incertus</i> M.A. Curtis	x		x		x	x						
* <i>Cynodon dactylon</i> (L.) Pers.		x	x	x								
* <i>Dactylus glomerata</i> L.	x	x	x	x	x	x	x	x	x	x		
* <i>Digitaria ischaemum</i> (Schreber) Muhl.	x					x	x	x				
<i>Distichlis spicata</i> (L.) Greene.	x	x	x	x	x	x	x	x		x	x	x
* <i>Echinochloa crus-galli</i> (L.) Beauv.				x	x		x					
* <i>Eleusine indica</i> (L.) Gaertn.				x	x	x	x					
* <i>Elytrigia repens</i> (L.) Nevski												
[<i>Agropyron repens</i> (L.) Beauv.]	x								x			
* <i>Eragrostis curvula</i> (Schrader) Nees.	x			x	x							x
<i>Eragrostis spectabilis</i> (Pursh) Steudel.	x			x								
<i>Eragrostis pectinacea</i> (Michx.) Nees. ex Steud.												
<i>Festuca arundinacea</i> Schreb.							x					
[<i>F. elatior</i> L. var. <i>arundinacea</i> (Schreb.) Wimmer.]												
* <i>Lolium perenne</i> L.	x											
<i>Muhlenbergia schreberi</i> J.F.Gmel.												
<i>Panicum accuminatum</i> Sw.	x	x	x			x						x
(sensu lato) [incl. <i>P. auburne</i> Ashe <i>P. leucothrix</i> Nash; <i>Dichantherium</i> <i>acuminatum</i> (Sw.) [Gould & Clark] <i>Panicum capillare</i> L.	x											
<i>Panicum dichotomiflorum</i> Michx.						x	x	x	x			
<i>Panicum lanuginosum</i> Elliott.							x					
<i>Panicum virgatum</i> L.	x	x		x	x	x	x	x	x		x	x
<i>Phragmites australis</i> (Cav.) Trin. [<i>P. communis</i> Trin.]	x	x	x	x	x	x	x	x	x	x	x	x
* <i>Poa annua</i> L.	x	x	x	x	x	x	x	x		x		
* <i>Poa compressa</i> L.	x											
* <i>Setaria glauca</i> (L.) Beauv.				x	x	x				x		
<i>Setaria viridis</i> (L.) Beauv.		x		x	x	x	x			x		
<i>Schizachyrium scoparium</i> (Michx.) Nash.	x	x	x	x	x	x	x	x	x			x
<i>Sorghastrum nutans</i> (L.) Nash							x					
<i>Spartina alterniflora</i> Loisel	x	x	x	x	x	x	x	x	x	x	x	x
<i>Spartina patens</i> (Ait.) Muhl.	x	x	x	x	x	x	x		x		x	x
<i>Tridens flavus</i> (L.) A.Hitchc. [<i>Triodia flava</i> (L.) Smyth.]	x	x	x	x	x	x	x	x				
<i>Triplasis purpurea</i> (Walter) Chapman	x	x		x		x						
<i>Tripsacum dactyloides</i> (L.) L.						x	x					
<i>Vulpia myuros</i> (L.) C.Gmelin	x											
<i>Vulpia octoflora</i> (Walt.) Rydb. [<i>Festuca octoflora</i> Walt.]												
Smilacaceae												
<i>Smilax rotundifolia</i> L.							x					

A Molecular Polymorphic Analysis Of Brassicaceae Outgroups For Use In Phylogeny Studies With *Arabidopsis thaliana*

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Abstract

A study was performed to examine the intraspecific distance relationships between *Arabidopsis thaliana* ecotypes and related brassicaceae species, as well as determine the optimum outgroups for use in future phylogenetic studies. Eleven populations of Brassicaceae species, related to *A. thaliana*, were studied using polymorphic DNA analyses. Experiments employed 15 microsatellite loci in 10 individual plants from each population. Microsatellite loci were amplified by PCR, electrophoresed and polymorphisms analyzed. The species *A. himalaica*, *A. griffithiana*, *Capsella bursa-pastoris*, *A. pumila* and *Draba nemoroza* were found to be too genetically distant from *A. thaliana* and not suitable as outgroups. *A. korshinskyi*, *A. lyrata*, *Cardamine flexuosa* and *Lepidium virginicum* were found to be genetically close to *A. thaliana* and potentially useful as outgroups. Conversely, *A. suecica* and *A. wallichii* were found to be too genetically similar to *A. thaliana* and impractical as outgroups. All outgroups, except *A. suecica*, demonstrated the presence of null alleles for the microsatellite markers employed; *A. himalaica* and *A. griffithiana* had the largest number of null alleles.

INTRODUCTION

Microsatellite loci have been successfully used to reconstruct intraspecific phylogenetic relationships in *Arabidopsis thaliana*^{1,2,3,4,5}. Unfortunately, these studies consistently have not included the use of an outgroup species for comparison to the intraspecific populations. In this paper, we report on a study to find an appropriate outgroup for use in intraspecific polymorphic analyses of microsatellite sequences with *A. thaliana*.

A. thaliana has become the scientific community's most common system for the study of physiology, genetics, and development of higher plants. Reasons for the continued popularity of *A. thaliana* are its short growth and seed-bearing cycle (~6 wk), small physical size, low chromosome number (n=5), small genome size (~120 Mb), almost non-repetitive DNA, numerous genetic markers and the recent genomic sequencing^{6,7,8}. *A. thaliana* is a member of the Brassicaceae family which grows wild as a winter annual in most temperate zones of the world.

Because *A. thaliana* has become such a

popular system of study, plant biologists need to have a better understanding of the relationships between ecological populations (ecotypes) of the plant throughout the world. Polymorphic microsatellite sequences⁹ have been a great benefit to the study of *Arabidopsis* phylogeny due to their consistently high levels of intraspecific variability. Researchers have taken advantage of these markers to create elaborate cladograms using most of the available *A. thaliana* populations. Unfortunately, a deficiency exists in many of the recently published studies in this area^{1,2,3,4,5}, because the intraspecific genetic distance of the *A. thaliana* species was not compared to a baseline outgroup.

Without the appropriate outgroups employed in the studies, their overall reliability is called into question. In order to clarify relationships between *A. thaliana* ecotypes, an outgroup comparison should be required. However, few studies have been performed to characterize appropriate Brassicaceae outgroups. Several surveys have examined *Arabidopsis* interspecific genetic distance^{10,11,12,13,14,15}, but most did not do so in a molecular context using

microsatellite polymorphisms. Clauss et al.¹¹ and Van Treuren et al.¹⁵ did utilize microsatellites to perform such interspecific studies, but they limited their analysis to *A. thaliana* outcrossing relatives *Arabis petraea*, *Arabis lyrata* and *Arabis halleri*.

By definition, an outgroup is assumed to retain the ancestral state of the ingroups. If no outgroup is used in a genetic distance analysis, it can not be clearly determined whether the characteristics used in an analysis were present at an earlier state of evolution. In this instance, an appropriate *Arabidopsis* outgroup is necessary in order to best systematize intraspecific ecotypes on a rooted tree.

The present study has examined eleven of the most appropriate outgroup species. The basis for selection of potential outgroups in this inquiry came from an examination of the literature. A number of genetically close species to *A. thaliana* have been described in the literature^{10,13,16,17}.

Once the Brassicaceae outgroup species were chosen for analysis, genetic distance comparisons were made among the various outgroups relative to their appropriate distance from our *A. thaliana* ecotypic populations examined in Vander Zwan et al.⁴.

MATERIALS AND METHODS

Plants and plant growth

A. griffithiana and *A.* (Table 1) were obtained from Randy Scholl (Ohio State University, Columbus, Ohio, USA). *A. lyrata* was donated by Charles Langley, University of California at Davis. All other species (Table 1) were obtained from Nobuharu Goto (Miyagi University of Education, Sendai, Japan). All seedlings were germinated under sterile conditions and grown under the methods previously described⁴.

DNA extraction

Ten individuals from each of the 11 populations were examined for the variation of microsatellites among populations for a total of ~110 plants. The DNA was extracted from 0.2-0.8 g of plant tissue grown 14 to 28 days of age. The tissue was frozen overnight at -70°C and homogenized in an ice-cooled mortar and pestle. The cetyltrimethyl ammonium bromide (CTAB) method⁴ was used for DNA extraction.

Polymerase chain reaction and detection of polymorphisms

Fifteen PCR Simple Sequence Length Polymorphism (SSLP) primer sets (Table 2) were purchased from Research Genetics, Inc. and used to amplify microsatellite loci from plant samples⁹. The

PCR amplification conditions principally followed the directions of Bell and Ecker⁹. All PCR amplification was performed in a Mastercycler gradient thermocycler (Eppendorf, Inc.). DNA fragments were then electrophoretically separated and analyzed by the method of Todokoro et al.¹. The PCR products were imaged with Scion computer software (Scion, Inc.). Molecular weights were analyzed and calculated with Collage Version 4.0 (Image Dynamics Corporation). The raw DNA allele lengths were used in distance analyses of the various populations. The source of the *A. thaliana* polymorphic data used to construct the pairwise trees was our earlier publication⁴.

Distance relationships were ascertained among the various populations using several computer software packages. Microsat⁴³ was used to calculate microsatellite-based genetic distances between populations: both Ddm and Dad distances. These genetic distances were then evaluated with Phylip²⁸ to calculate neighbor-joining relationships between populations. The neighbor-joining data generated by Phylip was formed into a graphic tree by Treeview⁴⁴. Bootstrapping was performed using Microsat and analyzed by Phylip. An arbitrary cut-off value was used for inclusion of any particular population in the neighbor-joining tree analysis. A minimum of eight of the fifteen microsatellite markers had to be amplified for inclusion in the phylogenetic trees, although all populations were included in the overall analysis. This limitation was employed to ensure accuracy in any neighbor-joining trees generated.

An unrooted, parsimonious tree was produced by the Phylip subroutine Clique²⁸, using a two-state character analysis. The two character states were determined by the presence or absence of primer annealing sites that allowed any amplification of the SSLP polymorphisms by PCR. This type of analysis allowed us to include all the studied species in a compatibility-based comparison.

RESULTS

Microsatellite variation among the outgroups

Ten plants from each of the 11 various species were examined by 15 microsatellite loci. Microsatellites were amplified successfully by PCR in 124 out of 165 locus-individual combinations tested. A single, major PCR product was obtained in 98 of those 165 combinations tested (Table 2). In those species/marker combinations in which there were multiple products of similar amplification magnitude, those alleles were not used in the pairwise analyses, although this amplification data was employed for the parsimonious analysis.

The average standard deviation for the allele

TABLE 1. Brassicaceae populations examined as outgroups.

*Seed source is Randy Scholl, Ohio State University. †Seed source is Nobuharu Goto, Miyagi University of Education, Japan.

‡Seed source is Charles Langley, University of California at Davis.

Species	Geographic Source
<i>Arabidopsis griffithiana</i> *	Tajikistan, Central Asia
<i>Arabidopsis himalaica</i> *	China
<i>Arabidopsis korshinskyi</i> †	Tajikistan, Central Asia
<i>Arabidopsis lyrata</i> ‡	McClendon Bluffs, North Carolina, USA
<i>Arabidopsis pumila</i> †	?
<i>Arabidopsis suecica</i> †	Finland, Northern Europe
<i>Arabidopsis wallichii</i> †	?
<i>Capsella bursa-pastoris</i> †	Guatemala, South America
<i>Cardamine flexuosa</i> †	Sendai, Japan
<i>Draba nemoroza</i> †	Sendai, Japan
<i>Lepidium virginicum</i> †	Sendai, Japan

size differences at each locus for each population examined was 1.61; although *A. wallichii*, *A. korshinskyi*, *A. pumila*, *C. flexuosa*, *D. nemoroza*, and *L. virginicum* demonstrated no intra-population variation with the markers examined. The remaining five populations all indicated some level of intra-population allelic differences.

The sizes of the PCR-amplified fragments of the 15 loci from the 11 species are shown in Table 2. All the loci were highly polymorphic across the 11 species. The number of alleles observed at each locus ranged from 2 to 9 with a mean of 5.2 (Table 3). Gene diversity was calculated using the method of Nei¹⁸.

Relative outgroup distances from *Arabidopsis thaliana*.

Some of our results supported conclusions of previously published research. The great genetic distance of the species *A. himalaica* and *A. griffithiana* from *A. thaliana* was demonstrated by the fact that so few of the markers used were able to amplify at all (Table 2). This result supports the recent conclusion that *himalaica* and *griffithiana* are no longer thought to be in the genus *Arabidopsis* at all and have now been assigned to the genera *Crucihimalaya* and *Olimarabidopsis*, respectively¹⁷. Also unsurprising was the data supporting a close relationship between *A. thaliana* and *A. suecica* (Fig. 1A, 1B). *A. suecica* is believed to have a genome derived through interspecies hybridization from *A. thaliana*¹⁹, so common genetic elements are to be expected.

A. wallichii appears to be closely related to *A.*

suecica (Fig. 1). The *wallichii* species may also have a genome that is the result of hybridization with *A. thaliana*, but the literature does not suggest whether this is actually the case²⁰.

Based on weak bootstrapping scores (~42%), the *A. korshinskyi* species also appears to be genetically near to *A. thaliana*. Although weak, the same level of bootstrapping is found in both neighboring trees (Fig. 1A and 1B). The *A. lyrata* species is believed to be one of the closest relatives to *A. thaliana* of the North American-Eurasian species^{11,13,14,15}. Our microsatellite data supports this conclusion and also suggests that *A. lyrata* may be one of the best candidates to act as an outgroup species in *A. thaliana* studies. Bootstrapping scores, using both the square mean difference and absolute difference calculations, support this result with values of over 50% in both trees (Fig. 1A and 1B). Our result is further supported by the data of both Van Treuren et al.¹¹ and Clauss et al.¹⁵ who obtained comparable results in their more limited studies.

The *Arabidopsis-Capsella* separation is estimated to have occurred six to ten million years ago²¹, so it is not surprising that *Capsella bursa-pastoris* is too distant to act as an outgroup for *A. thaliana*. The *Draba* genus is equally distant and, if *D. nemoroza* is a typical example, not acceptable for use as an *Arabidopsis* outgroup.

A. pumila appears to be so distant from *A. thaliana* that the question arises of whether it has been correctly categorized into the *Arabidopsis* genus. Perhaps, like *A. griffithiana* and *A. himalaica*, its systematics need to be re-examined. A n

TABLE 2. Size of PCR products (in bp) for 15 microsatellite loci from eleven Brassicaceae populations.
N/A=Not Amplified by primer used.
M = multiple alleles present.

Alleles	<i>A. lyrata</i>	<i>A. wallichii</i>	<i>A. korshinskyi</i>	<i>A. griffithiana</i>	<i>A. himalaica</i>	<i>A. suecica</i>	<i>A. pumila</i>	<i>C. flexuosa</i>	<i>C. bursa-pastoris</i>	<i>D. nemoroza</i>	<i>L. virginicum</i>
Nga158	90.0	101.0	N/A	128.0+4.1	N/A	111.0	680.0	M	135.0	M	109.0
Nga59	110.0	124.0	110.0	N/A	N/A	125.8+7.0	400.0	M	135.0	465.0	110.0
Nga168	146.0	131.0	134.0	141.0+3.0	N/A	120.0	146.0	115.0	124.0	115.0	146.0
ATHCHIB	N/A	N/A	76.0	N/A	N/A	51.6+7.0	N/A	N/A	N/A	N/A	N/A
Nga225	100.0	100.0	82.0	N/A	N/A	100.0	M	M	M	M	M
Nga8	M	M	152.0	289.0+8.0	N/A	185.0	M	95.0	M	M	95.0
Nga162	90.0	111.0	90.0	79.4+0.7	N/A	90.0	82.5+6.9	N/A	N/A	N/A	40.0
Nga151	100.0	100.0	141.0	N/A	N/A	134.0	M	50.0	100.0	100.0	100.0
Ca72	M	N/A	260.0	221.5+2.3	217.0+2.5	225.0	200.0	188.0	M	M	68.0
Nga172	60.0+5	152.0	160.0	N/A	N/A	160.0	M	45.0	M	M	M
Nga1145	220.0	200.0	228.0	N/A	N/A	220.0	228.0	252.0	238.0	1500.0	173.0
Nga707	M	123.0	622.0	N/A	N/A	132.0	M	112.0	M	M	46.0
ATHACS	46.0	275.0	280.0	N/A	N/A	268.0	N/A	50.0	N/A	995.0	50.0
Nga32	240.0	250.0	262.0	N/A	N/A	250.0	M	50.0	N/A	M	50.0
Nga1107	N/A	153.0	135.0	131.6+0.7	135.4+0.6	138.0	M	234.0	66.3+76.0	400.0	50.0

TABLE 3. Summary of microsatellite polymorphisms in Brassicaceae populations studied.

SEM= Standard Error of the Mean.

Loci	Number of Alleles	Mean		Gene Diversity
		Allele Size (bp)	SEM of Allele Size	
ATHACS	6	280.5	47.8	0.702
ATHCHIB	2	63.8	8.6	0.569
ca72	7	197.0	8.7	1.000
nga8	4	163.2	14.3	1.000
nga32	4	183.6	15.7	0.871
nga59	6	186.5	15.6	0.962
nga151	4	103.1	3.4	1.000
nga158	7	193.4	28.4	1.000
nga162	5	83.2	3.0	0.900
nga168	7	131.8	1.2	1.000
nga172	4	115.4	11.5	0.936
nga225	2	95.5	2.2	1.000
nga707	5	207.0	41.9	0.835
nga1107	9	166.8	9.3	0.975
nga1145	7	362.1	47.4	1.000

unexpected result is that *C. flexuosa* and *L. virginicum* do not appear to be as distantly related to *A. thaliana* as one may have predicted. Studies, using *rbcl* polymorphic sequence data²², suggest that the *Lepidium* genus is in the same clade as *A. thaliana*, but the *Cardamine* genus falls into a separate clade. Nevertheless, although they are the most distant species to be included on the neighbor-joining tree, they are not distant from the *A. lyrata* clade (Fig. 1A, 1B).

In this present study the square mean difference^{23,24} and absolute difference^{23,24,25} calculations do not appear to give results of similar utility. The Ddm, whose calculation is based on the stepwise mutation hypothesis, seems to give a more robust tree topology than the DAD calculation. This conclusion is based on the comparable number of branches with and without bootstrapping scores above

40% in each neighbor-joining tree (Fig. 1A, 1B).

A parsimonious analysis of species similarity

One weakness in the neighbor-joining analysis is that it does not include the data for the analyzed species that possess an abundance of null alleles and/or multiple alleles, however there is additional phylogenetic information to be evaluated in the probability of positive or negative amplification of SSLP loci. If primer annealing sites flanking polymorphic loci are altered so PCR amplification can not take place, or alternatively if multiple binding sites develop for various primers, this suggests that evolutionary changes are occurring in the genome. Both null alleles and multiple alleles²⁶ are difficult to include in a standard examination using neighbor-joining analysis, so we turned to a compatibility method of comparison^{27,28}.

Characters were scored as being present (1) or absent (0) based on primer annealing regions being present and allowing PCR amplification. Using this system, both null alleles (0) and multiple alleles (1) could be scored for each locus. The results of this method (Fig. 2) separate the eleven *Brassicaceae* populations on a rootless tree along with *A. thaliana*. The tree possesses a gradient of genetic distance, where at its distal end are the species least similar to *A. thaliana* (*A. griffithiana* and *A. himalaica*) and at its proximal end are those most similar (*A. suecica*, *A. korshinskyi*, and *A. lyrata*).

DISCUSSION

The null allele and multiple allele questions.

A troubling aspect of the analyses performed in this study is the number of “null alleles” found. Null alleles are defined as those that do not amplify by PCR^{29,30,31,32}. Such alleles have been found in a variety of eucaryotic taxa^{29,30,33,34,35,36,37} and the phenomenon does not appear to be an uncommon one, but what are the real implications of not obtaining PCR amplification for various microsatellite markers in a particular outgroup? Lack of microsatellite amplification is not generally a problem in *A. thaliana* when conducting intraspecific studies. It is common^{1,3,4,5} to find that a small percentage (5-10%) of SSLP markers that do not amplify. Because they are a small proportion of the total, researchers commonly ignore null allele markers and do not incorporate them into analyses since their inclusion as a length of zero basepairs would skew the final genetic distance. Such treatment follows from Wiens’ analysis of various difficulties in polymorphic studies²⁶, where he suggests that null alleles may not be used as evidence to group polymorphic species because they are essentially uninformative. Indeed, here we have also ignored null alleles for pairwise analysis, though this may equally be leading to distortions of genetic distances.

There are three possible sources for a lack of PCR amplification of microsatellite sequences^{29,30,31,32}. First, the sequences flanking the microsatellite repeats of interest may have been mutated or deleted over evolutionary time. This would leave the microsatellite insert present, but not detectable by PCR. Second, the flanking sequences may be present and unaltered over time, but the intervening microsatellite region may be so small as to remain essentially undetectable. Third, both flanking and microsatellite sequences may no longer be in the population at all due to great evolutionary divergence.

The second and third conditions may be defined as “allelic drop-outs”³¹ since the allele is undetectable because it is not present. It is only under these conditions that a value of zero basepairs may

legitimately be used to describe the examined locus.

Given the first condition of an undetectable, but present, insert, the choice is either ignore the data in favor of a different marker or somehow detect the insert size for inclusion. If the data in this situation is ignored, it again skews the result and creates a potential inaccuracy. If the microsatellite sequence was originally detected as an RFLP, then this may be an alternative method of size detection, although most SSLP sequences can only be detected by PCR.

This becomes a technical problem of detecting the presence of both the small flanking sequences of the priming sites as well as the actual microsatellite region. DNA sequencing may be employed to determine whether the flanking primer sequences are still present when the microsatellite region itself is absent.

If one did not detect the presence of the flanking sequences using DNA sequencing methods, it does not necessarily indicate the additional loss of the intervening polymorphic microsatellite region. If the flanking primer sequences themselves have altered or been deleted over time, DNA sequencing primers would not anneal and could not probe the region. Chambers and MacAvoy³² call null alleles “ghosts” since they exist, but are invisible to standard detection. They suggest trying to detect ghost markers with redesigned primers (called “ghost busters”) to match other parts of the flanking regions.

Another concern brought to light in our study is that, in many of the species examined, we consistently detected multiple DNA fragments of varying sizes being amplified with microsatellite primers. The obvious implication is that the primers are annealing to additional priming sites besides those predicted. The most likely source of the additional amplified fragments may be multiple allelic copies of the same microsatellite sequences within populations. These multiple alleles may have originated in intraspecific hybridization of populations. Heteroplasmy would have resulted if there were high levels of outcrossing concomitant with mutation. It is interesting to note that multiple alleles in microsatellite sequences are not observed in natural populations of *A. thaliana* which has very low outcrossing levels^{1,2,3,4,5}.

Another source of the multiple amplified loci may be allopolyploidy in the various species. Several of the species examined in this study are allopolyploids and hence may have multiple alleles of the SSLP priming sites. *A. suecica* is a known allotetraploid thought to be derived from an interspecific hybridization between *A. thaliana* and *A. arenosa*¹⁹. *Capsella bursa-pastoris*, *A. griffithiana*, *C. flexuosa*, *L. virginicum* and *A. pumila* are also allotetraploid species^{13,14,38,39,40}. *A. korshinskyi* is an allohexaploid species²⁰.

A. lyrata, *A. wallichii*, *A. himalaica* and *D. nemoroza* are diploid species^{13,14,20,41,42} so

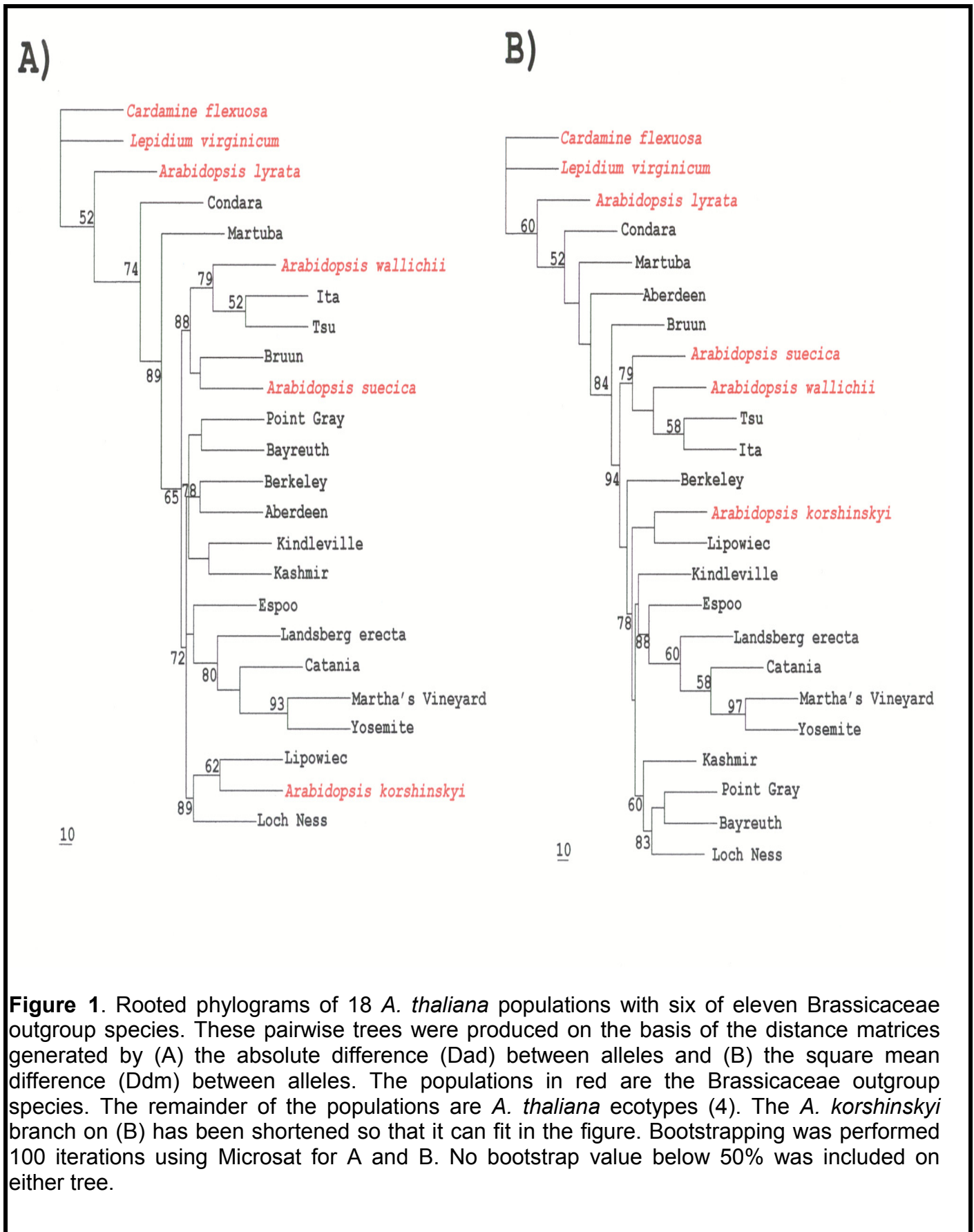


Figure 1. Rooted phylograms of 18 *A. thaliana* populations with six of eleven Brassicaceae outgroup species. These pairwise trees were produced on the basis of the distance matrices generated by (A) the absolute difference (Dad) between alleles and (B) the square mean difference (Ddm) between alleles. The populations in red are the Brassicaceae outgroup species. The remainder of the populations are *A. thaliana* ecotypes (4). The *A. korshinskyi* branch on (B) has been shortened so that it can fit in the figure. Bootstrapping was performed 100 iterations using Microsat for A and B. No bootstrap value below 50% was included on either tree.

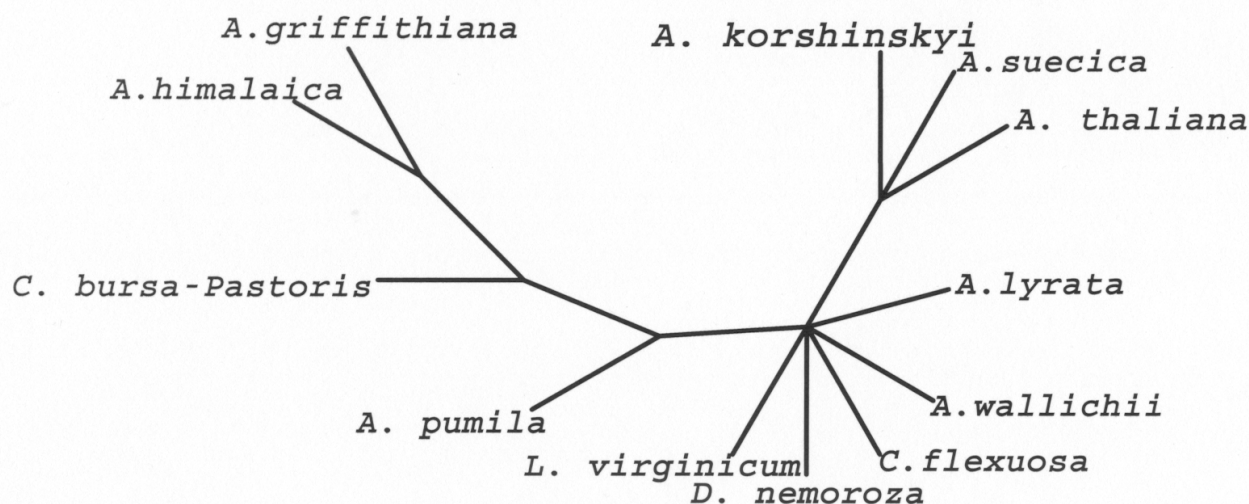


Figure 2. Unrooted radial tree of eleven Brassicaceae species along with *Arabidopsis thaliana*. This tree was produced using a parsimonious compatibility method to analyze the presence or absence of PCR amplification at each polymorphic locus. The data was analyzed by the Phylip subroutine Clique, and the tree was produced by Treeview.

allopolyploidy would not account for multiple alleles in these species. Three of these populations show few (*A. lyrata* and *A. wallichii*) or no (*A. himalaica*) multiple alleles, and only *A. pumila* displays a large number of these multiple allelic loci (six) (Table 2).

In our neighbor-joining analyses, we omitted multiple alleles from our genetic distance calculations. Unfortunately, this strategy poses the same difficulties as those stated earlier with the null allele problem. If there are data that can be extracted from the multiple allele situation, then ignoring that data only leads to inaccurate conclusions.

Using a method suggested by Wiens²⁶, we attempted to solve both the null and multiple allele problems by performing a cladistic analysis that examines the DNA polymorphisms on a two-state character basis. The method assumes that if there is any amplification of polymorphic primers, then species that have a greater majority of common amplifying polymorphic loci are more closely related. This type of test incorporates both the null alleles and multiple alleles and provides an important phylogenetic result. On the basis of this result (Fig. 2) *A. Korshinskyi* and *A. suecica* are in a "clique" with *A. thaliana* and may not be useful as outgroups; while the more distant *A. lyrata*, *A. wallichii*, *C. Flexuosa*, *D. nemoroza*, and *L. Virginicum* may be more appropriate outgroups for *thaliana* studies.

A Final Conclusion

Although very helpful in gene mapping and intraspecific distance studies, microsatellite polymorphisms have limited utility in determining interspecific distance. The markers, although highly polymorphic within a species, are not always accessible for analysis in related species. This lack of accessibility is problematic in some applications. Microsatellite markers were specifically employed in this study because we were endeavoring to find an interspecific outgroup that could be employed for comparative purposes with *A. thaliana*. It is clear that species-specific microsatellite markers have little utility in examination of other species. However, it must be emphasized that the method adopted here for distance analysis was the only technique that could be employed. If any other types of markers or genome comparison methods had been utilized, they would have invalidated the point of the study, which was to find an outgroup species that could be employed specifically with microsatellite markers. Despite the drawbacks inherent in the use of microsatellite polymorphisms, we were able to successfully find several species that may be used as outgroups in future interspecific studies of *A. thaliana*.

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Additional Abstracts from the MACUB Conference

Survey of Bacteriocin Activity in *Enterococcus* spp. Isolated From Canada Geese (*Branta canadensis*). Duong, D., J. Muscolino and J. Middleton. Dept. Biology, Fairleigh Dickinson University, Madison, NJ.

Bacteriocins are proteins produced by certain bacteria that inhibit or kill closely related strains and, occasionally, other species of bacteria. We were interested in determining whether the fecal enterococci of geese (*Branta canadensis*) produced bacteriocins and, if so, examining the activity spectrum of these proteins. Fresh feces from resident Canada geese were collected at Loantaka Brook Reservation in Morris County, NJ during June, July and August, 2003. Thermotolerant *Enterococcus* strains were cultured from the feces by standard techniques and screened against a panel of 10 antibiotics. Unique enterococcal strains (n=264) were selected on the basis of pigmentation, antibiotic resistance patterns, and colonial morphology. Isolates were screened for bacteriocin production by the method of Lauková *et al.* (Lett. Appl. Microbiol, 1998). Isolates were evaluated against other enterococci isolated from the same and from different goose fecal samples and against both G+ and G- reference strains. Bacteriocins were detected in 74 isolates and 23 different bacteriocin activity patterns were observed. We isolated 6 enterococcal strains that inhibited *S.aureus*, 3 that inhibited *E.cloacae* and 65 strains that inhibited only other *Enterococcus* strains. Reciprocal crosses were used to distinguish between bacteriocin producing strains and strains sensitive or resistant to each bacteriocin. Two global indicator strains were identified by the reciprocal cross method. Bacteriocin producing strains were identified as *E.casseliflavus*, *E. mundtii*, *E.faecium*, *E.faecalis* and *E. sulfureus*.

Investigation on Microbial Pollution Indicators in Jamaica Bay. May Antoine, Farah Jean, Samuel Greenidge, Carolle Bolnet Ph.D. Medgar Evers College. Faculty mentor: Dr. Carolle Bolnet.

Jamaica Bay is a popular recreational area in which many people fish and swim. This study made a water quality test at the Gateway National Park Marina in Rockaway and the Kingsborough Community College Marina in Sheepshead Bay for the presence of coliforms and fecal coliform bacteria, important indicators for the health of the environment. Although these bacteria rarely cause disease, their presence is an indicator for other disease causing agents including those responsible for typhoid, hepatitis A, cholera, and are indicators of the general health of the waters. Using multiple tube and membrane filter methods and selective and differential media, we obtained significant differences between the two sites: 7 coliforms/100ml sample was found at the Gateway Marina, but 1,100-coliforms/100 ml sample was found at Kingsborough Marina, much higher than the US standard, indicating caution in consuming fish and shellfish from the area. Through fingerprinting technique, we plan to differentiate human from non-human fecal coliform in order to determine the origin of the pollution at the Kingsborough site. This work was supported by grant 051601105 of the CSTEP of NYS Dept. of Education. This work was also supported by grant 020-39-66 of the UMEB Program of the NSF.

McGraw-Hill/MACUB Research Award

The Metropolitan Association of College and University Biologists is pleased to announce the **McGraw-Hill/MACUB Research Award**. This Annual award, with a maximum of \$1,000, sponsored in part through the generosity of the McGraw-Hill Publishing Company, is to be used to fund a research project in the area of biology or collegiate biology education that involves students as active participants. Any project funded by a **McGraw-Hill/MACUB Research Award**, must be led by, and supervised by a college faculty member who is a current, active member of MACUB.

Eligibility for the McGraw-Hill/MACUB Research Award:

All applicants for a **McGraw-Hill/MACUB Research Award**, and all students who will participate in the project funded by this award, must be current, active members of the Metropolitan Association of College and University Biologists at the time of application and announcement of the award. It should be noted that members of the Executive board and/or Awards Committee may apply for this award. However, if an applicant is a member of the Executive Board and/or Awards Committee, he or she shall not take part in the deliberations or the selection procedure for this award.

Application Procedure:

Applications for the **McGraw-Hill/MACUB Research Award** shall consist of:

- a. A cover page including the name(s) and affiliations(s) of the faculty applicant(s), and the title of the project;
- b. A brief description (not to exceed 4 pages, single spaced using 12 pt. type and 2.5 cm. margins) of the project, need for funding, and how the students will be involved in the project;
- c. A detailed project budget;
- d. A brief vita of the faculty applicant(s).

Deadline for Submission:

All applications for the 2004-2005 **McGraw-Hill/MACUB Research Award** must be received by July 1, 2004 to be considered. Send applications to:

Dr. Anthony DePass, Awards Chair
Department of Biology
Long Island University
1 University Plaza
Brooklyn, New York 11201-5372

Obligations of Recipients of Award:

It shall be the obligation of the award recipient(s) to:

Submit a report of how the funds were used and the status of the research funded by September 1, 2005.

Arrange for student participant in the project give a presentation of the funded research at the 38th Annual MACUB Conference.

Acknowledge the **McGraw-Hill/MACUB Research Award** in any publication that result fully, or in part, from research funded by this award.

Announcement and presentation of **McGraw-Hill/MACUB Research Award**, recommended by the Awards Committee, and awarded at the sole discretion of the Executive Board of MACUB shall be made at the 37th Annual MACUB Conference. In the event that no worthy applications are received, no award will be made.

CALL FOR NOMINATIONS

The terms of office for the following positions will be up for re-election to serve on the 2005 - 2006 Executive Board:

President
Treasurer
Corresponding Secretary
Member-at-Large (2 positions)

The duties of these officers will involve attending all Executive Board meetings in addition to specific duties as described below:

President: Presides over the annual business meeting of the Association; serves as the Chairperson of the Executive Board; appoints and charges, with the approval of the Executive Board, the chairperson and members of all committees; and carries out other activities usually pertaining to the office.

Treasurer: is responsible for the preparation of an annual fiscal report, processing of dues, preparing regular financial reports for the Executive Board meetings, income tax reports and other duties pertaining to this office.

Corresponding Secretary: shall receive and validate applications for membership, respond to all inquiries, assemble and update the directory, and any other duties that pertain to this office.

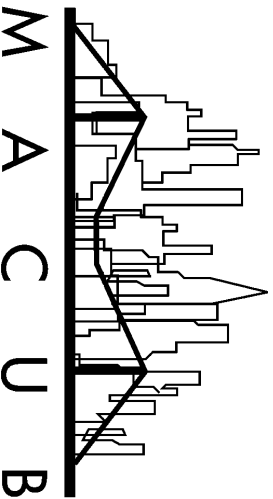
Members-at Large Chair committees (Articulation, Exhibition, etc.) and handles other assignments as directed by the Executive Board.

Normally, each candidate for President, Treasurer and Corresponding Secretary should have been a Member-at-Large for at least one term and each candidate for Member-at-Large should have attended at least on Annual Conference and be a member in good standing at the time of nomination.

DEADLINE FOR NOMINATIONS is July 30, 2004

If you are interested in running for office (or wish to nominate anyone else), please send a letter of nomination to:

Dr. Margaret Carroll
Department of Biology
Medgar Evers College
1150 Carroll Street
Brooklyn, New York 11225



Dr. Edward J. Catapane
Department of Biology
Medgar Evers College
1150 Carroll Street
Brooklyn, New York 11225

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