State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
Post Office Box 50130
Charleston, WV 25305-0130

Solicitation

NUMBER	PAGE
DNR214058	1

ADDRESS CORRESPONDENCE TO ATTENTION OF:
DEAN WINGERD
304-558-0468

RFQ COPY

VENDOR

West Virginia University
Divison of Forestry And Natural Resources
PO Box 6001
Morgantown, WV 26506-6001

SHIP TO

DIVISION OF NATURAL RESOURCES
PROCUREMENT OFFICE

324 4TH AVENUE
SOUTH CHARLESTON, WV
25303-1228 304-558-3397

DATE PRINTED: 02/19/2014

BID OPENING DATE: 03/20/2014 BID OPENING TIME 1:30PM

LINE	QUANTITY	UOP	CAT. NO.	ITEM NUMBER	UNIT PRICE	AMOUNT

THE WEST VIRGINIA PURCHASING DIVISION FOR THE AGENCY, WV DIVISION OF NATURAL RESOURCES, IS SOLICITING BIDS TO PROVIDE A PH.D. STUDENT TO ANALYZE A MAJOR GENETIC PROJECT ON WHITE-TAILED DEER IN WEST VIRGINIA, PER THE ATTACHED SPECIFICATIONS.

ATTACHMENTS INCLUDE:

1. INSTRUCTIONS TO VENDORS SUBMITTING BIDS.
2. GENERAL TERMS AND CONDITIONS.
3. DNR214058 SPECIFICATIONS.
4. CERTIFICATION AND SIGNATURE PAGE.
5. PURCHASING AFFIDAVIT.
6. RESIDENT VENDOR PREFERENCE (RVP) FORM.

0001	1	LS		956-70		

ANALYZE GENETIC PROJECT ON WHITE-TAILED DEER

****** THIS IS THE END OF RFQ DNR214058 ****** TOTAL:

03/19/14 08:57:24AM
West Virginia Purchasing Division

SIGNATURE Mary Jean Buckland TELEPHONE 304-293-3998 DATE 3/18/14

TITLE Interim Director FEIN 556000842 ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO SOLICITATION, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

State of West Virginia
Department of Administration
Purchasing Division
2019 Washington Street East
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Charleston, WV 25305-0130

Solicitation

NUMBER	PAGE
DNR214058	1

ADDRESS CORRESPONDENCE TO ATTENTION OF:
DEAN WINGERD
304-558-0468

VENDOR
RFQ COPY
TYPE NAME/ADDRESS HERE

SHIP TO
DIVISION OF NATURAL RESOURCES
PROCUREMENT OFFICE

324 4TH AVENUE
SOUTH CHARLESTON, WV
25303-1228 304-558-3397

DATE PRINTED: 03/11/2014

BID OPENING DATE: 03/20/2014 BID OPENING TIME 1:30PM

LINE	QUANTITY	UOP	CAT NO.	ITEM NUMBER	UNIT PRICE	AMOUNT

ADDENDUM NO. 1

ADDENDUM IS ISSUED:

1. TO PROVIDE RESPONSES TO VENDORS' QUESTIONS REGARDING THE ABOVE SOLICITATION. QUESTION AND ANSWER PAGES ARE ATTACHED.

2. TO PROVIDE ADDENDUM ACKNOWLEDGMENT. THIS DOCUMENT SHOULD BE SIGNED AND RETURNED WITH YOUR BID. FAILURE TO SIGN AND RETURN MAY RESULT IN THE DISQUALIFICATION OF YOUR BID.

********** END OF ADDENDUM NO.1 **********

SIGNATURE: Mary Jane Buckland TELEPHONE: 304-293-3998 DATE: 3/18/14

TITLE: Interim Director FEIN: 556000842 ADDRESS CHANGES TO BE NOTED ABOVE

WHEN RESPONDING TO SOLICITATION, INSERT NAME AND ADDRESS IN SPACE ABOVE LABELED 'VENDOR'

SOLICITATION NUMBER: DNR214058
Addendum Number: 1

The purpose of this addendum is to modify the solicitation identified as ("Solicitation") to reflect the change(s) identified and described below.

Applicable Addendum Category:

[ ] Modify bid opening date and time

[ ] Modify specifications of product or service being sought

[✓] Attachment of vendor questions and responses

[ ] Attachment of pre-bid sign-in sheet

[ ] Correction of error

[ ] Other

Description of Modification to Solicitation:

1. To provide copy of vendor questions and responses.
2. To provide Addendum Acknowledgment form.

Additional Documentation: Documentation related to this Addendum (if any) has been included herewith as Attachment A and is specifically incorporated herein by reference.

Terms and Conditions:

1. All provisions of the Solicitation and other addenda not modified herein shall remain in full force and effect.

2. Vendor should acknowledge receipt of all addenda issued for this Solicitation by completing an Addendum Acknowledgment, a copy of which is included herewith. Failure to acknowledge addenda may result in bid disqualification. The addendum acknowledgement should be submitted with the bid to expedite document processing.

ADDENDUM ACKNOWLEDGEMENT FORM
SOLICITATION NO.: DNR214058

Instructions: Please acknowledge receipt of all addenda issued with this solicitation by completing this addendum acknowledgment form. Check the box next to each addendum received and sign below. Failure to acknowledge addenda may result in bid disqualification.

Acknowledgment: I hereby acknowledge receipt of the following addenda and have made the necessary revisions to my proposal, plans and/or specification, etc.

Addendum Numbers Received:
(Check the box next to each addendum received)

[✓]	Addendum No. 1	[ ]	Addendum No. 6
[ ]	Addendum No. 2	[ ]	Addendum No. 7
[ ]	Addendum No. 3	[ ]	Addendum No. 8
[ ]	Addendum No. 4	[ ]	Addendum No. 9
[ ]	Addendum No. 5	[ ]	Addendum No. 10

I understand that failure to confirm the receipt of addenda may be cause for rejection of this bid. I further understand that any verbal representation made or assumed to be made during any oral discussion held between Vendor's representatives and any state personnel is not binding. Only the information issued in writing and added to the specifications by an official addendum is binding.

West Virginia University
Company

Authorized Signature

3/18/14
Date

NOTE: This addendum acknowledgement should be submitted with the bid to expedite document processing.
Revised 6/8/2012

ATTACHMENT A

DNR214058

Technical Questions

Q. 1. Section 3.1, page 20: What form of documentation do you need to confirm our university offers a Ph.D.? Does this require a statement on official university letterhead or would printing online information to prospective students suffice?

A. 1. Printing of online information to prospective students will suffice or bidders may copy their academic handbook or get a letter on official university letterhead.

Q. 2. Section 3.4, page 20: What form of documentation is necessary to be a "GIS Specialist." Does this require a university-granted "GIS Certificate" or would a researcher's manuscripts using GIS and years of experience in data analysis suffice?

A. 2. A researcher's manuscripts using GIS and years of experience in GIS data analysis will suffice.

Q. 3. Section 4.2.2.1. and 4.2.3.1. Is a report detailing completion of 4.2.3. really due by February 28, 2015 or should the year be 2016 or 17?

A. 3. The date in 4.2.3.1 was a mistake. It is corrected from February 28, 2015 to February 29, 2016.

Q. 4. Does the agency want a Report detailing landscape genetics analysis overlaying genetic and GIS assignment before the Report for 4.2.2. that actually includes the results of genetics analysis?

A. 4. No, see A.3. Date in 4.2.3.1 has been modified.

Q. 5. Specifications Article 5.2, Pricing Page (page 23) – "Vendor should complete the Pricing Page by filling in the Unit Price for each item listed, this Unit Price shall include all costs related to the project including salaries, fringe benefits, travel, supplies and any other incidentals required."

Given the RFQ language in the preceding paragraph, does the Agency expect to see a line item budget by year for salaries, fringe benefits, travel, etc. on the Pricing Page, or does the Agency want these types of costs rolled up into a yearly total and the yearly total is the "Unit Price".

A. 5. The Agency does not expect to see a line item budget. They want these types of costs rolled up into a yearly total and the yearly total is the "Unit Price."

Q. 6. If the "Unit Price" on the Pricing Page is not yearly total, then what is an acceptable "unit Price" measurement? See also Specifications Article 8. Travel that states "Any anticipated mileage or travel cost may be included in the flat fee or hourly rate listed on Vendor's bid..."

A. 6 The "Unit Price" is the yearly total on the pricing page.

Q. 7. Specifications, Article 7, Payment (page 23) – "Agency shall pay the annual unit price as shown on the Pricing Pages, for all Contract Services performed and accepted under this Contract. Vendor shall accept payment in the accordance with the payment procedures of the State of West Virginia. Vendor shall bill Agency monthly for costs accrued during that pay period."

Will this be a fixed price agreement or a cost reimbursement agreement? The language above could be interpreted to read as a fixed price agreement as well as a cost reimbursement.

A. 7. This will be a cost reimbursement not to exceed total bid amount.

Q. 8. Pricing Page: Would it be acceptable to have different Yearly totals? For example in out years, there may be cost of living increases, and other inflationary factors that might impact the costs of Years two (2) and three (3).

A. 8. Yes, it is accepted, and expected, that there would be different costs between years one (1), two (2), and three (3) depending upon supplies needed for that specific time period, cost of living inflation, etc.

INSTRUCTIONS TO VENDORS SUBMITTING BIDS

1. **REVIEW DOCUMENTS THOROUGHLY:** The attached documents contain a solicitation for bids. Please read these instructions and all documents attached in their entirety. These instructions provide critical information about requirements that if overlooked could lead to disqualification of a Vendor's bid. All bids must be submitted in accordance with the provisions contained in these instructions and the Solicitation. Failure to do so may result in disqualification of Vendor's bid.

2. **MANDATORY TERMS:** The Solicitation may contain mandatory provisions identified by the use of the words "must," "will," and "shall." Failure to comply with a mandatory term in the Solicitation will result in bid disqualification.

3. **PREBID MEETING:** The item identified below shall apply to this Solicitation.

☑ A pre-bid meeting will not be held prior to bid opening.

☐ A **NON-MANDATORY PRE-BID** meeting will be held at the following place and time:

☐ A **MANDATORY PRE-BID** meeting will be held at the following place and time:

All Vendors submitting a bid must attend the mandatory pre-bid meeting. Failure to attend the mandatory pre-bid meeting shall result in disqualification of the Vendor's bid. No one person attending the pre-bid meeting may represent more than one Vendor.

An attendance sheet provided at the pre-bid meeting shall serve as the official document verifying attendance. The State will not accept any other form of proof or documentation to verify attendance. Any person attending the pre-bid meeting on behalf of a Vendor must list on the attendance sheet his or her name and the name of the Vendor he or she is representing. Additionally, the person attending the pre-bid meeting should include the Vendor's E-Mail address, phone number, and Fax number on the attendance sheet. It is the Vendor's responsibility to locate the attendance sheet and provide the required information. Failure to complete the attendance sheet as required may result in disqualification of Vendor's bid.

All Vendors should arrive prior to the starting time for the pre-bid. Vendors who arrive after the starting time but prior to the end of the pre-bid will be permitted to sign in, but are charged with knowing all matters discussed at the pre-bid.

Questions submitted at least five business days prior to a scheduled pre-bid will be discussed at the pre-bid meeting if possible. Any discussions or answers to questions at the pre-bid meeting are preliminary in nature and are non-binding. Official and binding answers to questions will be published in a written addendum to the Solicitation prior to bid opening.

4. **VENDOR QUESTION DEADLINE:** Vendors may submit questions relating to this Solicitation to the Purchasing Division. Questions must be submitted in writing. All questions must be submitted on or before the date listed below and to the address listed below in order to be considered. A written response will be published in a Solicitation addendum if a response is possible and appropriate. Non-written discussions, conversations, or questions and answers regarding this Solicitation are preliminary in nature and are non-binding.

Question Submission Deadline: March 7, 2014 at 5:00pm

Submit Questions to: Dean Wingerd
2019 Washington Street, East
Charleston, WV 25305
Fax: 304-558-4115
Email: Dean.C.Wingerd@wv.gov

5. **VERBAL COMMUNICATION:** Any verbal communication between the Vendor and any State personnel is not binding, including that made at the mandatory pre-bid conference. Only information issued in writing and added to the Solicitation by an official written addendum by the Purchasing Division is binding.

6. **BID SUBMISSION:** All bids must be signed and delivered by the Vendor to the Purchasing Division at the address listed below on or before the date and time of the bid opening. Any bid received by the Purchasing Division staff is considered to be in the possession of the Purchasing Division and will not be returned for any reason. The Purchasing Division will not accept bids, modification of bids, or addendum acknowledgment forms via e-mail. Acceptable delivery methods include hand delivery, delivery by courier, or facsimile. The bid delivery address is:

Department of Administration, Purchasing Division
2019 Washington Street East
Charleston, WV 25305-0130

The bid should contain the information listed below on the face of the envelope or the bid maynot be considered:

SEALED BID
BUYER: _____
SOLICITATION NO.: _____
BID OPENING DATE: _____
BID OPENING TIME: _____
FAX NUMBER: _____

In the event that Vendor is responding to a request for proposal, the Vendor shall submit one original technical and one original cost proposal plus _____ convenience copies of each to the Purchasing Division at the address shown above. Additionally, the Vendor should identifythe bid type as either a technical or cost proposal on the face of each bid envelope submitted in response to a request for proposal as follows:

BID TYPE: ☐ Technical
☒ Cost

7. **BID OPENING:** Bids submitted in response to this Solicitation will be opened at the location identified below on the date and time listed below. Delivery of a bid after the bid opening date and time will result in bid disqualification. For purposes of this Solicitation, a bid is considered delivered when time stamped bythe official Purchasing Division time clock.

Bid Opening Date and Time: March 20, 2014 at 1:30pm

Bid Opening Location: Department of Administration, Purchasing Division
2019 Washington Street East
Charleston, WV 25305-0130

8. **ADDENDUM ACKNOWLEDGEMENT:** Changes or revisions to this Solicitation will be made by an official written addendum issued by the Purchasing Division. Vendor should acknowledge receipt of all addenda issued with this Solicitation by completing an Addendum Acknowledgment Form, a copy of which is included herewith. Failure to acknowledge addenda may result in bid disqualification. The addendum acknowledgement should be submitted with the bid to expedite document processing.

9. **BID FORMATTING:** Vendor should type or electronically enter the information onto its bid to prevent errors in the evaluation. Failure to type or electronically enter the information may result in bid disqualification.

GENERAL TERMS AND CONDITIONS:

1. **CONTRACTUAL AGREEMENT:** Issuance of a Purchase Order signed by the Purchasing Division Director, or his designee, and approved as to form by the Attorney General's office constitutes acceptance of this Contract made by and between the State of West Virginia and the Vendor. Vendor's signature on its bid signifies Vendor's agreement to be bound by and accept the terms and conditions contained in this Contract.

2. **DEFINITIONS:** As used in this Solicitation/Contract, the following terms shall have the meanings attributed to them below. Additional definitions may be found in the specifications included with this Solicitation/Contract.

   2.1 "Agency" or **"Agencies"** means the agency, board, commission, or other entity of the State of West Virginia that is identified on the first page of the Solicitation or any other public entity seeking to procure goods or services under this Contract.

   2.2 "Contract" means the binding agreement that is entered into between the State and the Vendor to provide the goods and services requested in the Solicitation.

   2.3 "Director" means the Director of the West Virginia Department of Administration, Purchasing Division.

   2.4 "Purchasing Division" means the West Virginia Department of Administration, Purchasing Division.

   2.5 "Purchase Order" means the document signed by the Agency and the Purchasing Division, and approved as to form by the Attorney General, that identifies the Vendor as the successful bidder and Contract holder.

   2.6 "Solicitation" means the official solicitation published by the Purchasing Division and identified by number on the first page thereof.

   2.7 "State" means the State of West Virginia and/or any of its agencies, commissions, boards, etc. as context requires.

   2.8 "Vendor" or **"Vendors"** means any entity submitting a bid in response to the Solicitation, the entity that has been selected as the lowest responsible bidder, or the entity that has been awarded the Contract as context requires.

3. **CONTRACT TERM; RENEWAL; EXTENSION:** The term of this Contract shall be determined in accordance with the category that has been identified as applicable to this Contract below:

☑ **Term Contract**

Initial Contract Term: This Contract becomes effective on Upon Award

and extends for a period of One (1) year(s).

Renewal Term: This Contract may be renewed upon the mutual written consent of the Agency, and the Vendor, with approval of the Purchasing Division and the Attorney General's office (Attorney General approval is as to form only). Any request for renewal must be submitted to the Purchasing Division Director thirty (30) days prior to the expiration date of the initial contract term or appropriate renewal term. A Contract renewal shall be in accordance with the terms and conditions of the original contract. Renewal of this Contract is limited to Two (2) successive one (1) year periods. Automatic renewal of this Contract is prohibited. Notwithstanding the foregoing, Purchasing Division approval is not required on agency delegated or exempt purchases. Attorney General approval may be required for vendor terms and conditions.

Reasonable Time Extension: At the sole discretion of the Purchasing Division Director, and with approval from the Attorney General's office (Attorney General approval is as to form only), this Contract may be extended for a reasonable time after the initial Contract term or after any renewal term as may be necessary to obtain a new contract or renew this Contract. Any reasonable time extension shall not exceed twelve (12) months. Vendor may avoid a reasonable time extension by providing the Purchasing Division Director with written notice of Vendor's desire to terminate this Contract 30 days prior to the expiration of the then current term. During any reasonable time extension period, the Vendor may terminate this Contract for any reason upon giving the Purchasing Division Director 30 days written notice. Automatic extension of this Contract is prohibited. Notwithstanding the foregoing, Purchasing Division approval is not required on agency delegated or exempt purchases, but Attorney General approval may be required.

Release Order Limitations: In the event that this contract permits release orders, a release order may only be issued during the time this Contract is in effect. Any release order issued within one year of the expiration of this Contract shall be effective for one year from the date the release order is issued. No release order may be extended beyond one year after this Contract has expired.

☐ **Fixed Period Contract:** This Contract becomes effective upon Vendor's receipt of the notice to proceed and must be completed within days.

☐ **One Time Purchase:** The term of this Contract shall run from the issuance of the Purchase Order until all of the goods contracted for have been delivered, but in no event shall this Contract extend for more than one fiscal year.

☐ **Other:** See attached.

4. **NOTICE TO PROCEED:** Vendor shall begin performance of this Contract immediately upon receiving notice to proceed unless otherwise instructed by the Agency. Unless otherwise specified, the fully executed Purchase Order will be considered notice to proceed

5. **QUANTITIES:** The quantities required under this Contract shall be determined in accordance with the category that has been identified as applicable to this Contract below.

☐ **Open End Contract:** Quantities listed in this Solicitation are approximations only, based on estimates supplied by the Agency. It is understood and agreed that the Contract shall cover the quantities actually ordered for delivery during the term of the Contract, whether more or less than the quantities shown.

☐ **Service:** The scope of the service to be provided will be more clearly defined in the specifications included herewith.

☑ **Combined Service and Goods:** The scope of the service and deliverable goods to be provided will be more clearly defined in the specifications included herewith.

☐ **One Time Purchase:** This Contract is for the purchase of a set quantity of goods that are identified in the specifications included herewith. Once those items have been delivered, no additional goods may be procured under this Contract without an appropriate change order approved by the Vendor, Agency, Purchasing Division, and Attorney General's office.

6. **PRICING:** The pricing set forth herein is firm for the life of the Contract, unless specified elsewhere within this Solicitation/Contract by the State. A Vendor's inclusion of price adjustment provisions in its bid, without an express authorization from the State in the Solicitation to do so, may result in bid disqualification.

7. **EMERGENCY PURCHASES:** The Purchasing Division Director may authorize the Agency to purchase goods or services in the open market that Vendor would otherwise provide under this Contract if those goods or services are for immediate or expedited delivery in an emergency. Emergencies shall include, but are not limited to, delays in transportation or an unanticipated increase in the volume of work. An emergency purchase in the open market, approved by the Purchasing Division Director, shall not constitute of breach of this Contract and shall not entitle the Vendor to any form of compensation or damages. This provision does not excuse the State from fulfilling its obligations under a One Time Purchase contract.

8. **REQUIRED DOCUMENTS:** All of the items checked below must be provided to the Purchasing Division by the Vendor as specified below.

☐ **BID BOND:** All Vendors shall furnish a bid bond in the amount of five percent (5%) of the total amount of the bid protecting the State of West Virginia. The bid bond must be submitted with the bid.

☐ **PERFORMANCE BOND:** The apparent successful Vendor shall provide a performance bond in the amount of . The performance bond must be issued and received by the Purchasing Division prior to Contract award. On construction contracts, the performance bond must be 100% of the Contract value.

☐ **LABOR/MATERIAL PAYMENT BOND:** The apparent successful Vendor shall provide a labor/material payment bond in the amount of 100% of the Contract value. The labor/material payment bond must be issued and delivered to the Purchasing Division prior to Contract award.

In lieu of the Bid Bond, Performance Bond, and Labor/Material Payment Bond, the Vendor may provide certified checks, cashier's checks, or irrevocable letters of credit. Any certified check, cashier's check, or irrevocable letter of credit provided in lieu of a bond must be of the same amount and delivered on the same schedule as the bond it replaces. A letter of credit submitted in lieu of a performance and labor/material payment bond will only be allowed for projects under $100,000. Personal or business checks are not acceptable.

☐ **MAINTENANCE BOND:** The apparent successful Vendor shall provide a two (2) year maintenance bond covering the roofing system. The maintenance bond must be issued and delivered to the Purchasing Division prior to Contract award.

☑ **WORKERS' COMPENSATION INSURANCE:** The apparent successful Vendor shall have appropriate workers' compensation insurance and shall provide proof thereof upon request.

☐ **INSURANCE:** The apparent successful Vendor shall furnish proof of the following insurance prior to Contract award and shall list the state as a certificate holder:

☐ **Commercial General Liability Insurance:**
or more.

☐ **Builders Risk Insurance:** builders risk – all risk insurance in an amount equal to 100% of the amount of the Contract.

☐

☐

☐

☐

☐

The apparent successful Vendor shall also furnish proof of any additional insurance requirements contained in the specifications prior to Contract award regardless of whether or not that insurance requirement is listed above.

☐ **LICENSE(S) / CERTIFICATIONS / PERMITS:** In addition to anything required under the Section entitled Licensing, of the General Terms and Conditions, the apparent successful Vendor shall furnish proof of the following licenses, certifications, and/or permits prior to Contract award, in a form acceptable to the Purchasing Division.

☐

☐

☐

☐

The apparent successful Vendor shall also furnish proof of any additional licenses or certifications contained in the specifications prior to Contract award regardless of whether or not that requirement is listed above.

9. **LITIGATION BOND:** The Director reserves the right to require any Vendor that files a protest of an award to submit a litigation bond in the amount equal to one percent of the lowest bid submitted or $5,000, whichever is greater. The entire amount of the bond shall be forfeited if the hearing officer determines that the protest was filed for frivolous or improper purpose, including but not limited to, the purpose of harassing, causing unnecessary delay, or needless expense for the Agency. All litigation bonds shall be made payable to the Purchasing Division. In lieu of a bond, the protester may submit a cashier's check or certified check payable to the Purchasing Division. Cashier's or certified checks will be deposited with and held by the State Treasurer's office. If it is determined that the protest has not been filed for frivolous or improper purpose, the bond or deposit shall be returned in its entirety.

10. **ALTERNATES:** Any model, brand, or specification listed herein establishes the acceptable level of quality only and is not intended to reflect a preference for, or in any way favor, a particular brand or vendor. Vendors may bid alternates to a listed model or brand provided that the alternate is at least equal to the model or brand and complies with the required specifications. The equality of any alternate being bid shall be determined by the State at its sole discretion. Any Vendor bidding an alternate model or brand should clearly identify the alternate items in its bid and should include manufacturer's specifications, industry literature, and/or any other relevant documentation demonstrating the equality of the alternate items. Failure to provide information for alternate items may be grounds for rejection of a Vendor's bid.

11. **EXCEPTIONS AND CLARIFICATIONS:** The Solicitation contains the specifications that shall form the basis of a contractual agreement. Vendor shall clearly mark any exceptions, clarifications, or

other proposed modifications in its bid. Exceptions to, clarifications of, or modifications of a requirement or term and condition of the Solicitation may result in bid disqualification.

12. **LIQUIDATED DAMAGES:** Vendor shall pay liquidated damages in the amount for

This clause shall in no way be considered exclusive and shall not limit the State or Agency's right to pursue any other available remedy.

13. **ACCEPTANCE/REJECTION:** The State may accept or reject any bid in whole, or in part. Vendor's signature on its bid signifies acceptance of the terms and conditions contained in the Solicitation and Vendor agrees to be bound by the terms of the Contract, as reflected in the Purchase Order, upon receipt.

14. **REGISTRATION:** Prior to Contract award, the apparent successful Vendor must be properly registered with the West Virginia Purchasing Division and must have paid the $125 fee if applicable.

15. **COMMUNICATION LIMITATIONS:** In accordance with West Virginia Code of State Rules §148-1-6.6, communication with the State of West Virginia or any of its employees regarding this Solicitation during the solicitation, bid, evaluation or award periods, except through the Purchasing Division, is strictly prohibited without prior Purchasing Division approval. Purchasing Division approval for such communication is implied for all agency delegated and exempt purchases.

16. **FUNDING:** This Contract shall continue for the term stated herein, contingent upon funds being appropriated by the Legislature or otherwise being made available. In the event funds are not appropriated or otherwise made available, this Contract becomes void and of no effect beginning on July 1 of the fiscal year for which funding has not been appropriated or otherwise made available.

17. **PAYMENT:** Payment in advance is prohibited under this Contract. Payment may only be made after the delivery and acceptance of goods or services. The Vendor shall submit invoices, in arrears, to the Agency at the address on the face of the purchase order labeled "Invoice To."

18. **UNIT PRICE:** Unit prices shall prevail in cases of a discrepancy in the Vendor's bid.

19. **DELIVERY:** All quotations are considered freight on board destination ("F.O.B. destination") unless alternate shipping terms are clearly identified in the bid. Vendor's listing of shipping terms that contradict the shipping terms expressly required by this Solicitation may result in bid disqualification.

20. **INTEREST:** Interest attributable to late payment will only be permitted if authorized by the West Virginia Code. Presently, there is no provision in the law for interest on late payments.

21. **PREFERENCE:** Vendor Preference may only be granted upon written request and only in accordance with the West Virginia Code § 5A-3-37 and the West Virginia Code of State Rules. A Resident Vendor Certification form has been attached hereto to allow Vendor to apply for the preference. Vendor's

failure to submit the Resident Vendor Certification form with its bid will result in denial of Vendor Preference. Vendor Preference does not apply to construction projects.

22. **SMALL, WOMEN-OWNED, OR MINORITY-OWNED BUSINESSES:** For any solicitations publicly advertised for bid on or after July 1, 2012, in accordance with West Virginia Code §5A-3-37(a)(7) and W. Va. CSR § 148-22-9, any non-resident vendor certified as a small, women-owned, or minority-owned business under W. Va. CSR § 148-22-9 shall be provided the same preference made available to any resident vendor. Any non-resident small, women-owned, or minority-owned business must identify itself as such in writing, must submit that writing to the Purchasing Division with its bid, and must be properly certified under W. Va. CSR § 148-22-9 prior to submission of its bid to receive the preferences made available to resident vendors. Preference for a non-resident small, women-owned, or minority owned business shall be applied in accordance with W. Va. CSR § 148-22-9.

23. **TAXES:** The Vendor shall pay any applicable sales, use, personal property or any other taxes arising out of this Contract and the transactions contemplated thereby. The State of West Virginia is exempt from federal and state taxes and will not pay or reimburse such taxes.

24. **CANCELLATION:** The Purchasing Division Director reserves the right to cancel this Contract immediately upon written notice to the vendor if the materials or workmanship supplied do not conform to the specifications contained in the Contract. The Purchasing Division Director may cancel any purchase or Contract upon 30 days written notice to the Vendor in accordance with West Virginia Code of State Rules § 148-1-7.16.2.

25. **WAIVER OF MINOR IRREGULARITIES:** The Director reserves the right to waive minor irregularities in bids or specifications in accordance with West Virginia Code of State Rules § 148-1-4.6.

26. **TIME:** Time is of the essence with regard to all matters of time and performance in this Contract.

27. **APPLICABLE LAW:** This Contract is governed by and interpreted under West Virginia law without giving effect to its choice of law principles. Any information provided in specification manuals, or any other source, verbal or written, which contradicts or violates the West Virginia Constitution, West Virginia Code or West Virginia Code of State Rules is void and of no effect.

28. **COMPLIANCE:** Vendor shall comply with all applicable federal, state, and local laws, regulations and ordinances. By submitting a bid, Vendors acknowledge that they have reviewed, understand, and will comply with all applicable law.

29. **PREVAILING WAGE:** On any contract for the construction of a public improvement, Vendor and any subcontractors utilized by Vendor shall pay a rate or rates of wages which shall not be less than the fair minimum rate or rates of wages (prevailing wage), as established by the West Virginia Division of Labor under West Virginia Code §§ 21-5A-1 et seq. and available at http://www.sos.wv.gov/administrative-law/wagerates/Pages/default.aspx. Vendor shall be responsible for ensuring compliance with prevailing wage requirements and determining when prevailing wage

requirements are applicable. The required contract provisions contained in West Virginia Code of State Rules § 42-7-3 are specifically incorporated herein by reference.

30. **ARBITRATION:** Any references made to arbitration contained in this Contract, Vendor's bid, or in any American Institute of Architects documents pertaining to this Contract are hereby deleted, void, and of no effect.

31. **MODIFICATIONS:** This writing is the parties' final expression of intent. Notwithstanding anything contained in this Contract to the contrary, no modification of this Contract shall be binding without mutual written consent of the Agency, and the Vendor, with approval of the Purchasing Division and the Attorney General's office (Attorney General approval is as to form only). **No Change shall be implemented by the Vendor until such time as the Vendor receives an approved written change order from the Purchasing Division.**

32. **WAIVER:** The failure of either party to insist upon a strict performance of any of the terms or provision of this Contract, or to exercise any option, right, or remedy herein contained, shall not be construed as a waiver or a relinquishment for the future of such term, provision, option, right, or remedy, but the same shall continue in full force and effect. Any waiver must be expressly stated in writing and signed by the waiving party.

33. **SUBSEQUENT FORMS:** The terms and conditions contained in this Contract shall supersede any and all subsequent terms and conditions which may appear on any form documents submitted by Vendor to the Agency or Purchasing Division such as price lists, order forms, invoices, sales agreements, or maintenance agreements, and includes internet websites or other electronic documents. Acceptance or use of Vendor's forms does not constitute acceptance of the terms and conditions contained thereon.

34. **ASSIGNMENT:** Neither this Contract nor any monies due, or to become due hereunder, may be assigned by the Vendor without the express written consent of the Agency, the Purchasing Division, the Attorney General's office (as to form only), and any other government agency or office that may be required to approve such assignments. Notwithstanding the foregoing, Purchasing Division approval may or may not be required on certain agency delegated or exempt purchases.

35. **WARRANTY:** The Vendor expressly warrants that the goods and/or services covered by this Contract will: (a) conform to the specifications, drawings, samples, or other description furnished or specified by the Agency; (b) be merchantable and fit for the purpose intended; and (c) be free from defect in material and workmanship.

36. **STATE EMPLOYEES:** State employees are not permitted to utilize this Contract for personal use and the Vendor is prohibited from permitting or facilitating the same.

37. **BANKRUPTCY:** In the event the Vendor files for bankruptcy protection, the State of West Virginia may deem this Contract null and void, and terminate this Contract without notice.

38. **[RESERVED]**

39. **CONFIDENTIALITY**: The Vendor agrees that it will not disclose to anyone, directly or indirectly, any such personally identifiable information or other confidential information gained from the Agency, unless the individual who is the subject of the information consents to the disclosure in writing or the disclosure is made pursuant to the Agency's policies, procedures, and rules. Vendor further agrees to comply with the Confidentiality Policies and Information Security Accountability Requirements, set forth in http://www.state.wv.us/admin/purchase/privacy/default.html.

40. **DISCLOSURE:** Vendor's response to the Solicitation and the resulting Contract are considered public documents and will be disclosed to the public in accordance with the laws, rules, and policies governing the West Virginia Purchasing Division. Those laws include, but are not limited to, the Freedom of Information Act found in West Virginia Code § 29B-1-1 et seq.

    If a Vendor considers any part of its bid to be exempt from public disclosure, Vendor must so indicate by specifically identifying the exempt information, identifying the exemption that applies, providing a detailed justification for the exemption, segregating the exempt information from the general bid information, and submitting the exempt information as part of its bid but in a segregated and clearly identifiable format. Failure to comply with the foregoing requirements will result in public disclosure of the Vendor's bid without further notice. A Vendor's act of marking all or nearly all of its bid as exempt is not sufficient to avoid disclosure and WILL NOT BE HONORED. Vendor's act of marking a bid or any part thereof as "confidential" or "proprietary" is not sufficient to avoid disclosure and WILL NOT BE HONORED. In addition, a legend or other statement indicating that all or substantially all of the bid is exempt from disclosure is not sufficient to avoid disclosure and WILL NOT BE HONORED. Vendor will be required to defend any claimed exemption for nondisclosure in the event of an administrative or judicial challenge to the State's nondisclosure. Vendor must indemnify the State for any costs incurred related to any exemptions claimed by Vendor. Any questions regarding the applicability of the various public records laws should be addressed to your own legal counsel prior to bid submission.

41. **LICENSING:** In accordance with West Virginia Code of State Rules §148-1-6.1.7, Vendor must be licensed and in good standing in accordance with any and all state and local laws and requirements by any state or local agency of West Virginia, including, but not limited to, the West Virginia Secretary of State's Office, the West Virginia Tax Department, West Virginia Insurance Commission, or any other state agency or political subdivision. Upon request, the Vendor must provide all necessary releases to obtain information to enable the Purchasing Division Director or the Agency to verify that the Vendor is licensed and in good standing with the above entities.

42. **ANTITRUST:** In submitting a bid to, signing a contract with, or accepting a Purchase Order from any agency of the State of West Virginia, the Vendor agrees to convey, sell, assign, or transfer to the State of West Virginia all rights, title, and interest in and to all causes of action it may now or hereafter acquire under the antitrust laws of the United States and the State of West Virginia for price fixing and/or unreasonable restraints of trade relating to the particular commodities or services purchased or acquired

by the State of West Virginia. Such assignment shall be made and become effective at the time the purchasing agency tenders the initial payment to Vendor.

43. **VENDOR CERTIFICATIONS:** By signing its bid or entering into this Contract, Vendor certifies (1) that its bid was made without prior understanding, agreement, or connection with any corporation, firm, limited liability company, partnership, person or entity submitting a bid for the same material, supplies, equipment or services; (2) that its bid is in all respects fair and without collusion or fraud; (3) that this Contract is accepted or entered into without any prior understanding, agreement, or connection to any other entity that could be considered a violation of law; and (4) that it has reviewed this RFQ in its entirety, understands the requirements, terms and conditions, and other information contained herein. Vendor's signature on its bid also affirms that neither it nor its representatives have any interest, nor shall acquire any interest, direct or indirect, which would compromise the performance of its services hereunder. Any such interests shall be promptly presented in detail to the Agency.

    The individual signing this bid on behalf of Vendor certifies that he or she is authorized by the Vendor to execute this bid or any documents related thereto on Vendor's behalf; that he or she is authorized to bind the Vendor in a contractual relationship; and that, to the best of his or her knowledge, the Vendor has properly registered with any State agency that may require registration.

44. **PURCHASING CARD ACCEPTANCE:** The State of West Virginia currently utilizes a Purchasing Card program, administered under contract by a banking institution, to process payment for goods and services. The Vendor must accept the State of West Virginia's Purchasing Card for payment of all orders under this Contract unless the box below is checked.

    ☐ Vendor is not required to accept the State of West Virginia's Purchasing Card as payment for all goods and services.

45. **VENDOR RELATIONSHIP:** The relationship of the Vendor to the State shall be that of an independent contractor and no principal-agent relationship or employer-employee relationship is contemplated or created by this Contract. The Vendor as an independent contractor is solely liable for the acts and omissions of its employees and agents. Vendor shall be responsible for selecting, supervising, and compensating any and all individuals employed pursuant to the terms of this Solicitation and resulting contract. Neither the Vendor, nor any employees or subcontractors of the Vendor, shall be deemed to be employees of the State for any purpose whatsoever. Vendor shall be exclusively responsible for payment of employees and contractors for all wages and salaries, taxes, withholding payments, penalties, fees, fringe benefits, professional liability insurance premiums, contributions to insurance and pension, or other deferred compensation plans, including but not limited to, Workers' Compensation and Social Security obligations, licensing fees, *etc.* and the filing of all necessary documents, forms and returns pertinent to all of the foregoing. Vendor shall hold harmless the State, and shall provide the State and Agency with a defense against any and all claims including, but not limited to, the foregoing payments, withholdings, contributions, taxes, Social Security taxes, and employer income tax returns.

46. **INDEMNIFICATION:** The Vendor agrees to indemnify, defend, and hold harmless the State and the Agency, their officers, and employees from and against: (1) Any claims or losses for services rendered

by any subcontractor, person, or firm performing or supplying services, materials, or supplies in connection with the performance of the Contract; (2) Any claims or losses resulting to any person or entity injured or damaged by the Vendor, its officers, employees, or subcontractors by the publication, translation, reproduction, delivery, performance, use, or disposition of any data used under the Contract in a manner not authorized by the Contract, or by Federal or State statutes or regulations; and (3) Any failure of the Vendor, its officers, employees, or subcontractors to observe State and Federal laws including, but not limited to, labor and wage and hour laws.

47. **PURCHASING AFFIDAVIT:** In accordance with West Virginia Code § 5A-3-10a, all Vendors are required to sign, notarize, and submit the Purchasing Affidavit stating that neither the Vendor nor a related party owe a debt to the State in excess of $1,000. The affidavit must be submitted prior to award, but should be submitted with the Vendor's bid. A copy of the Purchasing Affidavit is included herewith.

48. **ADDITIONAL AGENCY AND LOCAL GOVERNMENT USE:** This Contract may be utilized by and extends to other agencies, spending units, and political subdivisions of the State of West Virginia; county, municipal, and other local government bodies; and school districts ("Other Government Entities"). This Contract shall be extended to the aforementioned Other Government Entities on the same prices, terms, and conditions as those offered and agreed to in this Contract. If the Vendor does not wish to extend the prices, terms, and conditions of its bid and subsequent contract to the Other Government Entities, the Vendor must clearly indicate such refusal in its bid. A refusal to extend this Contract to the Other Government Entities shall not impact or influence the award of this Contract in any manner.

49. **CONFLICT OF INTEREST:** Vendor, its officers or members or employees, shall not presently have or acquire any interest, direct or indirect, which would conflict with or compromise the performance of its obligations hereunder. Vendor shall periodically inquire of its officers, members and employees to ensure that a conflict of interest does not arise. Any conflict of interest discovered shall be promptly presented in detail to the Agency.

50. **REPORTS:** Vendor shall provide the Agency and/or the Purchasing Division with the following reports identified by a checked box below:

☑ Such reports as the Agency and/or the Purchasing Division may request. Requested reports may include, but are not limited to, quantities purchased, agencies utilizing the contract, total contract expenditures by agency, etc.

☐ Quarterly reports detailing the total quantity of purchases in units and dollars, along with a listing of purchases by agency. Quarterly reports should be delivered to the Purchasing Division via email at purchasing.requisitions@wv.gov.

51. **BACKGROUND CHECK:** In accordance with W. Va. Code § 15-2D-3, the Director of the Division of Protective Services shall require any service provider whose employees are regularly employed on the grounds or in the buildings of the Capitol complex or who have access to sensitive or critical information

to submit to a fingerprint-based state and federal background inquiry through the state repository. The service provider is responsible for any costs associated with the fingerprint-based state and federal background inquiry.

After the contract for such services has been approved, but before any such employees are permitted to be on the grounds or in the buildings of the Capitol complex or have access to sensitive or critical information, the service provider shall submit a list of all persons who will be physically present and working at the Capitol complex to the Director of the Division of Protective Services for purposes of verifying compliance with this provision.

The State reserves the right to prohibit a service provider's employees from accessing sensitive or critical information or to be present at the Capitol complex based upon results addressed from a criminal background check.

Service providers should contact the West Virginia Division of Protective Services by phone at (304)558-9911 for more information.

52. **PREFERENCE FOR USE OF DOMESTIC STEEL PRODUCTS:** Except when authorized by the Director of the Purchasing Division pursuant to W. Va. Code § 5A-3-56, no contractor may use or supply steel products for a State Contract Project other than those steel products made in the United States. A contractor who uses steel products in violation of this section may be subject to civil penalties pursuant to W. Va. Code § 5A-3-56. As used in this section:

    a. "State Contract Project" means any erection or construction of, or any addition to, alteration of or other improvement to any building or structure, including, but not limited to, roads or highways, or the installation of any heating or cooling or ventilating plants or other equipment, or the supply of and materials for such projects, pursuant to a contract with the State of West Virginia for which bids were solicited on or after June 6, 2001.

    b. "Steel Products" means products rolled, formed, shaped, drawn, extruded, forged, cast, fabricated or otherwise similarly processed, or processed by a combination of two or more or such operations, from steel made by the open heath, basic oxygen, electric furnace, Bessemer or other steel making process.

    The Purchasing Division Director may, in writing, authorize the use of foreign steel products if:

    a. The cost for each contract item used does not exceed one tenth of one percent (.1%) of the total contract cost or two thousand five hundred dollars ($2,500.00), whichever is greater. For the purposes of this section, the cost is the value of the steel product as delivered to the project; or

    b. The Director of the Purchasing Division determines that specified steel materials are not produced in the United States in sufficient quantity or otherwise are not reasonably available to meet contract requirements.

53. PREFERENCE FOR USE OF DOMESTIC ALUMINUM, GLASS, AND STEEL: In Accordance with W. Va. Code § 5-19-1 et seq., and W. Va. CSR § 148-10-1 et seq., for every contract or subcontract, subject to the limitations contained herein, for the construction, reconstruction, alteration, repair, improvement or maintenance of public works or for the purchase of any item of machinery or equipment to be used at sites of public works, only domestic aluminum, glass or steel products shall be supplied unless the spending officer determines, in writing, after the receipt of offers or bids, (1) that the cost of domestic aluminum, glass or steel products is unreasonable or inconsistent with the public interest of the State of West Virginia, (2) that domestic aluminum, glass or steel products are not produced in sufficient quantities to meet the contract requirements, or (3) the available domestic aluminum, glass, or steel do not meet the contract specifications. This provision only applies to public works contracts awarded in an amount more than fifty thousand dollars ($50,000) or public works contracts that require more than ten thousand pounds of steel products.

The cost of domestic aluminum, glass, or steel products may be unreasonable if the cost is more than twenty percent (20%) of the bid or offered price for foreign made aluminum, glass, or steel products. If the domestic aluminum, glass or steel products to be supplied or produced in a "substantial labor surplus area", as defined by the United States Department of Labor, the cost of domestic aluminum, glass, or steel products may be unreasonable if the cost is more than thirty percent (30%) of the bid or offered price for foreign made aluminum, glass, or steel products.

This preference shall be applied to an item of machinery or equipment, as indicated above, when the item is a single unit of equipment or machinery manufactured primarily of aluminum, glass or steel, is part of a public works contract and has the sole purpose or of being a permanent part of a single public works project. This provision does not apply to equipment or machinery purchased by a spending unit for use by that spending unit and not as part of a single public works project.

All bids and offers including domestic aluminum, glass or steel products that exceed bid or offer prices including foreign aluminum, glass or steel products after application of the preferences provided in this provision may be reduced to a price equal to or lower than the lowest bid or offer price for foreign aluminum, glass or steel products plus the applicable preference. If the reduced bid or offer prices are made in writing and supersede the prior bid or offer prices, all bids or offers, including the reduced bid or offer prices, will be reevaluated in accordance with this rule.

REQUEST FOR QUOTATION
DNR214058 – Ph.D. Student to analyze white tailed deer

SPECIFICATIONS

1. **PURPOSE AND SCOPE:** The West Virginia Purchasing Division is soliciting bids on behalf of West Virginia Division of Natural Resources, hereinafter referred to as DNR, to establish a contract for reporting, analyzing, writing, and publishing scientific results of a major genetic project dealing with Chronic Wasting Disease (CWD) in white-tailed deer in West Virginia.

   The 2005 discovery of Chronic Wasting Disease (CWD) among white-tailed deer in Hampshire County West Virginia presents an unprecedented white-tailed deer management challenge to DNR. The disease does not cause an immediate wide spread die-off of white-tailed deer, but models indicate long-term damage to the white-tailed deer herd and human dimension surveys indicate adverse impacts to hunting recreation. Those that have tried to predict the outcome of the disease on deer populations have described the disease as a 30-50 year epizootic. Due to the uncertain ramifications that CWD may have on the state's white-tailed deer resource, and the protracted time during which these ramifications will occur, the DNR is seeking information on the population genetic structure of white-tailed deer. The resulting data will then be used to help plan and implement management strategies that may limit adverse impacts to both the resource and the associated recreation.

   As part of an ongoing multi-year effort by the DNR to monitor the distribution and prevalence of CWD in Hampshire and Hardy counties, tissue samples were collected from hunter harvested white-tailed deer. Therefore, the DNR wishes to contract with a university to fund a Ph.D. student in wildlife management to undertake a project to examine the landscape scale gene flow of white-tailed deer in the eastern panhandle of West Virginia by analyzing DNA from the aforementioned geo-referenced archived white-tailed deer tissue samples collected from 2006 through 2013 and additional samples from surrounding counties and states if necessary. From all the archived tissue samples, a stratified subsample of a minimum of 800 individuals will be analyzed.

   DNA samples from the infected CWD area and a minimum of 4 additional references areas from other regions of the state were collected by WVDNR personnel from 2005–2013. Sample sizes for each fall range from approximately 650 to 1200. Tissue samples consist of ear notches taken from the edge of the pinna or, if the hunter did not want to disfigure the deer, a small piece of skin with hair from the sternum. All tissue samples were place in a 57 x 16.5mm tube with O-ring cap containing 95% ethanol. Each sample is geo-referenced to 1 square kilometer grid and the age, sex, estimation of physical condition via rump fat, and if appropriate number of antler points and greatest outside spread in inches was recorded for

each deer. Eighty five percent of the samples taken from hunter harvested deer are from male deer with the majority being 2.5 years of age and older.

2. **DEFINITIONS:** The terms listed below shall have the meanings assigned to them below. Additional definitions can be found in section 2 of the General Terms and Conditions.

2.1 **"Contract Services"** means reporting, analyzing, writing, and publishing scientific results of a major genetic project dealing with Chronic Wasting Disease (CWD) in white-tailed deer in West Virginia

2.2 **"Pricing Page"** means the pages upon which Vendor should list its proposed price for the Contract Services. The Pricing Page is either included on the last page of this RFQ or attached hereto as Exhibit A.

2.3 **"RFQ"** means the official request for quotation published by the Purchasing Division and identified as DNR214058.

2.4 **"CWD"** means Chronic Wasting Disease.

2.5 **"Ph.D. STUDENT"** means Doctor of Philosophy in Wildlife Management student.

2.6 **"DNA"** means deoxyribonucleic acid which is a self-replicating material present in nearly all living organisms as the main constituent of chromosomes and it is the carrier of genetic information.

2.7 **"mtDNA"** means mitochondrial DNA.

2.8 **"GIS"** means geographic information system.

2.9 **"PRNP"** means prion protein.

2.10 **"SNP"** means single nucleotide polymorphism.

2.11 **"GenBank DATABASE"** is an open access, annotated collection of all publicly available nucleotide sequences and their protein translations.

3. **QUALIFICATIONS:** Vendor shall have the following minimum qualifications:

   3.1. Offer a Ph.D. in wildlife management, wildlife biology or wildlife ecology from an accredited university. Documentation will be done by supplying a list of majors within a respective unit of the accredited university.

   3.2. At least one faculty member within their college or university who has a Ph.D. specialty in genetics who is willing to serve on the student's committee. If the principal investigator is not the faculty with a specialty in genetics, a letter on University letterhead stating their willingness to serve on the committee will serve as documentation.

   3.3. Summaries of and references from at least five (5) studies dealing with natural barriers, genetic flow or microsatellite loci published in peer reviewed scientific journals by the vendor's anticipated academic advisor to the prospective student. Bidders shall provide the publication name, the date on which the study was published, the name of the study (as published), and the abstract (i.e., scientific citation plus abstract). A full version of each study must be available for review at request of the Agency at no cost (bidders' including full copies or reprints of each study with the bid is acceptable). Bidders should use Attachment B, Study References Sheet, to provide this information, or provide reprints.

   3.4. Documentation of a GIS specialist within their college or university who is willing to serve on the student's committee. If the principal investigator is not the faculty with a specialty in GIS, a letter on University letterhead stating their willingness to serve on the committee will serve as documentation.

   3.5. All documentation required to indicate the vendor meets these qualifications should be included with the vendor's bid, but must be supplied prior to award of the Contract.

4. **MANDATORY REQUIREMENTS:**

   4.1 Mandatory Contract Services Requirements and Deliverables: Contract Services must meet or exceed the mandatory requirements listed below.

      4.1.1 Genetic neighborhoods based on relatedness between individuals. Fifteen microsatellite loci and the control region of the mitochondrial DNA from female white-tailed deer will be used to measure genetic relatedness between individuals and to delineate neighborhoods of related

individuals. Mitochondrial DNA is maternally inherited and will be used to identify maternal lineages.

4.1.2 **Dispersal patterns of male white-tailed deer.** Male deer will be analyzed using 15 microsatellite loci and mitochondrial DNA to assign the deer to the neighborhood from which they most likely originated and dispersed.

4.1.3 **Barriers to white-tailed deer dispersal.** Genetic breaks in the landscape will be identified using GIS and the corresponding features in the landscape that are causing that break in gene flow will be determined.

4.1.4 **Relationship between genetic diversity at the PRNP gene and CWD prevalence.** There is a genotype at the prion protein gene (PRNP) that is associated with CWD resistance. Using a single nucleotide polymorphism (SNP) assay, all individuals will be genotyped at the 95th, 96th, and 116th codon of the PRNP gene. A correlation between frequency of the CWD-resistant genotype and CWD prevalence will be tested.

4.1.5 **Long-term storage of genetic data.** Generated genotypes and sequence data will need to be archived and compiled in a database for potential future use. Novel mitochondrial haplotypes should be inputted into the GenBank database. Data must be assessable for at least 30 years in case of future advancements in CWD work.

4.1.6 **Original research.** Because most universities require Ph.D. students to design and conduct their own research, the student will also be encouraged to develop and test additional hypotheses that examine how genetics may be used to further the advancement of management practices as they relate to CWD in white-tailed deer. However, the student's first priority will be to complete the major objectives as listed above.

4.2 Performance Schedule

4.2.1 August 2014 Ph. D. Student begins classes.

4.2.1.1 By December 2014, Ph.D. Student must have first committee meeting and select committee.

4.2.2 Microsatellite genotyping and mtDNA sequencing of all samples shall be performed, with:

4.2.2.1 Report detailing completion of 4.2.2 due by August 31, 2015. Report will be submitted to Supervisor of Game Management Services and Cervid Project Leader via e-mail in either Word or PDF format and will detail completion percentage of said task.

4.2.3 Landscape genetic analysis overlaying genetic & GIS assignment of male deer to neighborhoods shall be performed, with:

4.2.3.1 Report detailing completion of 4.2.3 due by February 28, 2015. Report will be submitted to Supervisor of Game Management Services and Cervid Project Leader via e-mail in either Word or PDF format and will detail completion percentage of said task.

4.2.4 SNP assay of all samples shall be performed, with:

4.2.4.1 Report detailing completion of 4.2.4 due by October 31, 2016. Report will be submitted to Supervisor of Game Management Services and Cervid Project Leader via e-mail in either Word or PDF format and will detail completion percentage of said task.

4.2.5 Complete analysis of genetic data and preparation of manuscripts shall be performed, with:

4.2.5.1 Final report detailing all contract services must be complete and turned into Agency by July 31, 2017. Final report will be submitted to Supervisor of Game Management Services and Cervid Project Leader via e-mail in either Word or PDF format and will document 100% completion of all requirements.

4.2.6 Assignment of committee.

4.2.6.1 Either or both of the West Virginia Division of Natural Resources Cervid Project Leader or the Supervisor of Game Management Services will be a member of the student's committee and invited to all committee meetings.

4.2.7 Publication of results.

4.2.7.1 Either or both of the West Virginia Division of Natural Resources Cervid Project Leader or the Supervisor of Game Management Services will be a co-author on all publications or presentations.

4.2.8 Annual reports.

4.2.8.1 Annual reports are due to the Cervid Project Leader by August 1 of each respective year. Report will be submitted to Supervisor of Game Management Services and Cervid Project Leader via e-mail in either Word or PDF format and will detail completion percentage of said task that were to be completed during the respective year.

5. CONTRACT AWARD:

5.1 Contract Award: The Contract is intended to provide Agency with a purchase price for the Contract Services. The Contract shall be awarded to the Vendor that provides the Contract Services meeting the required specifications for the lowest overall TOTAL BID (Combine All Years), as shown on the Pricing Page (Attachment A). The initial Contract shall be awarded for the Year One (1) Total only, effective for the first year of the Contract, with Years Two (2) and Three (3) added by subsequent annual renewal change orders.

5.2 Pricing Page: Vendor should complete the Pricing Page by filling in the Unit Price for each item listed, this Unit Price shall include all costs related to the project including salaries, fringe benefits, travel, supplies and any other incidentals required. Vendor should complete the Pricing Page in full as failure to complete the Pricing Page in its entirety may result in Vendor's bid being disqualified.

Notwithstanding the foregoing, the Purchasing Division may correct errors as it deems appropriate. Vendor should enter the information into the Pricing Page to prevent errors in the evaluation.

6. **PERFORMANCE:** Vendor and Agency shall agree upon a schedule for performance of Contract Services and Contract Services Deliverables, unless such a schedule is already included herein by Agency. In the event that this Contract is designated as an open-end contract, Vendor shall perform in accordance with the release orders that may be issued against this Contract.

7. **PAYMENT:** Agency shall pay the annual unit price as shown on the Pricing Pages, for all Contract Services performed and accepted under this Contract. Vendor shall accept payment in accordance with the payment procedures of the State of West Virginia. Vendor shall bill Agency monthly for costs accrued during that pay period.

8. **TRAVEL:** Vendor shall be responsible for all mileage and travel costs, including travel time, associated with performance of this Contract. Any anticipated mileage or travel costs may be included in the flat fee or hourly rate listed on Vendor's bid, but such costs will not be paid by the Agency separately.

9. **FACILITIES ACCESS:** Performance of Contract Services may require access cards and/or keys to gain entrance to Agency's facilities. In the event that access cards and/or keys are required:

   9.1. Vendor must identify principal service personnel which will be issued access cards and/or keys to perform service.

   9.2. Vendor will be responsible for controlling cards and keys and will pay replacement fee, if the cards or keys become lost or stolen.

   9.3. Vendor shall notify Agency immediately of any lost, stolen, or missing card or key.

   9.4. Anyone performing under this Contract will be subject to Agency's security protocol and procedures.

   9.5. Vendor shall inform all staff of Agency's security protocol and procedures.

10. VENDOR DEFAULT:

10.1. The following shall be considered a vendor default under this Contract.

10.1.1. Failure to perform Contract Services in accordance with the requirements contained herein.

10.1.2. Failure to comply with other specifications and requirements contained herein.

10.1.3. Failure to comply with any laws, rules, and ordinances applicable to the Contract Services provided under this Contract.

10.1.4. Failure to remedy deficient performance upon request.

10.2. The following remedies shall be available to Agency upon default.

10.2.1. Cancellation of the Contract.
10.2.2. Cancellation of one or more release orders issued under this Contract.
10.2.3. Any other remedies available in law or equity.

11. MISCELLANEOUS:

11.1. Contract Manager: During its performance of this Contract, Vendor must designate and maintain a primary contract manager responsible for overseeing Vendor's responsibilities under this Contract. The Contract manager must be available during normal business hours to address any customer service or other issues related to this Contract. Vendor should list its Contract manager and his or her contact information below. The Contract manager for this project will be the academic advisor of the Ph.D. Student.

Contract Manager: Amy Welsh
Telephone Number: 304-293-0718
Fax Number: 304-293-2441
Email Address: Amy.Welsh@mail.wvu.edu

Detection of natural barriers to movement of lake sturgeon (*Acipenser fulvescens*) within the Namakan River, Ontario

A.B. Welsh and D.T. McLeod

Abstract: Many populations of lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817) are below historic population sizes, and migration barriers have likely contributed to some of these population declines. Dams and natural barriers can potentially isolate populations along a single river and can have a strong effect on the ability of lake sturgeon to move upstream. Along the Namakan River in Ontario, Canada, a series of natural rapids could impede movement of lake sturgeon and fragment the sturgeon into several small populations. Movement patterns of lake sturgeon were assessed using genetics and acoustic telemetry. Samples were collected from five locations along the river, each one separated by a rapid or falls, and were analyzed at 12 microsatellite loci. No significant genetic differences were observed between the five segments, indicating that the groups of lake sturgeon are not isolated. There were no significant differences in genetic diversity between the five segments. Therefore, migration is likely occurring both upstream and downstream. The acoustic telemetry study also confirmed bidirectional movement of adult fish. The natural rapids and falls along the Namakan River do not appear to be a significant barrier to movement of lake sturgeon, and the lake sturgeon within this river represent a single population.

Résumé : Plusieurs populations d'esturgeons jaunes (*Acipenser fulvescens* Rafinesque, 1817) se retrouvent à des densités inférieures à celles du passé et il est vraisemblable que des barrières à la migration aient contribué au déclin de certaines de ces populations. Les barrages et les barrières naturelles peuvent potentiellement isoler les populations le long du cours d'une même rivière et affecter fortement la capacité des esturgeons jaunes à se déplacer vers l'amont. Le long de la rivière Namakan en Ontario, Canada, une série de rapides naturels pourrait entraver le déplacement des esturgeons jaunes et les séparer en plusieurs petites populations. Nous avons évalué les patrons de déplacement des esturgeons jaunes à l'aide de la génétique et de la télémétrie acoustique. Nous avons prélevé des esturgeons à cinq sites sur le cours de la rivière, chacun séparé par un rapide ou une chute, et procédé à une analyse génétique de 12 locus microsatellites. Il n'y a aucune différence génétique significative entre les cinq segments, ce qui indique que les groupes d'esturgeons jaunes ne sont pas isolés. Il n'y a pas non plus de différence significative de diversité génétique entre les cinq segments. Il se produit donc vraisemblablement de la migration tant vers l'amont que vers l'aval. La télémétrie acoustique confirme aussi les déplacements des poissons adultes dans les deux directions. Les rapides naturels et les chutes le long de la Namakan ne semblent pas constituer des barrières significatives aux déplacements des esturgeons jaunes qui forment donc une seule population dans cette rivière.

[Traduit par la Rédaction]

Introduction

The lake sturgeon (*Acipenser fulvescens* Rafinesque, 1817) has a wide range throughout North America, including the Great Lakes and St. Lawrence River, Lake Winnipeg, Hudson Bay, and the Mississippi River systems. Many populations throughout their range are reduced in size relative to historic population numbers mainly owing to overfishing and habitat modifications (Peterson et al. 2007). Although fishing has been restricted in many jurisdictions, habitat changes, such as the construction and operation of dams, continue to have an effect on some populations of lake sturgeon. On the Ottawa River, greater abundance and faster growth of lake sturgeon were observed on reaches that were not impounded (Haxton and Findlay 2008). On the Mattagami River, hydroelectric operations appeared to have an effect on reproductive development of lake sturgeon (McKinley et al. 1998). Flow regimes on the Sturgeon River had an impact on spawning activity of sturgeon (Auer 1996*a*). In the Red River of the North basin, lake sturgeon have been extirpated owing to dams blocking access to historic spawning grounds (Aadland et al. 2005).

Studies on movements of lake sturgeon can provide additional information on the potential impacts of natural and artificial barriers. Current studies offer conflicting information, with some reporting large migration distances and others reporting minimal movement. In the Great Lakes, a range of adult migration distances from 32 to 225 km have been reported (reviewed in Auer 1996*b*). Movements of

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juveniles appear to be shorter (Holtgren and Auer 2004; Smith and King 2005). In the upper Mississippi River, movements of lake sturgeon ranged from 3 to 198 km, with fish moving both upstream and downstream past dams (Knights et al. 2002). In the Ottawa River, movements of four radio-tracked lake sturgeon were limited (Haxton 2003). The sturgeon remained in their respective basins and traveled a maximum distance of 10 km.

The Namakan River in Ontario, Canada, contains several rapids and falls that may act as potential barriers to complete migration along the river. The river connects Lac La Croix to Namakan Lake and ultimately flows into the Rainy River – Lake of the Woods within the Lake Winnipeg drainage system (Fig. 1). At least nine natural rapids and four falls exist along the river and the objective of this study was to determine if any of these rapids significantly limit movement of lake sturgeon along different stretches of the river. Lake sturgeon are known to occur throughout the Namakan River from Lac La Croix downstream to Namakan Reservoir. An understanding of current movement of lake sturgeon along the river and an identification of natural barriers can help evaluate the potential effects of construction of proposed generation sites.

We tested the hypothesis that areas of rapid elevation change (rapids and falls) along the Namakan River would present significant barriers to adult lake sturgeon movement throughout the system. We predicted that the rapids may not impede downstream movement in the system, but upstream movement would be limited. This prediction was tested using genetic analysis and acoustic telemetry. Insignificant genetic differentiation between groups on either side of the rapids would result from downstream movement. However, higher levels of genetic diversity would be expected at downstream locations owing to the higher level of immigration (Jager et al. 2001). Upstream locations would be expected to have a lower number of alleles and lower heterozygosity. Significant differences in upstream and downstream movements of adult sturgeon tracked with acoustic telemetry would also confirm the prediction.

Materials and methods

Study site

The Namakan River is located immediately downstream of Lac La Croix and upstream of Namakan Reservoir (Fig. 1), approximately 80 km southeast of Fort Frances, Ontario. This mesotrophic river is found in the southern range of the boreal forest in North America and is typical of Canadian Shield lakes and rivers with soft water and little submerged aquatic vegetation. The Namakan River drains close to 8860 km^2 in Ontario with an elevation drop of 19.2 m over a distance of 30.5 km from Lac La Croix to Namakan Reservoir (Ojibway Power and Energy Group 2007).

A number of potential barriers to fish migration exist along the river from the outlet of Lac La Croix downstream to the Namakan Reservoir. The following elevation changes are reported for the various rapids or falls under average flow conditions: 3.2 m at Snake Falls (29 river kilometre (rkm) upstream), 4.0 m at Myrtle Falls and Ivy Falls (25 rkm upstream), 1.0 m at Twisted Rapids (20 rkm upstream), 0.7 m at Quetico Rapids (14.7 rkm upstream), 6.8 m at High Falls (11.7 rkm upstream), 7.0 m at the Back Channel (over 2 km and 8–9 rapids; 10.2 rkm upstream), 3.0 m at Hay Rapids (7.4 rkm upstream), and 1.6 m at Lady Rapids (4 rkm upstream) (Fig. 1) (Ojibway Power and Energy Group 2007).

Water levels and flows in the Namakan River are not regulated. A Meteorological Service of Canada (Environment Canada) water-level gauge at the outlet of Lac La Croix provides relevant information on inflows to the Namakan River since 1921 (Lake of the Woods Control Board 2008). A maximum flow of 771 m^3/s was recorded in June 1950, while a minimum flow of 15 m^3/s was recorded in February 1924 and January 1977. Annual flow metrics derived from a recent 20-year period (1980–1999) provided a mean and median flow of 118 and 87 m^3/s, respectively. Time exceeded (percentile) flows are estimated at 182 m^3/s (20%) and 51 m^3/s (80%).

Sample collection

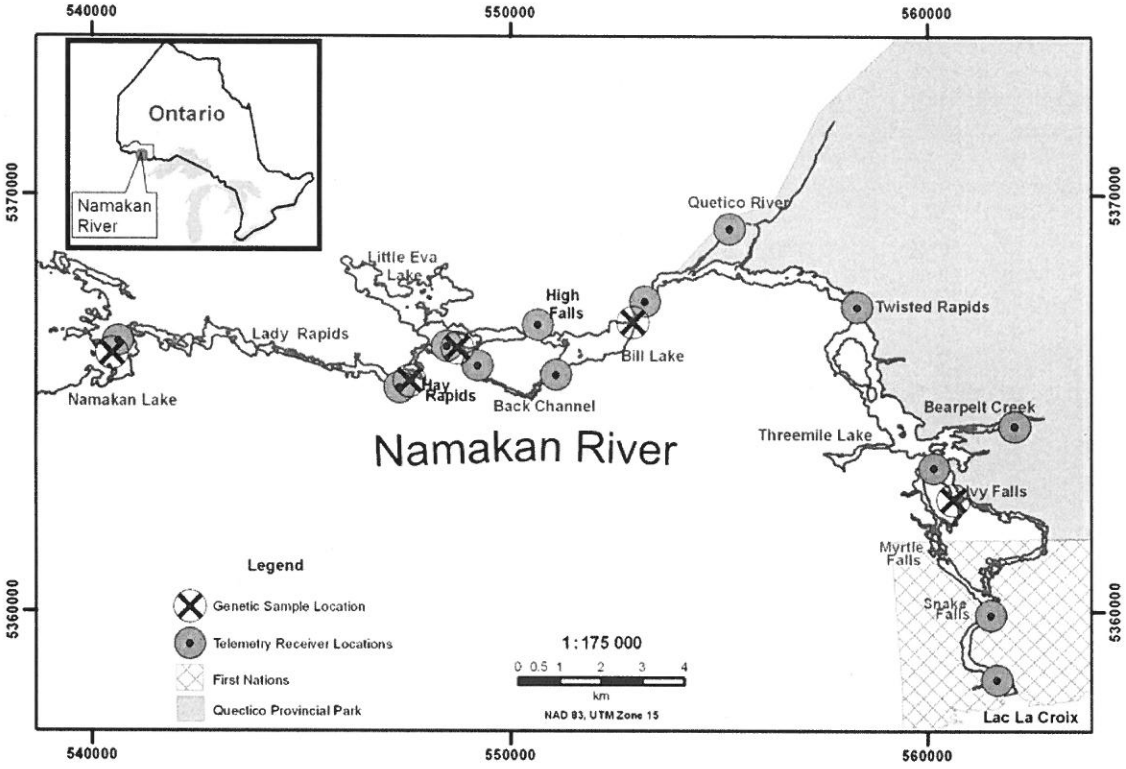
Fin clips from the tip of the pectoral fin were collected during spring 2007 from lake sturgeon at five suspected spawning sites along the Namakan River: (1) below Lady Rapids (n = 31), (2) below Hay Rapids (n = 30), (3) below the Back Channel (Little Eva Lake) (n = 31), (4) below Quetico Rapids (Bill Lake) (n = 14), and (5) below Ivy Falls (n = 23) (Fig. 1). Groups consisted of a mixture of mature and immature–developing fish, so not all the samples came from actively spawning adults. However, sampling was conducted during the active spawning season and water temperatures were within the range conducive for spawning.

Genetic analysis

Tissue samples were preserved in 95% ethanol. DNA was extracted using either the Promega Wizard SV 96 Genomic DNA Purification System or the Gentra Puregene Tissue Kit, according to manufacturers' protocols. Extracts were then quantified using either a microplate reader or a fluorometer. Twelve microsatellite loci were then amplified (*AfuG 9*, *AfuG 56*, *AfuG 63*, *AfuG 74*, *AfuG 112*, *AfuG 160*, *AfuG 195*, *AfuG 204*; *Afu 68*, *Afu 68b*; *Spl 120*; *Aox 27*; described in Welsh and May 2006). Polymerase chain reaction (PCR) reagents included 1 × PCR buffer, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L dNTPs, 0.2 μmol/L fluorescently labeled forward primer, 0.2 μmol/L unlabeled reverse primer, 0.25 U (1 U ≈ 16.67 nkat) Go*Taq* polymerase (Promega), and 20 ng of DNA. A BioRad iCycler was used and thermal cycling conditions for all loci (except *AfuG 56* and *Spl 120*) were as follows: 95 °C for 2 min; 40 cycles of 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 45 s; 72 °C for 7 min, ending with a 4 °C hold. Thermal cycling conditions for *AfuG 56* and *Spl 120* were 94 °C for 1 min; 20 cycles of 92 °C for 30 s and 70 °C for 40 s with a 0.5 °C decrease in the second step each cycle; 20 cycles of 92 °C for 30 s and 60 °C for 40 s with a 1 s increase in the second step each cycle; ending with a 4 °C hold. PCR products were then pooled into three groups and visualized on a Beckman Coulter CEQ 8000 Genetic Analysis System.

Each sampled group was tested for conformance to Hardy–Weinberg equilibrium (HWE) and for linkage disequilibrium between locus pairs using the software GDA (Lewis and Zaykin 2001). The five sampled groups were also pooled together to determine if the river as a whole

Fig. 1. Location of genetic sampling sites and VR2W acoustic telemetry receivers in the Namakan River, Ontario, Canada.



was in HWE. Genetic differentiation between groups along the Namakan River was measured using the Weir and Cockerham (1984) estimator of F_{ST}, which was estimated using the software Arlequin (Schneider et al. 2000), and pairwise contingency tests of allele frequency heterogeneity (Raymond and Rousset 1995), which was estimated using the software TFPGA (Miller 1997). F_{ST} values can range from 0 to 1, with 0 signifying no genetic differentiation and 1 indicating complete differentiation at all loci. The significance of the pairwise F_{ST} comparisons was based on 3024 permutations. For the pairwise contingency tests, 10 batches of 2000 permutations each were run, with 1000 dememorization steps. Significance of the HWE, linkage disequilibrium, pairwise F_{ST}, and contingency tests was assessed after a sequential Bonferroni correction (Rice 1989). Genetic distance was also calculated and a Mantel test (Mantel 1967) was performed to determine if there was a correlation between genetic distance and geographic distance and the number of potential barriers. Significance of the Mantel test was based on 999 permutations and the analysis was performed using the software GENALEX (Peakall and Smouse 2006).

Genetic diversity of each group along the Namakan River was also measured using heterozygosity and allelic richness (number of alleles corrected for differences in sample size; El Mousadik and Petit 1996). TFPGA was used to calculate heterozygosity and the software FSTAT (Goudet 2001) was used to measure allelic richness. Significant differences in genetic diversity were tested using Student's t tests. A regression was done to determine if there was a correlation between genetic diversity (i.e., heterozygosity and allelic richness) and distance upstream and number of potential barriers.

Acoustic telemetry

Lake sturgeon were first captured using large mesh (203–305 mm stretched mesh) multifilament gill nets in May 2007. All fish were sampled for total and fork length (mm), girth (mm), and round mass (g); tagged with an individually numbered Carlin disk dangler tag; and released live. A 3–4 cm section of the large, marginal ray of the left pectoral fin was removed for age determination. Thirty individual fish were selected for surgical implantation of acoustic transmitters (V16–4L; Vemco – Amirix Systems Inc., Halifax, Nova Scotia, Canada) at four different sample locations: below Hay Rapids (n = 10), below the Eva Island back channel in Little Eva Lake (n = 10), below Quetico Rapids in Bill Lake (n = 5), and below Ivy Falls in Three Mile Lake (n = 5). The transmitters operated at 69 kHz, were 68 mm × 16 mm in size, and weighed 10 g in water, therefore the transmitters did not exceed 2% of the total body mass for any given fish. Each transmitter emitted a unique code on a random interval of 60–120 s with a programmed operating life of 2190 days.

Surgical procedures followed guidelines by Hart and Summerfelt (1975). A 3–5 cm incision was made with a surgical scalpel on the ventral surface approximately 1 cm off the midline and 3–4 cm anterior to the pelvic girdle. The transmitter was inserted into the abdominal cavity with minimal pressure exerted on the internal organs. Following implantation, the peritoneum and associated muscle tissue were closed with a continuous modified Cushings suture technique (3–0

Ethicon PDS II, 1/2″ CT-2 needle) followed by five simple interrupted sutures (2–0 Ethicon Prolene, 1/2″ SH needle) to close the skin. Postoperative fish were immediately released at the surgical site, which was in close proximity to the capture site. Use of animals was reviewed and approved by the Ontario Ministry of Natural Resources following interim animal care protocols.

An array of 13 submersible acoustic receivers (VR2W; Vemco) with Bluetooth wireless download capability was used to collect data on locations and movements of lake sturgeon. The receivers were 308 mm × 73 mm in size and weighed 1450 g in air, with an 8 MB flash memory (1 million detections). Each receiver contained a 3.6 V lithium battery with an expected operating life of 12–15 months. At selected sample locations, each stationary receiver was suspended vertically approximately 1 m off bottom with a nylon rope, 15 kg cement anchor, and round net buoy in water depths of 3–6 m to avoid winter freeze-up. Anchors were also attached to an exposed shore anchor or treed shrub with 20–30 m of lead core rope to provide easy deployment and retrieval.

The vendor-provided interface software (Vemco User Environment, VUE) was used for initialization, configuration, data upload, and storage from each receiver. The VUE software package also allowed data from multiple receivers and transmitters to be combined into a single integrated database. Each submersible receiver detects and decodes the ultrasonic pulses from transmitters within approximately 500 m, logging the date, time, and individual transmitter code for each detection to internal storage.

Telemetry data obtained from fish implanted with transmitters were used to examine movement of fish through rapids or falls, and range of travel within the Namakan River. Movement of individual lake sturgeon was determined by recording the first daily detection at each station for every fish detected, and their range within the river was determined using detections from the two extreme receiver stations traveled. A χ^2 test was performed to determine if there were significant differences in upstream and downstream movement across potential barriers.

Results

Genetic analysis

All loci in all five sampled groups were in HWE and locus pairs showed no evidence of linkage disequilibrium. When the five sampled groups were pooled together as a single population, all loci remained in HWE. There were no significant genetic differences between the five spawning locations along the Namakan River (Table 1). Pairwise F_{ST} values indicated low levels of genetic variation, ranging from 0.00 to 0.03. All F_{ST} values and pairwise contingency values were not significant. Genetic distance was not correlated with either geographic distance ($R^2 = 0.002$, $p = 0.075$) or the number of potential barriers ($R^2 = 0.002$, $p = 0.065$) (Figs. 2A, 2B).

Mean observed heterozygosity across all 12 loci ranged from 0.31 to 0.35. Mean allelic richness ranged from 2.30 to 2.54. Heterozygosity and allelic richness values were similar among the five different groups (Figs. 3A, 3B). There were no significant differences in levels of genetic diversity between any of the five groups. Genetic diversity was not correlated to the distance upstream (heterozygosity: $R^2 = 0.000$, $p = 0.99$; allelic richness: $R^2 = 0.008$, $p = 0.88$) or to the number of potential barriers (heterozygosity: $R^2 = 0.015$, $p = 0.85$; allelic richness: $R^2 = 0.059$, $p = 0.69$).

Acoustic telemetry

Lake sturgeon implanted with transmitters had a mean total length of 1211 mm (863–1662 mm), mean girth of 426 mm (329–659 mm), mean round mass of 11 453 g (4 250 – 30 800 g), and mean age of 27.9 years (16–47 years). Based on the round mass of individual fish, implanted transmitters ranged from 0.03% to 0.23% of body mass. Sex could only be determined on 15 of the implanted fish (7 females and 8 males). All of the implanted lake sturgeon were adults at various stages of sexual development.

Eleven submersible receivers were deployed in the Namakan River on 15–25 May 2007. Two additional receivers were deployed on 30 April and 22 May 2008 below and above Snake Falls to investigate potential movements of telemetered fish through Myrtle, Ivy, and Snake falls in the upper most reaches of the Namakan River (Fig. 1). A total of 1 109 290 detections were recorded throughout the Namakan River over the 2007–2008 sampling period (to 21 October 2008). The maximum number of detections from a single fish was 196 709, while the minimum was 249. In addition, one individual fish was detected at 11 of the 13 stations, over a distance of 28.8 km. Three individuals were detected at one station only, and the mean number of receivers at which an individual fish was detected was 4.2 (SD = 2.8). Each receiver detected a mean of 15.0 fish (SD = 9.7) with a range of 0–33.

Movements of individual fish through shallow rapids and falls along the river were also evaluated based on detections from both upstream and downstream receivers (Table 2). Movements through proposed hydro development sites at Hay Rapids, Hay Falls, Back Channel, and Ivy–Myrtle falls were documented, as well as all other undeveloped sites along the Namakan River and Quetico River. The only exceptions were that no movements were recorded through Snake Falls or upstream at High Falls. The maximum number of movements ($n = 64$) was observed at Twisted Rapids at the outlet of Three Mile Lake, and were equally distributed between upstream and downstream over the sampling period. The most significant observations were seven recorded downstream movements of five individual fish over High Falls, an elevation drop of 6.8 m. In addition, both upstream and downstream movements of lake sturgeon through 8–9 shallow rapids in the Back Channel first occurred in October 2007, with a significantly greater number of upstream movements ($p < 0.05$) (Table 2). Of the 19 recorded fish movements, the majority (74%) were moving upstream from Little Eva Lake to Bill Lake.

Discussion

Lake sturgeon in this telemetry study represented a broad segment of the adult population, with total lengths ranging from 605 to 1746 mm and ages ranging from 16 to 47 years. Lake sturgeon can be highly mobile and exhibit complex behaviour patterns, especially in large systems where movements are not restricted. This study confirmed a smaller range of movements from 0 to 29 km, which represents the entire distance of the Namakan River to below Snake Falls.

Table 1. Genetic differentiation between lake sturgeon (*Acipenser fulvescens*) from five spawning locations along the Namakan River.

	Lady Rapids	Hay Rapids	Back Channel	Quetico Rapids	Ivy Falls
Lady Rapids (n = 31)		24.07	24.05	24.60	20.53
Hay Rapids (n = 30)	0.01 (0.19)		25.19	31.53	23.00
Back Channel (n = 31)	0.00 (0.27)	0.01 (0.13)		21.96	31.25
Quetico Rapids (n = 14)	0.00 (0.38)	0.03 (0.04)	0.00 (0.68)		29.38
Ivy Falls (n = 23)	0.01 (0.21)	0.01 (0.25)	0.01 (0.08)	0.02 (0.10)	

Note: Chi-square values from pairwise contingency tests (above the diagonal) and pairwise F_{ST} values (below the diagonal). Values in parentheses are the p values of F_{ST}. No comparisons were significant after a sequential Bonferroni correction.

Fig. 2. Correlation between genetic distance in lake sturgeon (*Acipenser fulvescens*) and spatial measures in the Namakan River. Correlations were not significant ($p > 0.05$). (A) Genetic distance versus geographic distance (km) ($R^2 = 0.002$). (B) Genetic distance versus number of potential barriers ($R^2 = 0.002$).

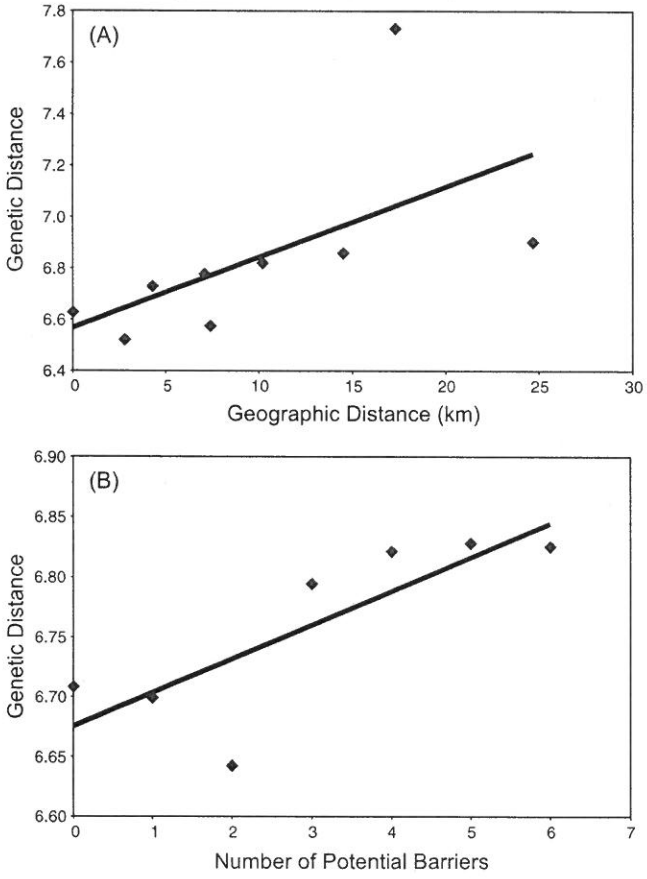
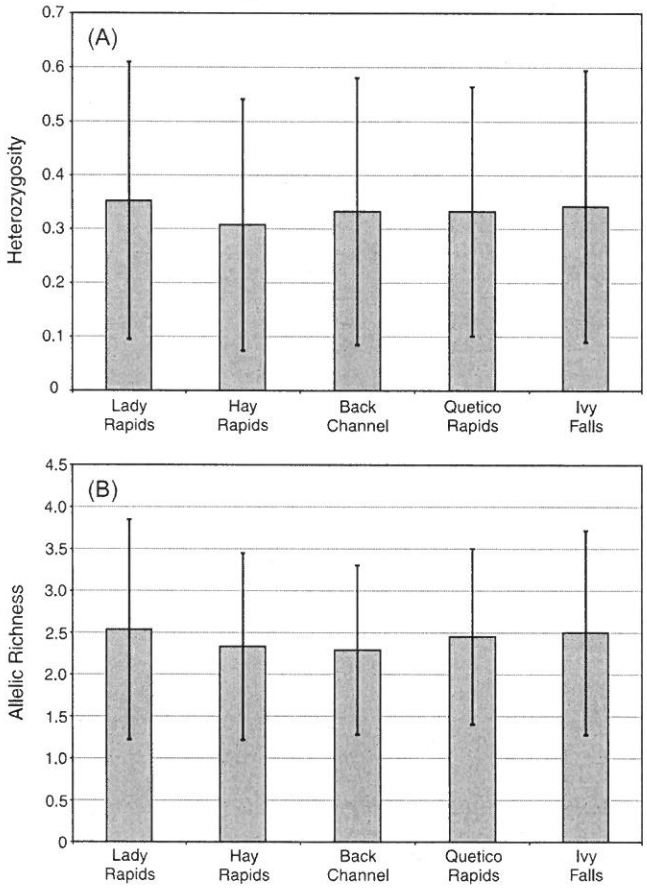


Fig. 3. Genetic diversity of the five putative spawning groups of lake sturgeon (*Acipenser fulvescens*) along the Namakan River. Standard deviations are also displayed. There were no significant differences in genetic diversity observed between the sampled groups. (A) Observed heterozygosity averaged across all 12 loci. (B) Allelic richness averaged across all 12 loci.



The total distance travelled by these fish will likely increase as additional movements are documented downstream in the Namakan Reservoir. Several fish (n = 6) in this study showed very limited movement with detections at only one receiver location. The extent of movement within a season was also highly variable among fish; some fish readily moved among habitats, while movements of others were more constrained. Monthly movements within the Namakan River indicate that individual lake sturgeon travelled over a range of 0 to 48.9 km/month from May to October. No movement of fish was observed between receiver locations from November to April over the two study years. In the Namakan River, lake sturgeon appear to move upstream in the late summer and fall to possibly forage and overwinter in lake environments, as well as in early spring to reach potential upstream spawning areas (McLeod and Debruyne 2009).

Both genetic and acoustic telemetry data suggest that the putative spawning groups along the Namakan River represent a single population. If the rapids or High Falls along the river have presented a long-term barrier to migration,

Table 2. Upstream and downstream movements of telemetered lake sturgeon (*Acipenser fulvescens*) through undeveloped rapids or falls in the Namakan River, Ontario, from 15 May 2007 to 21 October 2008.

Location	Elevation (m)	Upstream	Downstream	Total	χ^2
Lady Rapids	1.6	20	24	44	0.36
Hay Rapids	**3.0**	**21**	**17**	**38**	**0.42**
Back Channel (Eva Island)	**7.0**	**14**	**5**	**19**	**4.26***
High Falls	**6.8**	**0**	**7**	**7**	**7.00***
Quetico Rapids	0.7	20	20	40	0.00
Quetico River	—	1	1	2	0.00
Twisted Rapids	—	32	32	64	0.00
Bearpelt Creek	—	2	2	4	0.00
Ivy–Myrtle falls	**4.0**	**6**	**6**	**12**	**0.00**
Snake Falls	3.2	0	0	0	—

Note: Locations are listed from downstream to upstream, and proposed hydro development sites are in boldface type. Change in elevation based on a mean flow rate of 120 m^3/s. Significant χ^2 values ($p < 0.05$) are denoted with an asterisk.

genetic differences would have likely accumulated between the groups. Instead, no significant genetic differences were observed between the groups. However, the level of genetic differentiation was close to significant between Quetico Rapids and Hay Rapids (without a sequential Bonferroni correction) and may be due to the lack of statistical power resulting from the small sample size at Quetico Rapids. This lends support for the apparent lack of upstream movement directly at High Falls identified in the telemetry portion of the study. When the five sampled groups were pooled together, the lake sturgeon in the river as a whole were in HWE. If deviations from HWE were observed, it may have provided evidence for the presence of multiple populations (Wahlund effect; Wahlund 1928). The lack of HWE deviations indicates that the lake sturgeon at Namakan River represent a single population. Individuals also do not become more genetically distant at more geographically distant stretches of the river, indicating that movements of lake sturgeon are currently unimpeded along the Namakan River.

Additionally, there were no significant differences in genetic diversity between the five groups and genetic diversity did not decrease with increasing upstream distance, indicating that migration is likely occurring in both directions along the river. Asymmetrical movement would likely result in differences in genetic diversity along the river. If lake sturgeon were primarily moving downstream, upstream populations would be expected to have lower genetic diversity owing to the lack of migration into those populations (Jager et al. 2001).

Telemetry findings confirm movements through all natural constrictions in the system with the exception of Snake Falls and upstream movement at High Falls, which have an elevation drop of 3.8 and 6.8 m, respectively, under mean flow conditions. Although the Back Channel around Eva Island and High Falls have an elevation change of approximately 7.0 m, the numerous shallow rapids help dissipate this change over a distance of approximately 2 km. Lake sturgeon appear to use this natural bypass channel to migrate both upstream and downstream around High Falls. Upstream movement was greater than downstream movement in the Back Channel and this route likely compensates for the lack of upstream movement at High Falls. The return in spring 2008 of 8 fish that departed the river during summer–fall 2007, and the upstream movement of another 13 fish from the reservoir in 2008 indicates a high degree of preference to the Namakan River.

Effects of river fragmentation can vary depending on the type of barrier and the life histories of the species. In the Menominee River, Michigan, USA, the population of lake sturgeon was fragmented into sections by hydroelectric dams (Thuemler 1997). Knights et al. (2002) also found that dams appeared to be intermittent barriers to upstream passage. However, the genetic effects of fragmentation from dams may not be as apparent as the effects of natural barriers owing to differences in time since fragmentation (Deiner et al. 2007). The genetic effects of dams may also be temporarily masked in long-lived species with long generation times like the lake sturgeon. The putative natural barriers on the Namakan River have likely been in place for a sufficient amount of time to permit genetic divergence if the rapids were true migration barriers. Species characteristics also can provide insight into vulnerability to fragmentation. Haponski et al. (2007) suggested that nonmigratory fish may not become significantly isolated in the presence of a low-head dam. In contrast, habitat specialists and species inhabiting the edges of their range may be particularly vulnerable to fragmentation (Reid et al. 2008).

The genetic diversity observed in the Namakan River is lower than the diversity observed in other Hudson Bay – James Bay populations and Great Lakes populations (DeHaan et al. 2006; Welsh et al. 2008). The diversity is also lower than that observed for most freshwater fishes (mean heterozygosity = 0.46; DeWoody and Avise 2000). Possible reasons for low genetic diversity include reduced population size, inbreeding, or genetic drift. However, because the Namakan River population is in HWE, it is unlikely those attributes are responsible for the low levels of genetic diversity. Alternatively, the low levels of genetic diversity may be an artifact of the glacial history of the Hudson Bay drainage. Low genetic diversity in Hudson Bay populations has been observed in previous genetic studies (McQuown et al. 2003; Welsh et al. 2008). Evidence using mitochondrial DNA suggests that lake sturgeon in the Hudson Bay drainage may

have originated from a different glacial refugia than the current Great Lakes populations (Ferguson et al. 1993). Fewer postglacial dispersal routes into the Hudson Bay (Mandrak and Crossman 1992) and longer periods of glaciation may have resulted in lower genetic diversity in current populations relative to populations in the Great Lakes.

The rapids along the Namakan River do not represent reproductive barriers to lake sturgeon, and future management actions should preserve the integrity of this population. High Falls appears to be the only potential barrier to upstream fish passage. However, the Back Channel is providing a natural fish passage around High Falls, as the total elevation change is dissipated over 8–9 sets of shallow rapids. Fragmentation along rivers resulting from artificial barriers can lead to substantial genetic differentiation evolving within a few generations (e.g., Hänfling and Weetman 2006; Heggenes and Roed 2006). Continual upstream and downstream migration can maintain the genetic diversity along all the segments of the river (Jager et al. 2001; Reid et al. 2008) and prevent further erosion of the remaining genetic diversity in lake sturgeon along the Namakan River.

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Genetic Diversity of Striga hermonthica Populations in Ethiopia: Evaluating the Role of Geography and Host Specificity in Shaping Population Structure
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GENETIC DIVERSITY OF *STRIGA HERMONTHICA* POPULATIONS IN ETHIOPIA: EVALUATING THE ROLE OF GEOGRAPHY AND HOST SPECIFICITY IN SHAPING POPULATION STRUCTURE

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Striga hermonthica, a root hemiparasitic Orobanchaceae, severely constrains grain production in sub-Saharan Africa. Host specificity and geography may play important roles in shaping the population structure of *S. hermonthica*, with the Rift Valley potentially presenting a significant barrier to dispersal. Genetic diversity was assessed in 12 *S. hermonthica* populations from locations in Ethiopia. Of these, seven populations were parasitic on sorghum, two each on tef and maize, and one on finger millet. Genetic variation was detected using four amplified fragment length polymorphism (AFLP) primer combinations. After correcting for repeatability, 385 fragments were detected across all primer combinations. The percentage of polymorphic loci was relatively high, ranging from 53.2% to 76.4%. Expected heterozygosity ranged from 0.168 to 0.279. Genetic differentiation between populations was relatively high, and all populations were significantly different from each other. F_{ST} values ranged from 0.032 to 0.293 and averaged 0.146. Genetic differences between populations could not be attributed to host specificity. Instead, geography was the main determinant of population structure. There was a correlation between geographic and genetic distance. A significant portion of the genetic variance could be apportioned on either side of the Rift Valley (5%; $P = 0.001$). Also, a significant geographic barrier was identified in the southern portion of the sampled region.

Keywords: Striga hermonthica, genetic, AFLP, host specificity, geography, Ethiopia.

Introduction

Striga hermonthica (giant witchweed), a parasitic plant native to Ethiopia and Sudan (Musselman 1987), is known to cause substantial losses in cereal crop production across Africa and south Asia. Corn (*Zea mays* L.), sorghum (*Sorghum vulgare* Pers.), finger millet (*Eleusine coracana* [L.] Gaertn.), and tef (*Eragrostis tef* [Zucc.] Trotter) are among the staple foods threatened by giant witchweed. This parasitic plant currently affects up to 40% of Africa's crop production, and the annual crop yield losses in West African savannas alone account for $7 billion, affecting more than 100 million people (Emechebe et al. 2004).

The giant witchweed can adapt very quickly to different hosts and environments. Dawoud and Sauerborn (1994) showed that *S. hermonthica* can attain up to 50% germination under moisture regimes described as the permanent wilting point for its host, illustrating the potentially serious consequences this parasite can have in arid regions. Additionally, witchweed can tolerate wide ranges of day/night temperatures (25°/15°C–40°/30°C; Patterson et al. 1982), making it a successful parasite throughout its range. These characteristics render *S. hermonthica* a serious pest to cereal production, especially in the Sahel region (Senegal to Ethiopia), where it has developed two host-specific strains. The first is specific to millet, occurring in the drier and more northerly region of the Sahel, and the second attacks sorghum and is found farther south, in wetter regions (Musselman and Hepper 1986). In addition, this species has spread in Africa south to Angola and north to the Delta in Egypt, extending its range outside the continent to Yemen and Saudi Arabia (Mohamed et al. 2001).

The ability of *S. hermonthica* to withstand a wide range of climatic conditions (Patterson et. al. 1982; Dawoud and Sauerborn 1994) and parasitize different hosts (Ali et al. 2009) qualifies it to be considered among the most widely distributed known witchweeds with real invasive potential threatening cereal production worldwide (Mohamed et al. 2006). Therefore, it is difficult to develop universally resistant host crops, and crop breeding efforts toward obtaining resistant cultivars may need to take the view that *Striga* species are diverse at the intraspecific level. Instead, it may be better to focus efforts on controlling witchweed itself, particularly its spread. Host specificity and geography could potentially influence the spread of *S. hermonthica*. If host specificity and geography are significant evolutionary forces for witchweed, the population structure of the species should reflect genetic differences based on host or geographic barriers.

Limited studies on witchweed genetic diversity have been conducted, especially considering its wide range (Mohamed et al. 2007). *Striga hermonthica* is an obligate outbreeder (Safa et al. 1984), and its hybridization with *Striga aspera* has caused some taxonomic confusion (Aigbokhan et al. 2000). Allozyme electrophoresis of nine loci in two populations of *S. hermonthica* (pearl millet–adapted and sorghum-adapted populations) collected from Burkina Faso and one

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sorghum-adapted population from Sudan revealed high heterozygosity within each population ($H = 0.261 - 0.365$; Bharathalakshmi et al. 1990). That study showed that geographic distance played a greater role in genetic differentiation of *S. hermonthica* populations than host specialization. Similar evidence was presented for *S. hermonthica* parasitizing sorghum, pearl millet, maize, and wild grasses in Burkina Faso (Olivier et al. 1996, 1998), for six West African (Benin, Mali, and Burkina Faso) and nine Kenyan populations of *S. hermonthica* (Kuiper et al. 1996), and for *S. hermonthica* parasitizing maize, millet, and sorghum in Sudan (Ali et al. 2009). These studies indicate that low selectivity for hosts may be the trend in *S. hermonthica*. Genetic differentiation based on host specificity was shown to be greater between the millet and sorghum strains than between any of the maize strains (Ali et al. 2009). In summary, the genetic studies done to date show a general correlation between geographic distance and genetic distance and little evidence for host-specific witchweed populations.

The Rift Valley is a potential geographic barrier to the dispersal of *S. hermonthica* and may result in genetic differences between populations on the east and west sides of the valley. The Rift Valley is thought to have formed more than 2.5 million years ago, bisecting Ethiopia and essentially creating two land masses (Kingdon 1990). Previous studies on various plant and animal species have demonstrated the significance of this barrier in determining population structure in Ethiopia (e.g., Belay and Mori 2006; Kebede et al. 2007; Silvestrini et al. 2007).

Amplified fragment length polymorphisms (AFLPs; Vos et al. 1995) have been widely used in plant species, especially in crop plants (Bensch and Akesson 2005). The advantage of AFLPs is that hundreds of loci can be targeted, and no prior genetic information about the species is required. Additionally, many statistical methods and software packages are now available for AFLP analysis, allowing for the exploration of many population genetic questions (Bonin et al. 2007). Gethi et al. (2005) demonstrated the utility of AFLP analysis in *S. hermonthica* and identified four primer combinations that generated a large number of diverse fragments in the species.

The objective of our study was to identify which evolutionary force—host specificity or geography—is playing the greatest role in shaping genetic diversity within *S. hermonthica* in Ethiopia. By assessing the level of genetic differentiation between different geographic locations and between witchweed using different hosts, the influences of host specificity and geographic isolation on *S. hermonthica* evolution can be evaluated. We hypothesize that geographic barriers to dispersal, specifically the Rift Valley as a significant barrier, played a major evolutionary role in genetic differentiation in *S. hermonthica* populations.

Material and Methods

Sample Collection

Samples were collected in Ethiopia at the end of the growing season in November 2006. Samples were collected randomly from different *Striga* individuals parasitizing different individual host plants (table 1). Each sample came from a single *Striga* growing on a different individual host within the population. Young leaves were collected in the field, immediately placed in paper bags, and kept on dry silica gel. Upon return to the laboratory, the samples were placed on new and dry silica and were then refrigerated at 5°C. We collected samples from 12 populations of *Striga hermonthica* from 10 locations in central, northern, and eastern Ethiopia (table 1; fig. 1). Of these 12 populations, seven were parasitizing sorghum (*Sorghum vulgare* Pers.), two were parasitizing tef (*Eragrostis tef* [Zucc.] Trotter), two were parasitizing maize (*Zea mays* L.), and one was parasitizing finger millet (*Eleusine coracana* [L.] Gaertn.).

Laboratory Procedures

DNA was extracted from 10 randomly selected individuals from each of the sampled populations, using a standard CTAB extraction procedure (Doyle and Doyle 1987; Cullings 1992). Several representative samples were quantified, using a fluorometer to ensure that the extraction procedure worked and to optimize subsequent reactions. AFLPs were then analyzed. Each 10-μL digestion-ligation reaction consisted of 1X buffer, 0.05 M NaCl, 0.045 M BSA, 2 μM MseI adapter, 0.2 μM EcoRI adapter, 5 U MseI restriction enzyme, 5 U EcoRI restriction enzyme, 1 U T4 DNA ligase, and ~100 ng DNA. Samples were incubated at 37°C for 2 h. Each 20-μL preselective PCR reaction consisted of 15 μL of AFLP core mix (Applied Biosystems), 0.5 μM MseI-C primer, 0.5 μM EcoRI-A primer, and 4 μL of the digestion-ligation product. Thermocycler conditions were 72°C for 2 min; 20 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 2 min; 72°C for 2 min; and 60°C for 30 min. Each 10-μL selective PCR reaction consisted of 7.5 μL of AFLP core mix (Applied Biosystems), 0.25 μM MseI selective primer, 0.05 μM dye-labeled EcoRI selective primer, and 1.5 μL of the preselective PCR reaction. Selective primer combinations were EcoACT/MseCTC, EcoAGC/MseCTC, EcoACC/MseCTC, and EcoACC/MseCAT

Table 1

***Striga hermonthica* Collection Sites in Ethiopia**

Sample name	Locality and elevation (m)	Sorghum cultivar sampled
Sorghum1	10°03′N, 38°11′E, 1703	Land race
Sorghum2	12°59′N, 37°46′E, 1860	Land race
Sorghum3	13°17′N, 37°51′E, 1449	Land race
Millet4	13°32′N, 38°07′E, 1489	Land race
Tef5	13°32′N, 38°07′E, 1496	Improved variety
Tef6	14°12′N, 39°15′E, 1884	Land race
Sorghum7	13°05′N, 39°44′E, 1910	Meko, an improved variety
Maize8	12°11′N, 39°44′E, 2194	Farmer's variety
Sorghum9	10°17′N, 40°02′E, 1792	Land race
Sorghum10	08°44′N, 40°33′E, 1551	Land race
Maize11	09°08′N, 42°25′E, 1067	Farmer's variety
Sorghum12	09°08′N, 42°25′E, 1067	Gubiye, an improved variety

Note. Land races were farmer's domesticated indigenous sorghum, pearl millet, and tef varieties. Improved varieties were high-yielding varieties selected through breeding for certain areas. Farmer's varieties were nonindigenous maize cultivars but were left to hybridize naturally.

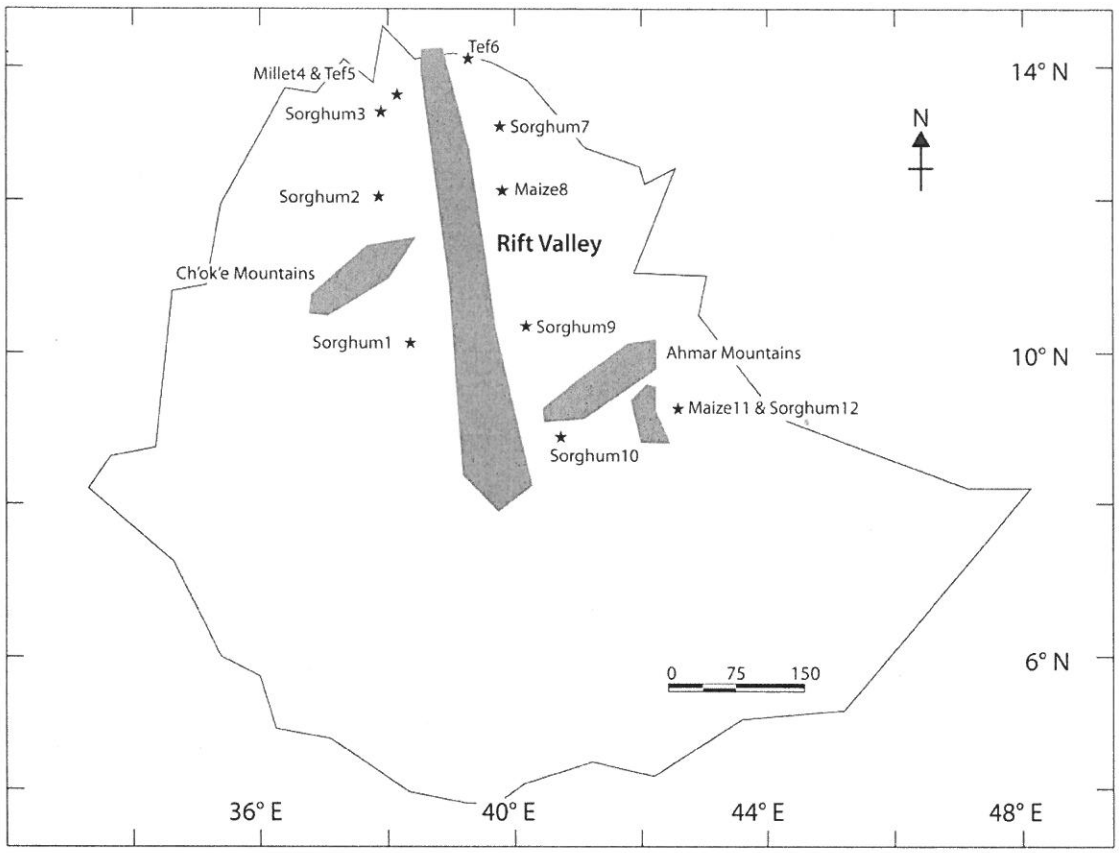

Fig. 1 Map showing locations of sampling sites (represented by stars) and hosts from which *Striga hermonthica* samples were collected in Ethiopia. Samples 4 and 5 were separated by 8 km, and samples 11 and 12 were collected from one site. Shaded areas represent potential geographic barriers.

(Gethi et al. 2005). Thermocycler conditions were 94°C for 2 min; 10 cycles of 94°C for 20 s, 66°C for 30 s with a 1°C decrease each cycle, and 72°C for 2 min; 20 cycles of 94°C for 20 s, 56°C for 30 s, and 72°C for 2 min; and 60°C for 30 min.

Fragments were visualized on a Beckman Coulter CEQ8000 Genetic Analysis System. Analysis of resulting fragments was conducted with the Beckman Coulter CEQ software, where the presence of a fragment of a particular size was denoted by 1, and the absence of the fragment was denoted by 0. Three samples (one sorghum sample, one tef sample, and one maize sample) were replicated with each primer combination to assess repeatability. Fragments that had a mismatch in scores in any of the replicated samples were dropped from subsequent analyses. All designations by the software were manually checked by a single individual.

Statistical Analyses

Levels of genetic diversity within each population were measured by calculating expected heterozygosity (assuming no inbreeding) and percentage of polymorphic loci, based on a Bayesian method with uniform prior distribution of allele frequencies (Zhivotovsky 1999). Genetic differences between populations were measured by F_{ST} (Wright 1978). These values can range between 0 (no differentiation) and 1 (complete differentiation). Significance of the F_{ST} values was assessed by permutation tests (1000 replicates). Nei's genetic distance (after Lynch and Milligan 1994) was also computed, and these genetic distances were used to construct a neighbor-joining tree to evaluate which populations were most genetically similar. Bootstrapping (based on 1000 replicates) was conducted to determine the statistical support for each group present in the tree. All these genetic measures were calculated using the software AFLP-SURV (Vekemans 2002). The tree was constructed using the software PHYLIP (Felsenstein 1993).

To test for the role of host specificity in determining population structure, an analysis of molecular variance (AMOVA) was conducted to determine the proportion of variance attributable to differences in host species, with significance based on 999 permutations. To test the role of geography in determining structure, a Mantel test was performed to determine if there was a significant correlation between geographic and genetic distances. Significance was determined based on 999 permutations. An AMOVA (with 999 permutations) was conducted to see if a significant proportion of genetic variance could be apportioned between populations on

the east and the west sides of the Rift Valley. We also calculated Φ_{ST} values, a genetic differentiation measure similar to F_{ST} (Peakall et al. 1995), during the AMOVA analyses. The software GenAlEx was used for the Mantel and AMOVA tests (Peakall and Smouse 2006). An additional test on the role of geography was also performed using the software BARRIER (Manni and Guérard 2004). This is a spatial autocorrelation approach that uses Monmonier's maximum difference algorithm (Monmonier 1973) to identify genetic barriers in a landscape, specifically those locations where genetic differences are largest.

Results

A sufficient amount of genetic variation was detected using the described AFLP primer combinations (fig. 2). The total number of bands detected across all primer combinations after correcting for repeatability was 385 fragments. The average percentage of polymorphic loci was 60.9%, ranging from 53.2% to 76.4% (table 2). Average expected heterozygosity was 0.204, ranging from 0.168 to 0.279 (table 2).

Genetic differentiation between populations was relatively high, and all populations were significantly different from each other ($P < 0.05$; table 3). F_{ST} values ranged from 0.032 (Sorghum10 vs. Maize11) to 0.293 (Sorghum9 vs. Maize11). The average F_{ST} was 0.146.

Genetic Diversity due to Geography

Geography appeared to play a significant role in shaping the genetic diversity of *S. hermonthica*. The neighbor-joining tree showed three distinct groupings with high statistical support that corresponds to geographic location (fig. 3). The first group included Sorghum2, Sorghum3, and Sorghum4, lying in the northwestern portion of the study area to the west of the Rift Valley. The second group consisted of Tef5, Tef6, Sorghum7, Maize8, and Sorghum9, all to the east of the Rift Valley, with the exception of Tef5, which was collected 8 km north of Sorghum4. The third group included Sorghum1, Sorghum10, Maize11, and Sorghum12, all in the southern portion of the study area. Samples 10–12 were collected in highlands in the Ahmar Mountain Range to the east of the Rift Valley.

The Rift Valley appeared to be a significant barrier to *S. hermonthica* dispersal. The AMOVA results suggest that a significant proportion of the variance can be attributed to the Rift Valley, with the rest of the genetic variance being partitioned among populations on either side of the barrier and within populations (table 4). Spatial autocorrelation also indicates that there is a significant genetic barrier in the southern portion of the study area that separates populations Sorghum1, Sorghum10, Maize11, and Sorghum12 from the rest of the populations (fig. 4), corroborating one of the three groups in the neighbor-joining tree.

There appears to be a small isolation-by-distance effect. Genetic distance slightly increased with geographic distance.

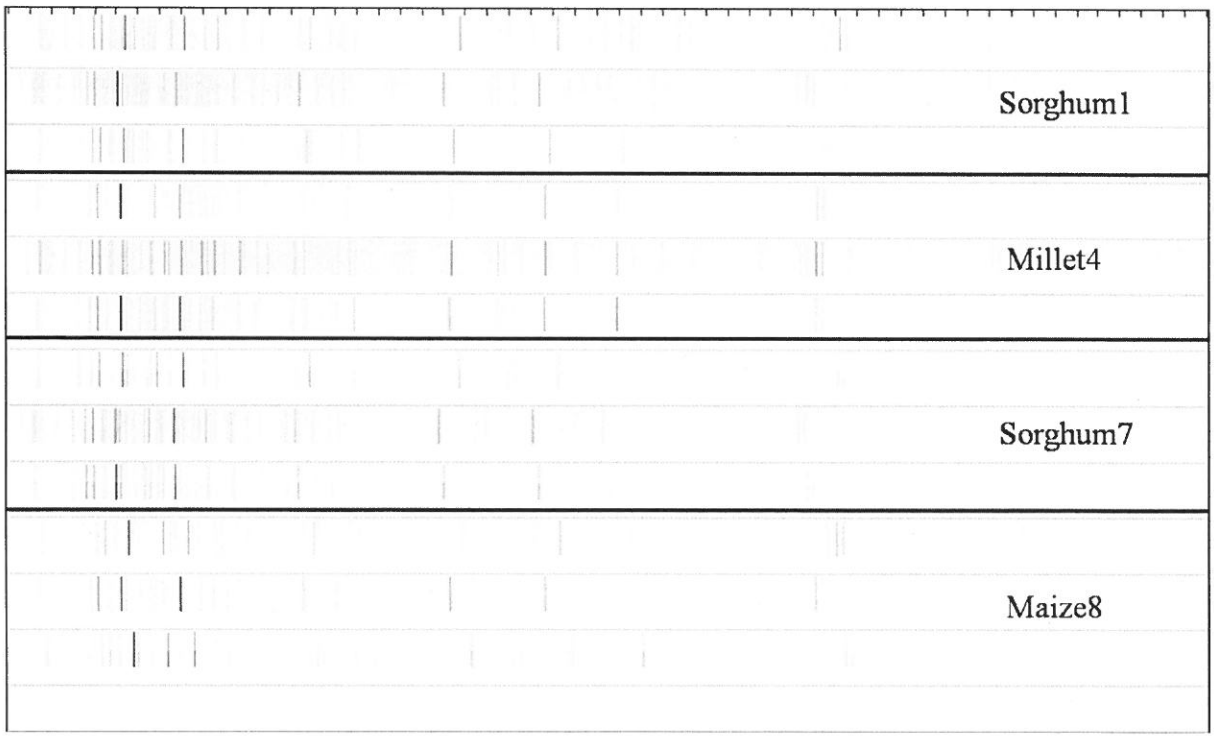

Fig. 2 Sample AFLP gel image. Three individuals from four populations (Sorghum1, Millet4, Sorghum7, Maize8) are represented. Image is a pseudogel created from raw capillary electrophoresis data using the software GelQuest (SequentiX Digital DNA Processing).

Table 2

Genetic Diversity of Each *Striga hermonthica* Population

Population	Sample size (*n*)	% polymorphic loci	Expected heterozygosity (SE)
Sorghum1	10	53.8	.168 (.009)
Sorghum2	8	76.4	.279 (.009)
Sorghum3	9	67.3	.233 (.009)
Sorghum7	8	55.6	.172 (.009)
Sorghum9	10	64.7	.200 (.009)
Sorghum10	9	53.2	.175 (.009)
Sorghum12	10	59.5	.194 (.009)
Millet4	8	66.5	.254 (.010)
Tef5	9	60.3	.200 (.009)
Tef6	9	59.7	.196 (.009)
Maize8	8	59.7	.204 (.010)
Maize11	9	53.8	.177 (.010)

Note. Percentage of polymorphic loci calculated based on analyzed fragments across all primer combinations (EcoACT/MseCTC: 102 fragments; EcoAGC/MseCTC: 79 fragments; EcoACC/MseCTC: 97 fragments; EcoACC/MseCAT: 107 fragments).

There was a weak but significant correlation between genetic and geographic distance ($R^2 = 0.025$, $P = 0.001$). In general, those populations that were farthest from each other geographically were also the most genetically distinct.

Genetic Diversity due to Host Specificity

A correlation between host specificity and genetic differentiation was not detected. The groupings on the neighbor-joining tree did not correspond to hosts (fig. 3). The results of the AMOVA suggested that host specificity was not a significant factor in explaining genetic differences between populations (table 4). The variance that could be attributed to host differences was 0%, while differences among populations contributed 27% and among individuals contributed 73%.

Discussion

The results from this study show that the sampled *Striga hermonthica* populations were characterized by high levels of genetic diversity, as indicated by the high level of polymorphism and moderate levels of heterozygosity. These values are comparable to the values obtained using allozymes for *S. hermonthica* parasitizing different hosts (Bharathalakshmi et al. 1990; Kuiper 1996; Olivier et al. 1996, 1998). Our results are also consistent with the genetic diversity of plants that share a similar life-history and ecology with *S. hermonthica*. Hamrick et al. (1979) and Loveless and Hamrick (1984) indicate that the heterozygosity for annuals is 0.116; for dicot species, 0.113; for outcrossed species, 0.185; and for weedy species, 0116.

The levels of genetic differentiation observed between the sampled populations span a broad range. According to the standards presented by Wright (1978), populations with F_{ST} values ranging from 0.15 to 0.25 are highly differentiated, and populations with values ranging from 0.05 to 0.15 are moderately differentiated. Genetic differentiation in our samples was relatively high, and all populations were significantly different from each other. Thirty-two of the 66 (48%) population comparisons were highly differentiated, and the average F_{ST} between all populations represents a high level of genetic differentiation. Lower genetic distance values (range = 0.007–0.025, mean = 0.015), using similar AFLP techniques, were obtained for 24 populations of *S. hermonthica* in Kenya (Gethi et al.

Table 3

Genetic Differentiation between the Populations, as Measured by F_{ST}

	Sorghum1	Sorghum2	Sorghum3	Millet4	Tef5	Tef6	Sorghum7	Maize8	Sorghum9	Sorghum10	Maize11
Sorghum1											
Sorghum2	.188										
Sorghum3	.241	.039									
Millet4	.144	.041	.062								
Tef5	.147	.121	.151	.041							
Tef6	.185	.138	.185	.077	.044						
Sorghum7	.270	.192	.244	.146	.123	.092					
Maize8	.202	.104	.146	.076	.062	.046	.063				
Sorghum9	.283	.186	.193	.139	.127	.113	.172	.100			
Sorghum10	.137	.154	.217	.133	.172	.167	.216	.167	.281		
Maize11	.180	.127	.189	.129	.188	.200	.240	.171	.293	.032	
Sorghum12	.161	.120	.188	.085	.107	.087	.118	.084	.205	.088	.091

Note. All values are significant at $P < 0.05$.

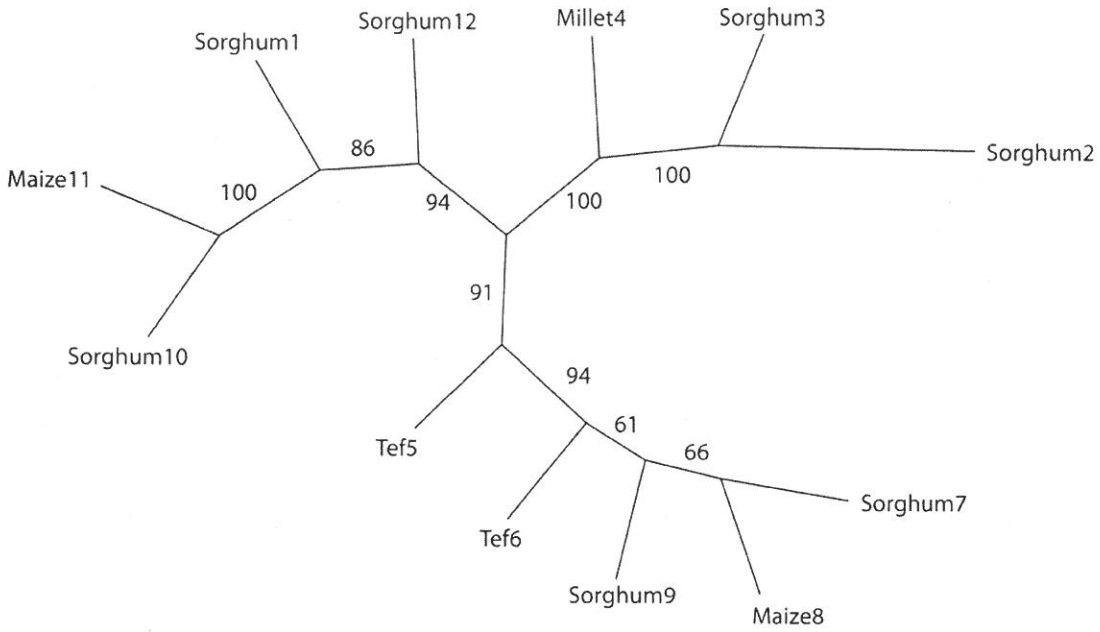

Fig. 3 Neighbor-joining tree based on Nei's genetic distance (after Lynch and Milligan 1994). Numbers on the tree represent bootstrap percentages, based on 1000 replicates. Labels correspond to populations in fig. 1.

2005). Those authors attributed the homogeneity of the Kenyan populations of *S. hermonthica* to recent colonization (a founder event) from the Lake Victoria basin east into Kenya and to its allogamous breeding system. The lack of evidence for genetic differentiation in the Kenyan populations could also be due to the small area sampled, which covered only 0.5° latitude and <1° longitude, in contrast to our study, which extends over a wider and more variable geographical area covering ~5° latitude and longitude.

Genetic Diversity due to Geography

Geography appears to play the greatest role in determining genetic differences between *S. hermonthica* populations, with the most substantial genetic barrier being in the southern portion of the study area. The southern barrier corresponds to the populations collected on the Ahmar Mountains. These mountains have an elevation of up to 2134 m and are one of the many mountain islands resulting from the topography in Ethiopia. The populations collected from the Ahmar Mountains also have the lowest genetic diversity, indicating that these populations may be isolated on the highlands and may experience a loss of diversity due to genetic drift. Sorghum1 also grouped with samples 10–12, despite the presence of the Rift Valley between the groups and the geographic distance. Sorghum1 also had lower genetic diversity compared to the other groups, perhaps due to its isolation between the Rift Valley and the Ch'ok'e Mountains with a peak of 2470 m. The genetic similarity between Sorghum1 and samples 10–12 may be due to the dispersal route of *S. hermonthica* in the southern region, with Sorghum1 being the potential source for samples 10–12.

The Rift Valley also appears to be a genetic barrier for *S. hermonthica*, resulting in genetic differences between popula-

Table 4

Results of AMOVA Analyses

	Variance	% variation	Φ_{ST}[a]	Probability[b]
Among Rift Valley groups[c]	2.21	5	.05	.001
Among populations within Rift Valley groups	10.08	23	.24	.001
Among host groups[d]	0.00	0	0.00	.993
Among populations within host groups	12.19	27	.27	.001
Within populations	32.46	73	.28	.001

Note. AMOVA = analysis of molecular variance.

[a] Significant Φ_{ST} values ($P < 0.05$) are underlined.

[b] Probability values are based on 999 permutations.

[c] Rift Valley groups consist of two groups: populations to the east of the valley and populations to the west.

[d] Host groups consist of four groups: sorghum, tef, maize, and millet.

tions on the east and west sides of the valley. Differentiation from this geographic barrier is probably a recent phenomenon and reflects the spread of *S. hermonthica* in recent evolutionary time. Although the proportion of genetic variation that could be attributed to the presence of the Rift Valley was significant, it was relatively low (5%), indicating a recent genetic divergence and probably reflecting the current spread of *S. hermonthica*. In contrast, the giant lobelia (*Lobelia giberroa*) had 58% of the genetic variance attributable to geography, suggesting that the populations diverged long ago and likely survived several glacial episodes (Kebede et al. 2007). One exception to the differentiation between east and west is the grouping of Tef5 with the east side of the Rift Valley, despite its location to the west of the Rift Valley and its close proximity to Millet4. This also may reflect the colonization history and recent divergence of the populations. Colonization may have occurred from the east side of the Rift Valley to the west side, with sufficient time not having passed for complete divergence. The series of highlands in this region may maintain some level of connectivity, allowing for higher levels of dispersal.

There also appears to be a slight isolation-by-distance effect. This would indicate that the most likely mode of dispersal is a stepping-stone model, with those populations that are geographically proximate providing the source for colonizers. However, the isolation-by-distance effect is not as strong as the effect of geographic barriers to dispersal. In contrast, a strong correlation between geographic and genetic distance ($R^2 = 0.61$) was observed by Botanga et al. (2002) for *Striga asiatica*, an autogamous species. In the absence of significant geographic barriers, geographic distance alone may be an even stronger determinant in population structure.

Genetic Diversity due to Host Specificity

In this study, host specificity does not appear to be the primary factor shaping the population structure of *S. hermonthica*. This is consistent with the results obtained for *S. hermonthica* by Bharathalakshmi et al. (1990). Other studies have also demonstrated that geography plays a greater role than host specialization in determining genetic differences between *Striga* populations (Bharathalakshmi et al. 1990; Musselman et al. 1991; Koyama 2000). Previous studies that were taken as strong evidence of host specificity in *S. hermonthica* may in fact be attributed to geographical distance. Among these was the study conducted in Sudan by Musselman and Hepper (1986). They concluded that *S. hermonthica* has two host-specific strains, one for sorghum and another for pearl millet. This is particularly true in areas where only sorghum or pearl millet is used as a food crop, either because of limitations imposed by climatic conditions or because of humans' food preferences. For example, in Sudan, sorghum is most commonly grown in the south, but pearl millet is grown in the north, where it is too dry for sorghum. In these regions, *S. hermonthica* populations developed host specificity to either sorghum or millet. However, in areas where both sorghum and pearl millet were grown, host specificity was not observed (Musselman and Hepper 1986), providing potential evidence for a weak genetic basis for host specificity.

The apparent lack of genetic differentiation based on host specificity may suggest that specialization of *S. hermonthica* to its host may be a recent phenomenon, with insufficient time for genetic differences to arise. This is consistent with the observation that when a sorghum field infested with *S. hermonthica* is replaced by millet, the new crop will be infested by *Striga* after a few years; this is dependent on the intensity with which a particular crop is grown in the absence of others in a given area (Olivier et al. 1998).

Origins of host species may also play a role in genetic differences between populations. Sorghum is native to Ethiopia, and sorghum *S. hermonthica* populations are characterized by fairly high genetic differentiation. *Striga hermonthica* populations associated with historically important and widely grown host crops in Africa, such as sorghum and pearl millet, may have coexisted with their hosts for a longer period than populations associated with unconventional hosts (e.g., finger millet, tef) or with introduced hosts such as maize. To our knowledge, tef and finger millet rarely were grown for food and were not conventional hosts for *S. hermonthica* in Africa; our study is among the first for *S. hermonthica* parasitizing finger millet and tef in Africa. Consistent with this low host specificity in tef and finger millet, the parasite does not seem to seriously damage its hosts in Ethiopia. Furthermore, *S. hermonthica* plants on these two hosts were much smaller and less branched compared to those collected from sorghum fields.

Stronger evidence of host specificity has been observed in autogamous *Striga* species such as *Striga gesnerioides*, where each host-specific strain is adapted to a narrow host range. Mohamed et al. (2001) demonstrated that these host-specific strains have somewhat unique morphology. AFLP markers confirmed genetic differences based on host specificity in populations of *S. gesnerioides* parasitic on *Indigofera hirsuta* in central Florida and populations parasitic on *I. hirsuta* and cowpea from West Africa. The Florida strain and the West African strain parasitic on indigo were more closely related to one another compared to the Florida strain and the West African strain parasitic on cowpea. Race formation in cowpea *S. gesnerioides* has been shown to be largely due to host-driven selection (Botanga and Timko 2005). This high level of genetic differentiation coupled with the species' ability to parasitize different hosts made *S. gesnerioides* the most widely distributed species in the genus, extending its range even to arid habitats in North and East Africa and Arabia, where it is parasitic on *Euphorbia abyssinica*.

Although the results suggest that host specificity is not as significant as geography in shaping *S. hermonthica* population structure, additional studies need to be conducted to confirm the lack of correlation regarding host specificity. Experimental studies in the laboratory involving infection of host species with *S. hermonthica* from different geographic locations are necessary in order to conclusively assess the role of host specialization in shaping genetic diversity. Additionally, in our study, the host species do not necessarily represent the same cultivar, and this could potentially confound the results.

Management Implications

The high genetic diversity of *S. hermonthica* presents a challenge for the development of resistance in its crop

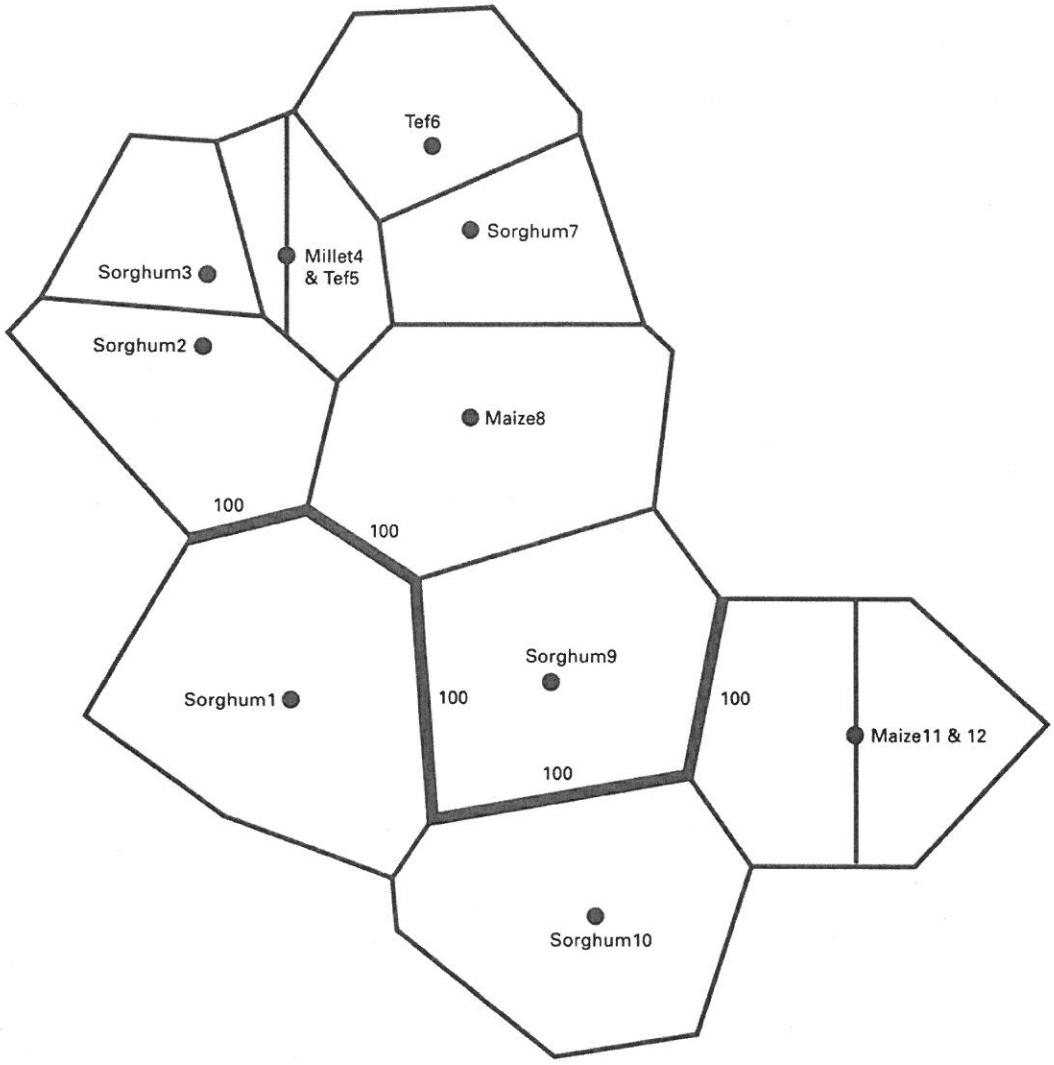

Fig. 4 Results of spatial autocorrelation analysis showing location of sampling sites (labels correspond to fig. 1) and the location of a potential genetic barrier (represented by red line). Numbers along the line represent bootstrap percentages, based on 1000 replicates.

hosts. Our results show that differences among individuals of *S. hermonthica* within the same population contributed to 73% ($P = 0.001$) of the genetic differences. This high level of genetic variability is consistent with obligate outcrosser species (Safa et al. 1984; Bharathalakshmi et al. 1990). The high genetic variability within populations of *S. hermonthica* may make it difficult, if not impossible, to produce reliable resistant varieties. In addition, a single *Striga* plant produces up to half a million seeds that add to the seed bank from previous years. Combined with the fact that seed of *Striga* can live up to 20 years in the soil, plant breeders have a great challenge in developing resistant varieties. These resistant varieties will soon be challenged by the diverse seed bank of *S. hermonthica*. Soils in Ethiopia were highly contaminated with *Striga* seed, and this may preclude cereal cultivation in some areas. The broader genetic background of *S. hermonthica* may enable the species to parasitize a number of cereal crops under different climatic conditions. *Striga hermonthica* and *Striga aspera* were among the few species proven to hybridize and produce viable seeds (Aigbokhan et al. 2000). This genetic exchange with nonweedy *Striga* species could provide a gene reservoir via hybridization.

Farmers in Ethiopia were instructed to pull and burn *Striga* plants to prevent seed set. However, in practice, the parasites were pulled after seed set and dumped by the roadside. Considering the topography of Ethiopia (i.e., lowlands and highlands), this practice of plant disposal is very harmful and results in spreading *Striga* to new and faraway places through runoff. A second source for the spread of *Striga* seed is the contamination of cereal grains with parasitic plant seed because cereals are threshed on the ground inside contaminated fields using oxen and donkeys. When grains were transported elsewhere, they carried *Striga* seeds with them. The oxen and donkeys provide a secondary source of seed dissemination. These

means of dispersal result in long-distance seed spread of *S. hermonthica* throughout much of Africa and across the Red Sea to neighboring Arabia. In these areas, *S. hermonthica* was able to develop genetically structured populations based on geography.

The most effective method to combat *S. hermonthica* is containment and then eradication of the parasite. There appear to be geographic barriers to *S. hermonthica* migration, providing natural containment areas. Proper disposal of *Striga* pulled by farmers in Ethiopia will also help in containing *Striga* and preventing its spread to new areas. Our study demonstrates the need for a more detailed analysis of genetic diversity in *S. hermonthica* at the level of local populations as well as on a large scale in Africa, in order to understand the parasite well enough for effective management.

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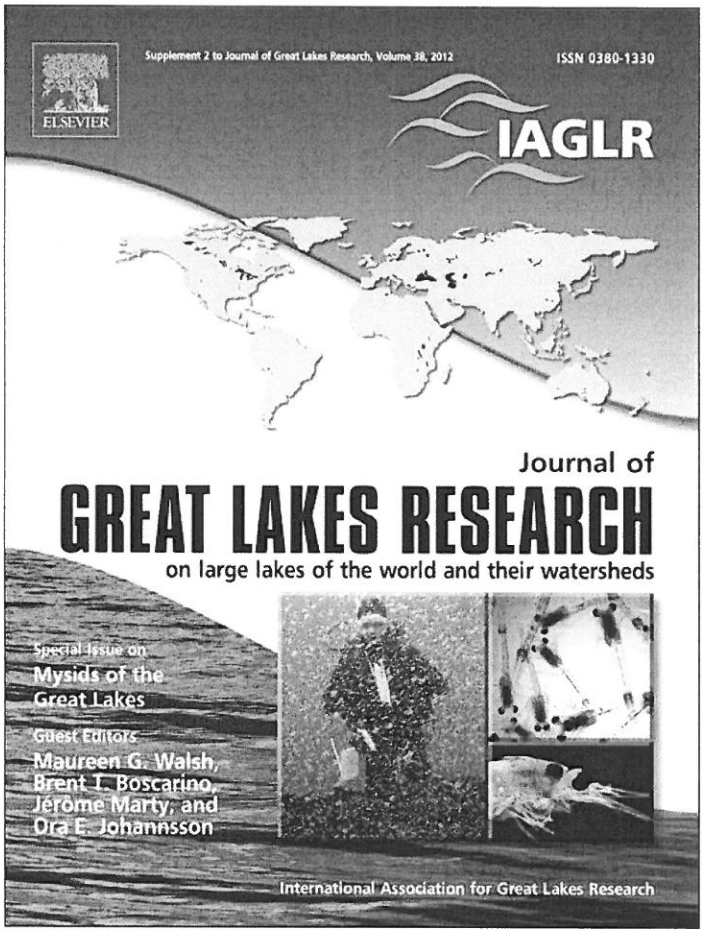

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New data on mitochondrial diversity and origin of *Hemimysis anomala* in the Laurentian Great Lakes

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ABSTRACT

The most recent Ponto-Caspian species to invade the Laurentian Great Lakes is the crustacean *Hemimysis anomala*, first reported in 2006. A previous study described three haplotype groups (A, B, C) of *H. anomala* in native and invaded areas within Europe, but only one haplotype (A1) in a sample from Lake Michigan. Our study expands these results to additional populations in the Great Lakes basin, and evaluates relationships among North American and European populations. A 549-bp fragment of the mitochondrial cytochrome oxidase I (COI) gene was analyzed from populations of *H. anomala* in Lakes Ontario, Erie, Huron, and the St. Lawrence River. Two different haplotypes, A1 and B1, were observed in the sampled populations of *H. anomala* and in a previous analysis from *H. anomala* in Oneida Lake (New York). Our results, in contrast with a previous study, detect an additional haplotype in North America.

Introduction

Since the 1800s, one of the biggest threats to freshwater ecosystems has been the introduction of nonindigenous species (NIS) via human activity (Mills et al., 1993). The most recognized vector for NIS into the Laurentian Great Lakes is through discharged ballast water from transoceanic ships (Holeck et al., 2004). Ballast water can harbor planktonic organisms capable of tolerating salinity changes associated with ballast water exchange, which has contributed to the more than 170 NIS established in the Laurentian Great Lakes (Grigorovich et al., 2003; Roman, 2006; Ellis and MacIsaac, 2009). Over 75% of the NIS recorded in the Great Lakes are endemic to the Ponto-Caspian region (Black, Caspian and Azov Seas; Ricciardi and MacIsaac, 2000). Some of the most widely recognized Ponto-Caspian invaders are the molluscs *Dreissena polymorpha* (Hebert et al., 1989) and *D. bugensis* (May and Marsden, 1992), fish such as round goby (*Neogobius melanostomus*; Jude et al., 1991) and ruffe (*Gymnocephalus cernuus*; Stepien et al., 1998), the cladoceran *Cercopagis pengoi* (MacIsaac et al., 1999; Ojaveer et al., 2001; Panov et al., 2007), and the amphipod *Echinogammarus ischnus* (Witt et al., 1997). Understanding the pathways by which an organism enters a new environment, and where that organism originated from, can aid in strengthening current regulations to prevent future invasions.

The most recent Ponto-Caspian species to invade the Laurentian Great Lakes is the crustacean *Hemimysis anomala* G. O. Sars, 1907. *H. anomala* is native to coastal areas and river deltas in the Black Sea, Sea of Azov, and northern and eastern Caspian Sea (Wittmann, 2007). In the 1960s *H. anomala* was intentionally introduced into the Kaunas reservoir in Lithuania to improve fish stocks (Ketelaars et al., 1999). This introduced population is the likely source of the species' spread to the Baltic Sea with subsequent expansion to the Rhine Delta. A second possible invasion route originated from the Danube Delta, spreading along the Danube River down to the Rhine Delta, where intermixing between the various lineages has occurred (Audzijonyte et al., 2008). During the 1990s and 2000s the species spread throughout western Europe. In 1992 it was discovered in Finnish coastal waters of the Baltic Sea, Sweden in 1995, Poland in 2002, and the UK in 2004 (Salemaa and Hietalahti, 1993; Holdich et al., 2006; Audzijonyte et al., 2008). Additional populations were documented in inland waterways of Germany in 1998 and the Czech Republic during 2003 (Audzijonyte et al., 2008).

In North America, evidence to date indicates widespread invasion and ongoing colonization within the Great Lakes basin. The first North American records of *H. anomala* came from Lakes Michigan and Ontario in 2006 (Pothoven et al., 2007; Walsh et al., 2010). Subsequent

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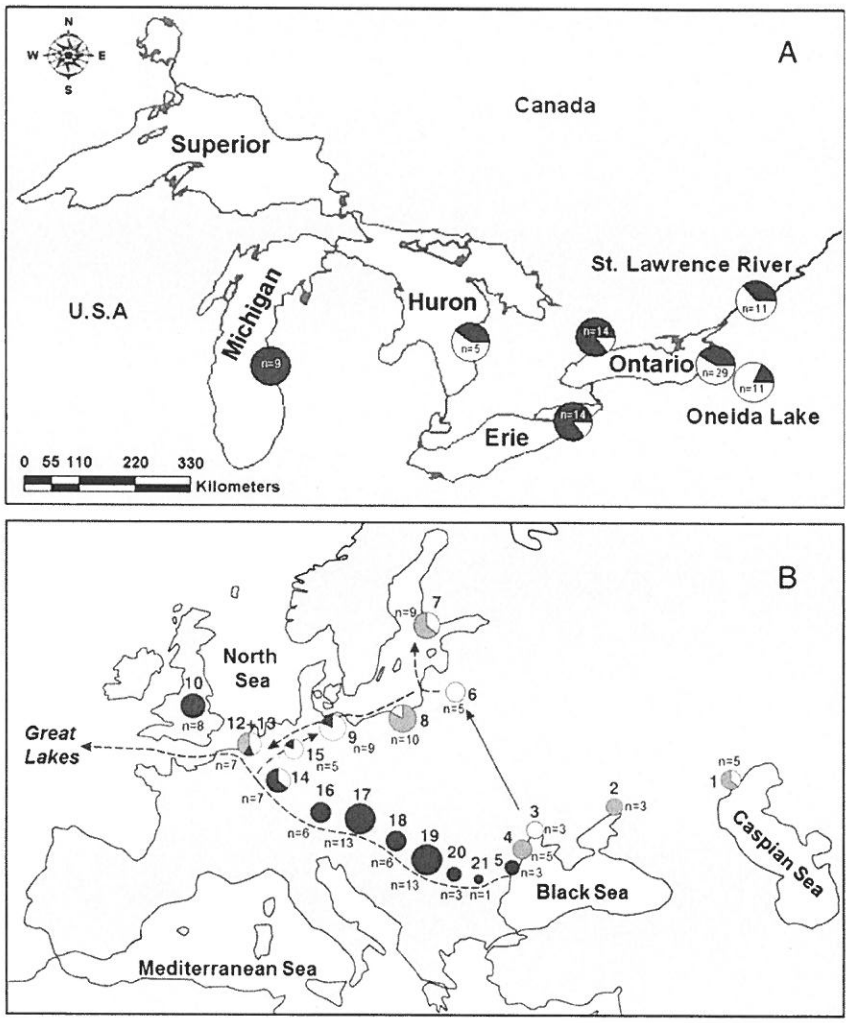

Fig. 1. A) Distribution of the two observed *H. anomala* haplotypes from the mitochondrial COI gene in the Laurentian Great Lakes. Data for Lake Michigan from Audzijonyte et al. (2008) and Oneida Lake from Brooking et al. (2010). B) Distribution of the three observed *H. anomala* haplotype clusters across Europe (modified from Audzijonyte et al., 2008). Dark gray pie slices represent the A1 haplotype, light gray represent the C haplotypes and white pie slices represent the B haplotypes. In all locations (except 1, 3, and 6 in Fig. 1B), the B cluster consisted solely of the B1 haplotype. Position of pie charts represents approximate sample locations.

sampling during 2007–2009 has confirmed *H. anomala* populations in Lakes Erie and Huron (Marty et al., 2010) and documented the spread of the species within the Lake Ontario watershed (Kestrup and Ricciardi, 2008; Brooking et al., 2010). A lack of positive findings of *H. anomala* in Lake Superior or other inland lakes in the Great Lakes basin may be attributed to a lack of sampling effort.

Advances in molecular techniques have created tools useful in phylogeography and population genetics for interpreting the mechanisms and biology behind pre- and post-settlement of NIS. Within the Laurentian Great Lakes, genetic methods have been applied in a number of studies to evaluate the origin and invasion history of NIS including sea lamprey (*Petromyzon marinus*, Bryan et al., 2005), round gobies (Dillon and Stepien, 2001) and ruffe (*Gymnocephalus*, Stepien et al., 1998). Mitochondrial DNA (mtDNA) is a widely accepted tool for assessing genetic patterns and structure. Comparison of sequences from mtDNA to native and nonindigenous populations has aided in identifying invasion pathways, source populations, and the number of separate introduction events, as well as recognizing key variables that help to enhance the success of establishment and spread of NIS in a novel environment (Kelly et al., 2006; Stepien and Tumeo, 2006; Facon et al., 2008).

Invasion history and relationships among *H. anomala* populations across Europe, the UK, and into Lake Michigan were evaluated in a previous study by Audzijonyte et al. (2008) using information from the mitochondrial cytochrome oxidase I (COI) gene. Nine haplotypes among three groups (A, B, C) were identified (GenBank accession numbers EU02162–EU029170) from the species' native range and the Kaunas Reservoir (site of first intentional introduction; Audzijonyte et al., 2008). Presence and proportions of these nine haplotypes among populations in the invaded areas were used to track the spread of *H. anomala* in western Europe. Only haplotype A1 was found throughout the Danube River drainage, and this was also the only haplotype found from the Lake Michigan population, leading Audzijonyte et al. (2008) to report the Danube River lineage as the likely source of introduction into the Great Lakes. Our work expands upon the study of North American populations to i) identify any additional haplotypes for *H. anomala* in Lakes Ontario, Erie, Huron and the St. Lawrence River and ii) compare haplotype frequencies observed in these areas with those previously observed in Lake Michigan, Oneida Lake (New York), and European populations of *H. anomala*.

Methods

Sample collection

We analyzed a total of 73 *H. anomala* collected during 2007–2009 at 5 localities encompassing Lake Ontario (northwestern: n = 14,

Table 1
Observed haplotype frequencies of mtDNA COI gene in *Hemimysis anomala* across the Laurentian Great Lakes. Haplotypes B2–C4 were not observed in the North American populations. *n*, number of individuals sequenced per population for the COI gene; year, year sampled. Data for Lake Michigan from Audzijonyte et al. (2008) and Oneida Lake from Brooking et al. (2010).

Location	Latitude (°N)	Longitude (°W)	Year	Haplotype frequencies A1	B1	*n*
Southeastern Lake Ontario						
Sunset Bay area (USA)	43.53	−76.38	2007	0.41	0.59	29
Northwestern Lake Ontario						
Cobourg, Ontario (Canada)	43.95	−78.15	2007	0.71	0.29	14
Lake Erie						
Port Maitland (Canada)	42.85	−79.58	2008	0.86	0.14	14
Lake Huron						
Kincardine (Canada)	44.18	−81.64	2008	0.40	0.60	5
St. Lawrence River						
Montreal Port (Canada)	45.50	−73.55	2009	0.36	0.64	11
Lake Michigan						
Muskegon Channel (USA)	43.23	−83.34	2006–07	1.00	0.00	9
Oneida Lake (USA)	43.20	−75.90	2009	0.18	0.82	11
Total						93

southeastern: n = 29), the St. Lawrence River (n = 11), Lake Erie (n = 14), and Lake Huron (n = 5; Fig. 1, Table 1). Samples from southeastern Lake Ontario (east of Oswego, New York) were collected by horizontal tows at night using a 505-μm mesh zooplankton net and a benthic sled with a 1-m^2 frame, 1000-μm mesh zooplankton net (as in Walsh et al., 2010). Samples from the other sites were collected using vertical tows with a 400-μm mesh zooplankton net (as in Marty et al., 2010). Samples from Oneida Lake, New York (n = 11) were analyzed concurrently with this study but reported previously as the first record of that population (Brooking et al., 2010). All *H. anomala* collected were preserved in 95% ethanol. All samples were collected during ongoing studies of the species and provided by collaborators; at the time, we were not able to obtain any additional collections from Lake Michigan.

DNA extraction and COI sequencing

Total genomic DNA was extracted from chopped whole specimens using Gentra Puregene tissue kits, according to the manufacturer's protocol. Isolated DNA was quantified using a fluorometer. A 549-bp fragment of the 3′ part of the mitochondrial cytochrome oxidase I (COI) subunit gene was amplified. PCR amplification was performed using primers HemiHatF and HemiHatR (Audzijonyte et al., 2008). In samples of lower quality, internal primers were used as described in Audzijonyte et al. (2008). Amplifications were performed in 20 μL volumes containing 0.5 U of Hot Start *Taq* DNA polymerase (Qiagen), 1× buffer, 3 mM $MgCl_2$, 0.2 mM dNTP, 10 pmoL of each primer, and 10 ng of genomic DNA. Thermal cycler conditions included a 95 °C activation for 15 min followed by 35 cycles for 1 min at 95 °C, 1 min at 50 °C, and 1 min at 72 °C, with a final extension for 5 min at 72 °C.

PCR products were purified using either Agencourt AMPure (Beckman Coulter Genomics) or QIAquick PCR cleanup kit (Qiagen) and directly sequenced in either the forward direction or both directions (depending on sequence quality), using the DTCS Quick Start Kit (Beckman Coulter) in 10 μL reaction volumes, according to manufacturer's protocol. Sequencing products were purified with either ethanol precipitation or CleanSEQ (Beckman Coulter Genomics) and capillary electrophoresis was conducted on a Beckman Coulter CEQ8000 genetic analysis system.

Data analysis

Mitochondrial COI sequences were aligned using BIOEDIT Ver. 7.0.9.0 (Hall, 1999) and compared to the 9 haplotypes observed by Audzijonyte et al. (2008). Frequencies of haplotypes were then compared to published data from Lake Michigan (Audzijonyte et al., 2008), Oneida Lake (Brooking et al., 2010) and European populations (Audzijonyte et al., 2008), with the Danube River and Rhine Delta locations in the European populations pooled to increase sample sizes. Population differentiation between North American and European populations was measured using an exact test of population differentiation (Raymond and Rousset, 1995) using 30,000 Markov steps and a chain length of 100,000 steps. A sequential Bonferroni correction was used to account for multiple comparisons (Rice, 1989). The analysis

Table 2
Probability values for exact tests of population differentiation between North American and Ponto-Caspian locations. Site locations correspond to numbers on Fig. 1B; sites 1–5 represent the native range of *H. anomala*, 6–21 represent range expansion. Bold values are significant ($p < 0.0006$) after a sequential Bonferroni correction.

Site location		SE Lake Ontario	NW Lake Ontario	St. Lawrence	Oneida Lake	Lake Erie	Lake Huron	Lake Michigan
	SE Lake Ontario							
	NW Lake Ontario	0.125						
	St. Lawrence	1.000	0.094					
	Oneida Lake	0.275	0.020	0.636				
	Lake Erie	0.007	1.000	0.016	0.001			
	Lake Huron	1.000	0.266	1.000	0.542	0.086		
	Lake Michigan	0.002	0.469	0.004	**0.000**	0.503	0.028	
1	Volga Delta	**0.000**	0.001	**0.000**	**0.000**	**0.000**	0.015	0.001
2	Don Delta	**0.000**	0.009	0.003	0.006	0.003	0.054	0.004
3	Dnieper Delta	0.048	0.045	0.225	0.245	0.017	0.642	0.004
4	Dniester Delta	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.008	0.001
5	Danube Delta	0.092	1.000	0.192	0.026	1.000	0.193	1.000
6	Kaunas WR	0.042	0.011	0.214	0.374	0.002	0.449	0.001
7	Gulf of Finland	**0.000**	**0.001**	**0.001**	0.004	**0.000**	0.027	**0.000**
8	Gulf of Gdansk	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	0.007	**0.000**
9	Lake Schwerin	0.439	0.059	0.642	1.000	0.006	0.580	0.002
10	Nottingham	0.004	0.469	0.012	0.001	0.515	0.034	1.000
12 & 13	Rhine Delta	0.007	0.020	0.074	0.084	0.002	0.434	0.001
14	Lower Rhine	0.217	1.000	0.335	0.048	0.575	0.558	0.177
15	Mittellandkanal	0.628	0.091	1.000	1.000	0.018	1.000	0.005
16–21	Danube	**0.000**	0.028	**0.000**	**0.000**	0.058	**0.001**	1.000

was conducted using the software ARLEQUIN (Excoffier and Lischer, 2010).

Results

Two different haplotypes, A1 and B1, were observed for *H. anomala* from Lakes Ontario, Erie, Huron, Oneida, and the St. Lawrence River (Table 1; Fig. 1). *H. anomala* in Lake Michigan (n = 9) were reported by Audzijonyte et al. (2008) to exhibit only the A1 haplotype. Our study found northwestern Lake Ontario (n = 14) and Lake Erie (n = 14) to have a higher frequency of the A1 haplotype than the B1 haplotype. In contrast, the St. Lawrence River (n = 11), southeastern Lake Ontario (n = 29) and Lake Huron (n = 5) had a higher frequency of the B1 haplotype than the A1 haplotype, as did Oneida Lake (n = 11, 0.82; Brooking et al., 2010). However, exact tests of population differentiation revealed Lake Michigan and Oneida Lake as the only North American populations to be statistically different ($p < 0.0006$) (Table 2). Although other comparisons had low p-values, they were not significant after correcting for multiple comparisons.

Based on exact tests of population differentiation, several Ponto-Caspian populations appeared to be unlikely sources for introduction into the Great Lakes (Table 2). The Volga Delta, Don Delta, Dniester Delta, Gulf of Finland, and Gulf of Gdansk locations each had significant genetic differences from the sampled North American locations (Table 2). The Danube, the purported source lineage for introductions into the Great Lakes (Audzijonyte et al., 2008), had significant genetic differences from the St. Lawrence River, southeastern Lake Ontario, Oneida Lake and Lake Huron (Table 2). High probability values indicate which of the sampled locations were most similar and highlight potential source populations. Within the Great Lakes, general patterns based on high probability values were observed, grouping southeastern Lake Ontario, the St. Lawrence River, Oneida Lake, and Lake Huron together; and grouping northwestern Lake Ontario, Lake Erie, and Lake Michigan together. When compared to Ponto-Caspian sources, relatively high probability values were observed between southeastern Lake Ontario/St. Lawrence River/Oneida Lake/Lake Huron and Lake Schwerin/the Mittellandkanal; and between northwestern Lake Ontario/Lake Erie and the Danube Delta/Nottingham/Lower Rhine. Low probability values were observed between the groups described above; however, most are not significant after correcting for multiple comparisons.

Discussion

Our results document the occurrence of two haplotypes (A1 and B1) in the Great Lakes and associated watersheds, in contrast to the single haplotype (A1) that was detected in Lake Michigan (Audzijonyte et al., 2008). The presence of the B1 haplotype demonstrates that the Danube River lineage, also characterized by only the A1 haplotype, was unlikely the sole source of introduction into the Great Lakes (Audzijonyte et al., 2008). Instead, based on existing data, locations where the B1 haplotype occurs (e.g., the Rhine and Baltic drainages in Germany) may be potential source populations. However, definitive source population(s) have yet to be confirmed with available data. *H. anomala* has expanded its range throughout Europe and all potential source populations were not represented in this analysis. More variable genetic markers, such as microsatellites, and larger sample sizes (to ensure sampling of all haplotypes present in the population) are necessary to identify source populations and understand migration pathways.

A possible invasion scenario to explain our results is that multiple, independent introductions of *H. anomala* into the Laurentian Great Lakes occurred from separate Ponto-Caspian populations or other regions where they had become established. Differences in haplotype frequencies would then be a result of the genetic differences between the various source populations. An alternative invasion scenario would be a single introduction from a source other than the Danube River lineage, followed by subsequent spread throughout the Great Lakes. Given the large populations observed to date in the Lake Ontario watershed (Walsh et al., 2010) and their tendency to swarm, it seems most likely that populations within the Great Lakes basin are being established from a large number of founding individuals as populations expand and colonize new areas through either active movement or transport by currents. It might be possible for small numbers of individuals to be transported to new areas accidentally via recreational boating, but little is known about the likelihood of this vector for dispersing *H. anomala*, and the large geographic distance between some populations evaluated in this study reduces the plausibility of that scenario. The southeastern Lake Ontario site is the area where the species was first reported in that Lake (Walsh et al., 2010), and movement of animals from this site to Oneida Lake and the St. Lawrence River via active or passive movement of animals, or transport by recreational or commercial boat traffic, is plausible because of the geographic proximity and location of all sites in the same watershed.

Understanding invasion pathways and migration patterns of *H. anomala* may help predict whether other nonnative species will become invasive. If introductions from multiple sources have occurred, the invasive species may be more likely to become established. Multiple introductions from different source populations can potentially enhance the establishment and spread of an invasive species by reducing the effect of a population bottleneck and increasing gene flow (Kelly et al., 2006; Suarez and Tsutsui, 2007). Genetic data can then help determine the risk of *H. anomala* to the Great Lakes ecosystem, thereby enhancing the risk analysis processes for NIS (as in Stepien and Tumeo, 2006).

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Development and standardization of disomic microsatellite markers for lake sturgeon genetic studies

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Summary

Lake sturgeon (*Acipenser fulvescens*) are of conservation concern in North America. To facilitate the recovery of this fish species, an understanding of their population genetic structure is necessary to develop and implement spatially and temporally appropriate management actions. Until recently, few genetic data using nuclear loci have been collected, primarily due to the paucity of suitable genetic markers because most microsatellite loci in lake sturgeon appeared to be tetrasomic. The authors identified nine microsatellite loci (from 254 examined) that were putative polymorphic disomic loci and tested their conformance to a disomic mode of inheritance using three lake sturgeon families. The objectives of the study were to: (i) confirm the disomic status of the nine loci through inheritance testing, and (ii) standardize the genetic markers among participating laboratories. At all nine loci, disomic inheritance were confirmed, and all nine loci segregated independently in the 26 of 36 loci pairs possible to test. One of the nine loci showed non-Mendelian segregation, possibly due to meiotic drive and/or selection. Three progeny had peak patterns inconsistent with disomy at one or more loci. The nine loci when combined with four microsatellite loci previously confirmed in other studies as disomic in lake sturgeon now yield a suite of 13 microsatellite markers. These 13 markers have been standardized among four other laboratories to facilitate building an inter-laboratory genetic database for lake sturgeon.

Introduction

Lake sturgeon (*Acipenser fulvescens*) historically ranged throughout the Great Lakes basin, Hudson Bay drainage, and Mississippi River of North America (Harkness and Dymond, 1961). Their numbers have been reduced by overfishing, dams and other migration impediments and diminished habitat quality and/or loss (Auer, 1999). Lake sturgeon are now listed as either endangered, threatened, or of special concern in most states within their historic range, as an Appendix II species under the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), and as a species of concern under the United States Endangered Species Act. As with many other sturgeon species, late sexual maturity and an intermittent spawning pattern (Harkness and Dymond, 1961) slow the ability of lake sturgeon to quickly rebound.

To facilitate the recovery of lake sturgeon, management efforts have focused on regulating or, in most cases, eliminating harvest (Welsh, 2004), providing fish passage over dams (Peake et al., 1997; Amaral et al., 2002), and restoring spawning habitat (Bruch, 1999). Reintroduction of lake sturgeon to locations where they have been extirpated, as well as stocking to increase the abundance of existing stocks, has been implemented to a limited extent (Schram et al., 1999; Runstrom et al., 2002). Approaches employed in conservation genetics can help to prioritize populations for active management, to identify suitable donor and recipient populations for stocking, and to guide interjurisdictional coordination by delineating appropriate management units.

Microsatellite loci are useful genetic markers for intraspecific population genetic studies because they are codominant, biparentally inherited, putatively neutral, and have a relatively high mutation rate (Goldstein and Schlotterer, 1999). Evidence of duplicated microsatellite loci has been observed in many sturgeon species (e.g. Jenneckens et al., 2001; Rodzen and May, 2005; Shao et al., 2005), as well as other fish species (e.g. David et al., 2003). The lake sturgeon genome appears to be tetraploid-derived and in the early stages of the diploidization process, with a mix of tetrasomic and disomic microsatellite loci (e.g. Pyatskowit et al., 2001; McQuown et al., 2002; Welsh et al., 2003). Duplicated loci can complicate population genetic analyses because of the difficulty in determining gene dosages and the assumption of diploidy is incorporated into many statistical tests.

In 1999, lake sturgeon geneticists and managers in the United States met to address priorities for the collection and analysis of genetic data that could be incorporated into management plans. Critical needs that were identified included the development of additional genetic markers and the standardization of those markers among the various laboratories (Lowie, 1999). To eliminate the inherent difficulties associated with tetrasomic loci, future lake sturgeon genetic marker development required identifying nuclear microsatellite loci that are disomic. Following selection of appropriate genetic markers, standardization of marker use and allelic designations was deemed critical for building a genetic database among the laboratories.

Building a North American genetic database of lake sturgeon is difficult because of differences among laboratories in analytic techniques and scoring procedures. Integration of genetic data collected at different laboratories will permit a more comprehensive understanding of lake sturgeon population structure. Many laboratories are using different genotyping platforms and apparent allele sizes can vary depending on electrophoretic conditions (Haberl and Tautz, 1999). Standardization of microsatellite loci also requires consistent allele scoring within and between laboratories. With the development of new microsatellite markers, the timing for such

PCL XL Error

Subsystem: KERNEL

Error: IllegalTag

File Name: kernel.c

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Request for Quotation #DNR214058
Research Project for Chronic Wasting Disease on White-Tailed Deer
Attachment A: Pricing Page

YEAR ONE (1)			YEAR TWO (2)			YEAR THREE (3)		
		Unit Price			Unit Price			Unit Price
Year One (1) Total		$62,669	Year Two (2) Total		$66,158	Year Three (3) Total		$46,518

TOTAL BID (Combine All Years)	$175,345

CERTIFICATION AND SIGNATURE PAGE

By signing below, I certify that I have reviewed this Solicitation in its entirety, understand the requirements, terms and conditions, and other information contained herein; that I am submitting this bid or proposal for review and consideration; that I am authorized by the bidder to execute this bid or any documents related thereto on bidder's behalf; that I am authorized to bind the bidder in a contractual relationship; and that to the best of my knowledge, the bidder has properly registered with any State agency that may require registration.

West Virginia University

(Company)

Mary Jane Buckland

(Authorized Signature)

Mary Jane Buckland, Interim Director

(Representative Name, Title)

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3/18/14

(Date)

RFQ No. DNR214058

STATE OF WEST VIRGINIA
Purchasing Division

PURCHASING AFFIDAVIT

MANDATE: Under W. Va. Code §5A-3-10a, no contract or renewal of any contract may be awarded by the state or any of its political subdivisions to any vendor or prospective vendor when the vendor or prospective vendor or a related party to the vendor or prospective vendor is a debtor and: (1) the debt owed is an amount greater than one thousand dollars in the aggregate; or (2) the debtor is in employer default.

EXCEPTION: The prohibition listed above does not apply where a vendor has contested any tax administered pursuant to chapter eleven of the W. Va. Code, workers' compensation premium, permit fee or environmental fee or assessment and the matter has not become final or where the vendor has entered into a payment plan or agreement and the vendor is not in default of any of the provisions of such plan or agreement.

DEFINITIONS:

"Debt" means any assessment, premium, penalty, fine, tax or other amount of money owed to the state or any of its political subdivisions because of a judgment, fine, permit violation, license assessment, defaulted workers' compensation premium, penalty or other assessment presently delinquent or due and required to be paid to the state or any of its political subdivisions, including any interest or additional penalties accrued thereon.

"Employer default" means having an outstanding balance or liability to the old fund or to the uninsured employers' fund or being in policy default, as defined in W. Va. Code § 23-2c-2, failure to maintain mandatory workers' compensation coverage, or failure to fully meet its obligations as a workers' compensation self-insured employer. An employer is not in employer default if it has entered into a repayment agreement with the Insurance Commissioner and remains in compliance with the obligations under the repayment agreement.

"Related party" means a party, whether an individual, corporation, partnership, association, limited liability company or any other form or business association or other entity whatsoever, related to any vendor by blood, marriage, ownership or contract through which the party has a relationship of ownership or other interest with the vendor so that the party will actually or by effect receive or control a portion of the benefit, profit or other consideration from performance of a vendor contract with the party receiving an amount that meets or exceed five percent of the total contract amount.

AFFIRMATION: By signing this form, the vendor's authorized signer affirms and acknowledges under penalty of law for false swearing (*W. Va. Code* §61-5-3) that neither vendor nor any related party owe a debt as defined above and that neither vendor nor any related party are in employer default as defined above, unless the debt or employer default is permitted under the exception above.

WITNESS THE FOLLOWING SIGNATURE:

Vendor's Name: West Virginia University

Authorized Signature: MaryJane Buckland Date: 3/18/2014

State of West Virginia

County of Monongalia, to-wit:

Taken, subscribed, and sworn to before me this 18 day of March, 2014.

My Commission expires September 27, 2022.

AFFIX SEAL HERE

NOTARY PUBLIC Molly Superfesky

Official Seal
Notary Public, State Of West Virginia
Molly A Superfesky
625 Southern Avenue
Morgantown WV 26501
My commission expires September 27, 2022

Rev. 07/12

State of West Virginia
VENDOR PREFERENCE CERTIFICATE

Certification and application* is hereby made for Preference in accordance with ***West Virginia Code***, §5A-3-37. (Does not apply to construction contracts). ***West Virginia Code***, §5A-3-37, provides an opportunity for qualifying vendors to request (at the time of bid) preference for their residency status. Such preference is an evaluation method only and will be applied only to the cost bid in accordance with the ***West Virginia Code***. This certificate for application is to be used to request such preference. The Purchasing Division will make the determination of the Resident Vendor Preference, if applicable.

1. **Application is made for 2.5% resident vendor preference for the reason checked:**
____ Bidder is an individual resident vendor and has resided continuously in West Virginia for four (4) years immediately preceding the date of this certification; **or,**
____ Bidder is a partnership, association or corporation resident vendor and has maintained its headquarters or principal place of business continuously in West Virginia for four (4) years immediately preceding the date of this certification; or 80% of the ownership interest of Bidder is held by another individual, partnership, association or corporation resident vendor who has maintained its headquarters or principal place of business continuously in West Virginia for four (4) years immediately preceding the date of this certification; **or,**
____ Bidder is a nonresident vendor which has an affiliate or subsidiary which employs a minimum of one hundred state residents and which has maintained its headquarters or principal place of business within West Virginia continuously for the four (4) years immediately preceding the date of this certification; **or,**

2. **Application is made for 2.5% resident vendor preference for the reason checked:**
____ Bidder is a resident vendor who certifies that, during the life of the contract, on average at least 75% of the employees working on the project being bid are residents of West Virginia who have resided in the state continuously for the two years immediately preceding submission of this bid; **or,**

3. **Application is made for 2.5% resident vendor preference for the reason checked:**
____ Bidder is a nonresident vendor employing a minimum of one hundred state residents or is a nonresident vendor with an affiliate or subsidiary which maintains its headquarters or principal place of business within West Virginia employing a minimum of one hundred state residents who certifies that, during the life of the contract, on average at least 75% of the employees or Bidder's affiliate's or subsidiary's employees are residents of West Virginia who have resided in the state continuously for the two years immediately preceding submission of this bid; **or,**

4. **Application is made for 5% resident vendor preference for the reason checked:**
____ Bidder meets either the requirement of both subdivisions (1) and (2) or subdivision (1) and (3) as stated above; **or,**

5. **Application is made for 3.5% resident vendor preference who is a veteran for the reason checked:**
____ Bidder is an individual resident vendor who is a veteran of the United States armed forces, the reserves or the National Guard and has resided in West Virginia continuously for the four years immediately preceding the date on which the bid is submitted; **or,**

6. **Application is made for 3.5% resident vendor preference who is a veteran for the reason checked:**
____ Bidder is a resident vendor who is a veteran of the United States armed forces, the reserves or the National Guard, if, for purposes of producing or distributing the commodities or completing the project which is the subject of the vendor's bid and continuously over the entire term of the project, on average at least seventy-five percent of the vendor's employees are residents of West Virginia who have resided in the state continuously for the two immediately preceding years.

7. **Application is made for preference as a non-resident small, women- and minority-owned business, in accordance with *West Virginia Code* §5A-3-59 and *West Virginia Code of State Rules*.**
____ Bidder has been or expects to be approved prior to contract award by the Purchasing Division as a certified small, women- and minority-owned business.

Bidder understands if the Secretary of Revenue determines that a Bidder receiving preference has failed to continue to meet the requirements for such preference, the Secretary may order the Director of Purchasing to: (a) reject the bid; or (b) assess a penalty against such Bidder in an amount not to exceed 5% of the bid amount and that such penalty will be paid to the contracting agency or deducted from any unpaid balance on the contract or purchase order.

By submission of this certificate, Bidder agrees to disclose any reasonably requested information to the Purchasing Division and authorizes the Department of Revenue to disclose to the Director of Purchasing appropriate information verifying that Bidder has paid the required business taxes, provided that such information does not contain the amounts of taxes paid nor any other information deemed by the Tax Commissioner to be confidential.

Under penalty of law for false swearing (West Virginia Code, §61-5-3), Bidder hereby certifies that this certificate is true and accurate in all respects; and that if a contract is issued to Bidder and if anything contained within this certificate changes during the term of the contract, Bidder will notify the Purchasing Division in writing immediately.

Bidder: West Virginia University **Signed:** Mary Jane Cuckland

Date: 3/18/14 **Title:** Interim Director

This bid is being submitted for consideration in establishing a contract to conduct genetic analysis on collected samples from white-tailed deer to better understand Chronic Wasting Disease (CWD). To accomplish this task, laboratory work will be completed on 800 samples from white-tailed deer. Processing of additional samples would require a contract amendment. All samples will be analyzed using 15 microsatellite loci, the control region of the mitochondrial DNA (mtDNA), and the prion protein gene (PRNP). All the laboratory work will be conducted at Dr. Amy Welsh's genetics laboratory at West Virginia University. Dr. Welsh has an active laboratory that is conducting similar research projects and is fully equipped to do all the laboratory techniques in-house. The resulting data will then be analyzed to achieve the following objectives:

1. **Identify genetic neighborhoods based on relatedness between individuals.**
   Microsatellite and mtDNA data from female deer will be used to achieve this objective . Genetic relatedness between individuals will be measured and neighborhoods of related individuals will be delineated.
2. **Determine dispersal patterns of male white-tailed deer.**
   Microsatellite and mtDNA data from male deer will be used to determine male dispersal patterns. Male deers will be assigned to the neighborhood from which they most likely originated.
3. **Identify barriers to white-tailed deer dispersal.**
   Genetic data and GIS data will be overlaid. Genetic breaks in the landscape will be identified and the corresponding features in the landscape that are causing that break in gene flow will be determined.
4. **Evaluate the relationship between genetic diversity at the PRNP gene and CWD prevalence.**
   A single nucleotide polymorphism (SNP) assay will be used to genotype individuals at the 95th, 96th, and 116th codon of the PRNP gene. A correlation between frequency of the CWD-resistant genotype and CWD prevalence will be tested.

Following manuscript submission, all generated microsatellite and SNP genotypes and mtDNA haplotypes will be archived in a database for potential future use. Mitochondrial sequences will be inputted into the publicly available database GENBANK (http://www.ncbi.nlm.nih.gov/genbank/). The genotype data (i.e., microsatellite loci, SNPs) will be stored in a data archive such as DRYAD (datadryad.org). In addition, an electronic file with all generated genotypes and haplotypes will be provided to WVDNR.

A Ph.D. student will work on the project under the direction of Dr. Amy Welsh. A student Darren Wood has been identified for the project. The student is completing his M.S. degree under Dr.

Welsh, studying the landscape genetics of brook trout. He has significant laboratory experience and experience analyzing landscape genetic data, therefore ensuring timely completion of the project. The student also has the necessary creativity and scientific reasoning skills to develop his own hypotheses related to the project. The student's committee will consist of Dr. Welsh, Dr. Michael Strager (GIS specialist at WVU), Dr. James Crum or Dr. Christopher Ryan from WVDNR, and two other members.

Timeline

January 2014 – December 2014	Microsatellite genotyping & mtDNA sequencing of all samples
January 2015 – June 2015	Landscape genetic analysis overlaying genetic & GIS data
	Assignment of male deer to neighborhoods
July 2015 – March 2016	SNP assay of all samples
April 2016 – December 2016	Complete analysis of genetic data
	Prepare dissertation and manuscripts for publication

Deliverables

A minimum of two presentations will be made at regional and national conferences. Annual reports will be submitted documenting progress and a final report describing the results and their management implications will be prepared. A minimum of one publication will be submitted to a peer-reviewed journal.

Documentation of Qualifications:

1. Offer a Ph.D. in wildlife management, wildlife biology or wildlife ecology from an accredited university.
   West Virginia University has a highly respected wildlife and fisheries program through which students can obtain their Ph.D. The program is housed within the Division of Forestry and Natural Resources; therefore, the actual name of the Ph.D. degree is Forestry and Natural Resources. Exhibit A contains a list of all Ph.D. within the Davis College.
2. At least one faculty member within their college or university who has a Ph.D. specialty in genetics who is willing to serve on the student's committee.
   The principal investigator, Dr. Amy Welsh, has a Ph.D. degree with a specialty in genetics. Exhibit B contains Dr. Welsh's CV.
3. Summaries of and references from at least 5 related studies.

Exhibit C contains reprints of 5 publications from Dr. Welsh dealing with natural barriers, genetic flow, or microsatellite loci that were all published in peer-reviewed scientific journals.

4. Documentation of a GIS specialist.

   Dr. Michael Strager is the GIS specialist in the College and has agreed to serve on the student's committee. Exhibit D is a memo from Dr. Strager stating such.

West Virginia University

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- Design & Merchandising
- Forestry & Natural Resources
- Plant & Soil Sciences
- Resource Management
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Doctoral Program Areas

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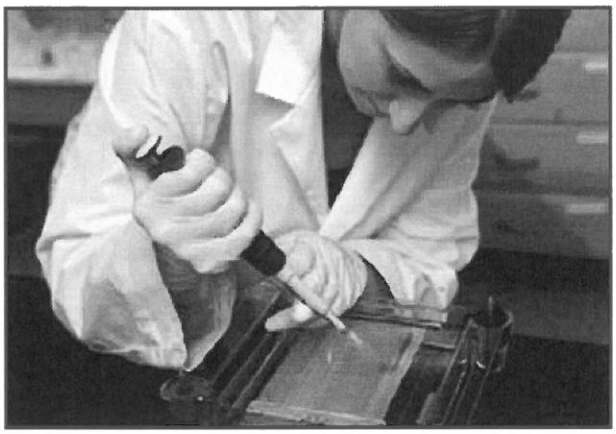
Agricultural Biochemistry	Forest Resource Management	Recreation, Parks & Tourism Resources
Agronomy	Genetics & Developmental Biology	Reproductive Physiology
Animal Nutrition	Horticulture	Resource Management
Animal Physiology	Human & Community Development	Soil Science
Animal Production	Human Nutrition	Wildlife & Fisheries Resources
Applied & Environmental Microbiology	Natural Resource Economics	Wood Science & Technology
Entomology	Organic Agriculture	

Agricultural & Extension Education

PhD in Resource Management & Sustainable Design

Agricultural & Extension Education website

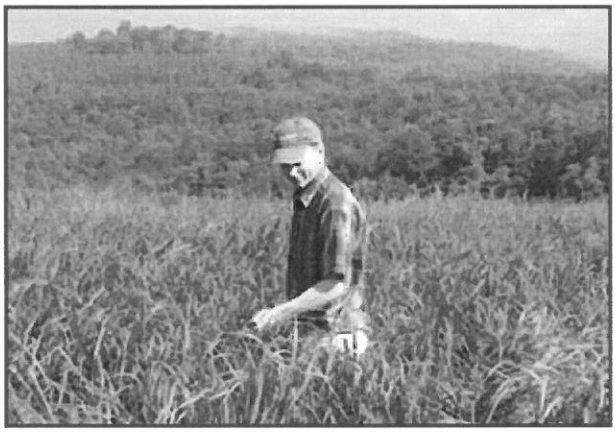
- major in agricultural and extension education (AGEE) OR
- human and community development (HCD)



Agricultural Biochemistry

PhD in Agricultural Sciences

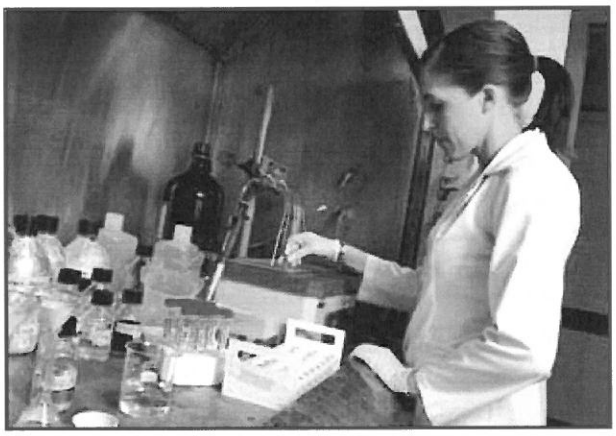
Biochemistry website



Agronomy

PhD in Agricultural Sciences

Agronomy website



Animal Nutrition

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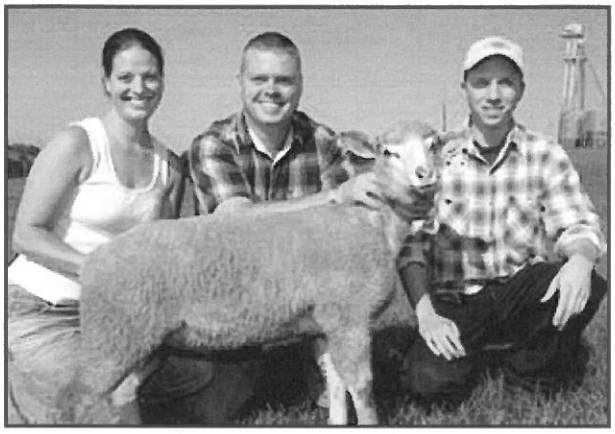
Animal & Nutritional Sciences website

Animal Physiology

PhD in Agricultural Sciences

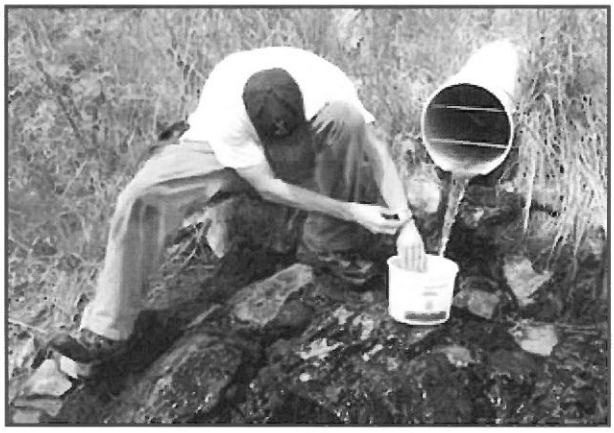
Animal & Nutritional Sciences website



Animal Production

PhD in Agricultural Sciences

Animal & Nutritional Sciences website



Applied & Environmental Microbiology

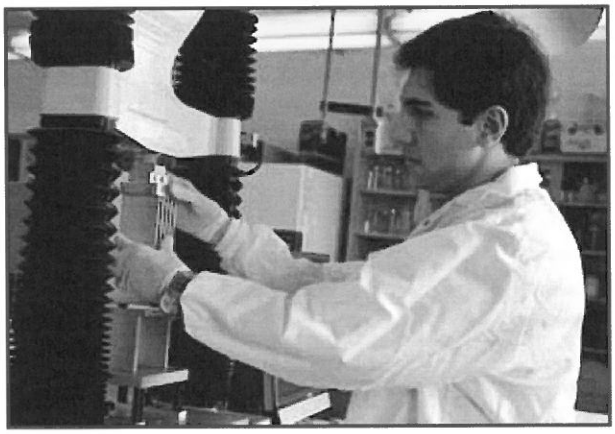
PhD in Agricultural Sciences

Applied & Environmental Microbiology website

Entomology

PhD in Agricultural Sciences

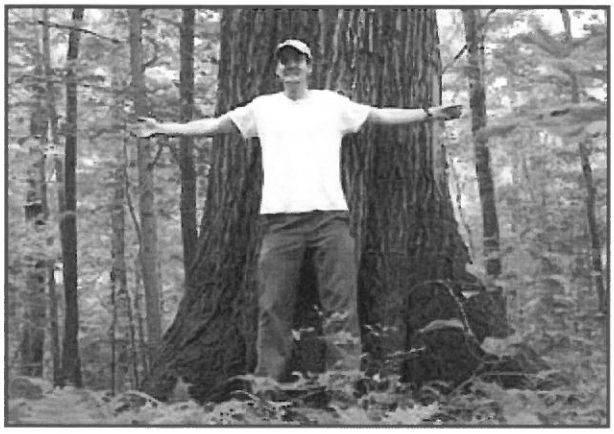
Entomology website



Food Science

PhD in Agricultural Sciences

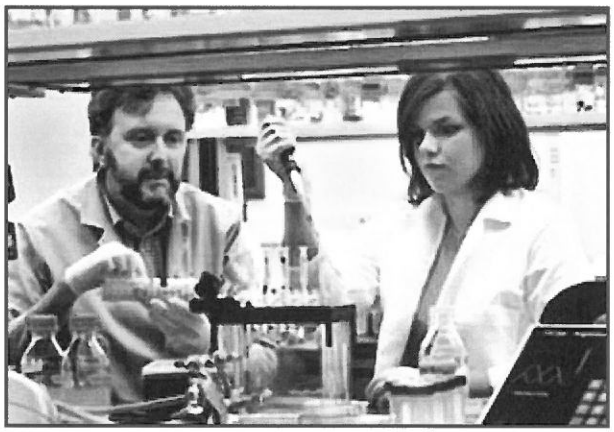
Human Nutrition & Foods website



Forest Resource Management

PhD in Forest and Resource Sciences

Forest Resources Management website



Genetics & Developmental Biology

PhD in Genetics & Developmental Biology

Genetics & Developmental Biology website

Horticulture

PhD in Agricultural Sciences

Horticulture website

Human & Community Development

PhD in Resource Management & Sustainable Development

Design Studies website

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Human Nutrition

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Human Nutrition & Foods website

Natural Resource Economics

PhD in Resource Management & Sustainable Development

Agricultural and Resource Economics Program

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Agroecology website

Plant Pathology

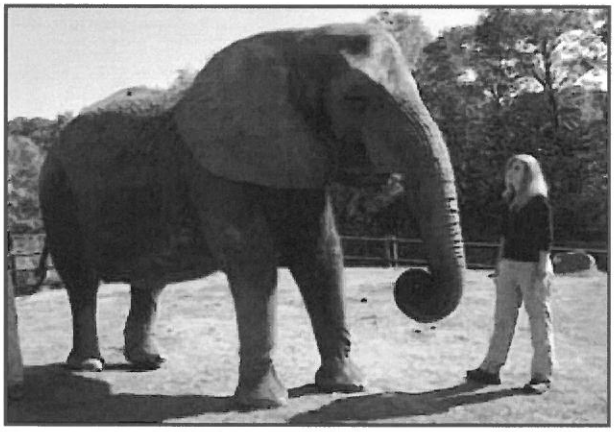
PhD in Agricultural Sciences

Plant Pathology website

Recreation, Parks & Tourism Resources

PhD in Forest and Resource Sciences

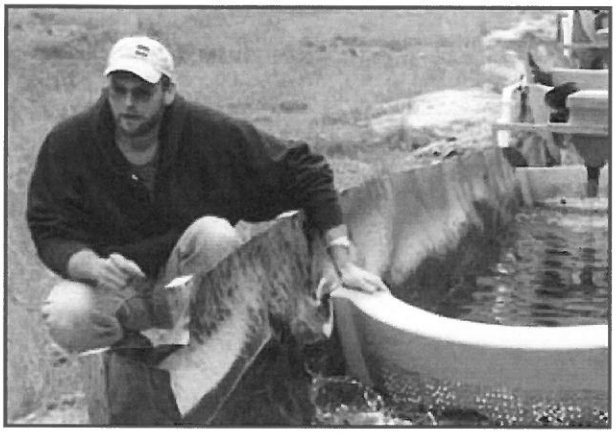
Recreation, Parks and Tourism Resources website



Reproductive Physiology

PhD in Reproductive Physiology

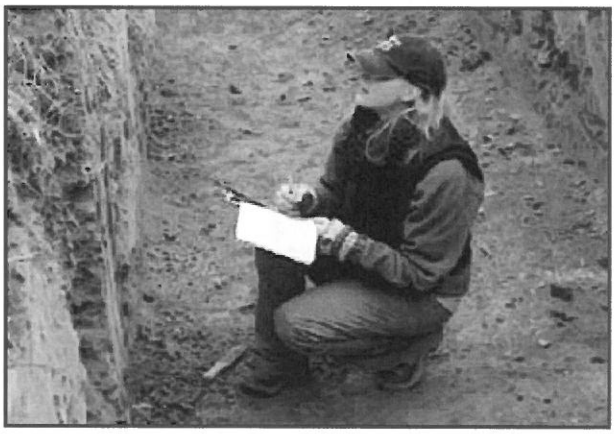
Reproductive Physiology website



Resource Management

PhD in Resource Management & Sustainable Development

PhD in Resource Management & Sustainable Development webpage



Soil Science

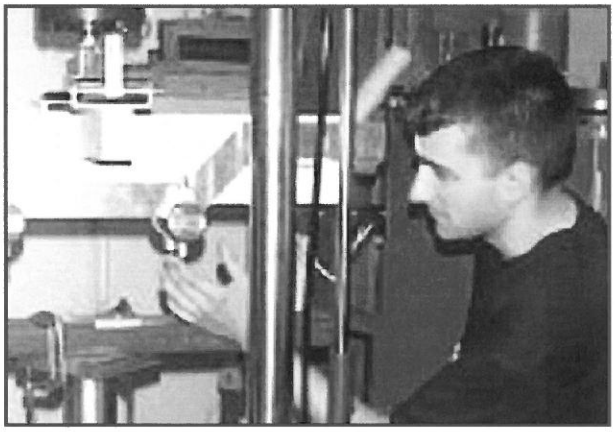
PhD in Agricultural Sciences

Soil Science website

Wildlife & Fisheries Resources

PhD in Forest and Resource Sciences

Wildlife & Fisheries Resources website



Wood Science & Technology

PhD in Forest and Resource Sciences

Wood Science and Technology website

Davis College of Agriculture, Natural Resources & Design | 1168 Agricultural Sciences Building
PO Box 6108 | Morgantown, WV 26506-6108 | Phone: 304-293-2395 | Fax: 304-293-3740 | **Contact Us**

400 Quarrier Street Charleston, WV 25301-2010

BrickStreet Mutual Insurance Company
A Mutual Company

Workers Compensation and Employers Liability Insurance Policy

Policy Number	Policy Period From	To
WCB1019700	07/01/2013	07/01/2014
	(12:01 AM at the insured location)	

Information Page	Renewal/Rewrite of Policy Number
	NEW

1. Named Insured and Address	Agency Information
West Virginia University One Waterfront Place Morgantown, WV 26506	2013 Wells Fargo Insurance Services of West Virginia Inc. Wells Fargo Morgantown WV 1075 Van Voorhis Road Ste 200 Morgantown, WV 26505-3587

Carrier No.	FEIN	Risk ID	Entity Type
15762	55-6000842	470150890	Government Agency

Additional Workplaces not shown above:
Refer to Schedule of Locations Endorsement WC 99 06 02 (07-09)

2. The Policy Period is from 07/01/2013 to 07/01/2014 12:01am Standard Time at the insured's mailing address.

3. A. Workers Compensation Insurance: Part One of the policy applies to the Workers Compensation Law of the states listed here: WV

   B. Employers Liability Insurance: Part Two of the policy applies to work in each state listed in Item 3.A. The limits of our liability under part Two are:

Bodily Injury by Accident:	$100,000.00	Each Accident
Bodily Injury by Disease:	$500,000.00	Policy Limit
Bodily Injury by Disease:	$100,000.00	Each Employee

   C. Other States Insurance: Part Three of the policy applies to the states, if any, listed here: All states and U.S. territories except North Dakota, Ohio, Washington, Wyoming, Puerto Rico, and the U.S. Virgin Islands, and states designated in Item 3.A. of the Information Page.

   D. This policy includes these endorsements and schedules: SEE ATTACHED SCHEDULE

4. The premium for this policy will be determined by our Manuals of Rules, Classifications, Rates and Rating Plans. All Information required below is subject to verification and change by audit.

SEE ATTACHED CLASSIFICATIONS OF OPERATIONS

Minimum Premium: $500.00

Total Estimated Annual Premium:	$488,285.00
Premium Discount:	
Expense Constant:	$175.00
Deposit Premium:	$104,987.00

Issue Date: 06/28/2013
Issuing Office: Charleston, WV

WestVirginiaUniversity.
Davis College of Agriculture, Natural Resources and Design

MEMORANDUM

March 8, 2014

This memo is to acknowledge my willingness to serve on the graduate committee of Darren Wood to assist with GIS data collection, analysis, and modeling as part of a landscape genetics project on white tail deer with WVDNR.

I have had extensive work and research experience using GIS and advanced spatial analysis techniques applied to terrestrial wildlife databases and models.

Sincerely,

Michael P. Strager

Michael P. Strager
Associate Professor of Spatial Analysis
304-293-6463
mstrager@wvu.edu

Division of Resource Management

Phone: 304-293-4832
Fax: 304-293-3752
www.davis.wvu.edu

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