

LIVING AT THE EDGE



October 2016

Do interstate corridors serve as linear dispersal routes for terricolous lichens?

2016 Fund for LSSU Grant Report

Living at the edge

DO INTERSTATE CORRIDORS SERVE AS LINEAR DISPERSAL ROUTES FOR TERRICOLOUS LICHENS?

ORIGINAL FUNDING DETAILS

Funding amount: \$500

Funding received: February 2016

Original Proposal Timeline:

Project Length 1 year: Spring 2016 until Spring 2017

- Genome Sequencing, and Microsatellite DNA Marker Design – Spring 2016
- Initial primer trials and optimization – Late spring 2016 – Early Summer 2016
- Field Collection and Fragmentation Trials – Summer 2016
- DNA Isolation and analysis – Fall 2016 (partially integrated into BIOL220 as lab exercises)
- Completion of Analysis and presentation of results – Spring 2017

CURRENT PROGRESS

Thanks to the funding from the LSSU Foundation's *Fund for LSSU* grant we were able to purchase *Cladonia rangiferina* (grey reindeer lichen) tissue cultures from ATCC.org (the American Type Culture Collection). These initial cultures were used to establish an ongoing axenic (pure) tissue culture of *C. rangiferina*'s mycobiont. This was a critical step for the eventual goal of sequencing the lichens genome because *C. rangiferina*, like all lichens, normally exists as a composite organism that combines a fungus (the mycobiont) with an algal and/or bacterial photosynthetic partner (the photobiont – a species of green algae in this case) (figure 1). This symbiosis imparts numerous advantages to the lichens such as incredible hardiness and the ability to colonize habitats long before other form of life. However, this also complicates matters, from a genetics point of view, as it makes it very difficult to know whose DNA you are isolating from a wild collected sample for sequencing.

Lichens are also notoriously difficult to establish as pure tissue cultures from wild samples, and even if you manage to isolate the right organism they are excruciatingly slow growing. So, to increase efficiency and save one to two years of lab work, we ordered a sample of a previously established pure culture. It still took over three months to culture sufficient tissue mass to isolate enough DNA for genome sequencing.

As the complete genome of *C. rangiferina* had never been sequenced previously, we chose to use the Illumina MiSeq Next Generation Sequencing platform at the Michigan State University Genomics Core facility for *de novo* (unreferenced) DNA sequencing. This generated a raw output of over 19 million sequence reads amounting to 2.83 billion base pairs of unassembled genomic data. From that raw data, a draft genome assembly of approximately 36 million base pairs by our co-investigator Amanda Charbonneau at MSU over the summer. As far as we are aware this is the first genome ever sequenced by LSSU.

While waiting for the genome assembly, my research student, Ian Mangold, began his field work in earnest. Over the summer he collected over 250 samples from across Michigan's Upper Peninsula (figure 2). I also collected additional samples for Ian on my various trips down state. By late summer, Ian also began isolating DNA from the collected samples, this is currently ongoing.

From the genome assembly Ian isolated 65 potential microsatellite DNA markers¹. Ian was then able to design PCR primer pairs for 44 of the potential microsatellite loci. He is currently in the process of trialing the primer pairs for amplification and polymorphism. Any markers that pass these trials must then be fluorescently labeled to enable genotyping via capillary electrophoresis. At this point, all collected samples will be analyzed with up to 15 labelled primers to generate the final dataset for analysis.

Once that final data set is assembled, true population genetic analysis can begin. We will be able to look at the levels of genetic diversity at the regional and local level, look for population structuring, investigate patterns of gene flow between populations and ultimately answer our core research questions – Do interstate corridors serve as linear dispersal routes or gene flow corridors for the grey reindeer lichen?

ADDRESSING STUDENT LEARNING

Other than the initial genome sequencing, this entire project has been student driven. I have been able to guide Ian Mangold through the various stages of the research in what will ultimately be his senior thesis project. This project will provide advanced conceptual and practical experience that will set the student ahead of the competition when applying to graduate school and in future conservation genetics. It is also expected that this project will spawn various spin off projects on topics related to the biology and ecology of *C. rangiferina*.

This project will already provide an excellent opportunity to the BIOL220 Genetics course to have real research material to utilize in lab when learning about DNA isolation and data collection. Using voucher samples collected in parallel with Ian's samples, I have been able to provide every student in the 2016 Genetics class with a unique sample of "unidentified" (identified to the student at least) lichen for them to work on identifying using DNA barcoding techniques. Involving a class in real research has a high potential for student engagement than "canned labs" as they have the benefit of knowing they are contributing to real science. This project is also expected to generate several opportunities for the research students to gain experience with manuscript preparation and conference presentation.

ADDRESSING THE "FOUR PILLARS OF INSTITUTIONAL NEED

The bulk of this project would fall under the pillar of "Student Professional Development" as it will directly contribute to the development of the professional research skills that will help set the students apart when they move onto the next level of employment or graduate school. Formal research outcomes such as journal articles and conference presentations will also help support the "Enrollment" pillar as a means of passive advertisement – spreading the LSSU name. That should in turn help draw in future students.

¹ Microsatellite DNA markers are regions of short sequences of DNA (2-6 base pairs) repeated a variable number of times. These markers are valuable for population genetics because they tend to follow predictable mutation and inheritance patterns while being easy to numerically score, which in turn allows for detailed statistical analysis of populations.

GRANT FUNDED EXPENDITURES

Expenditure	Vender	Cost
Axenic Tissue culture	ATCC	\$280.25
Sample Tubes	USA Scientific	\$123.61
	Total expenditure:	\$403.86

Receipts attached in appendix.

FIGURES



Figure 1- *Cladonia rangiferina*. Natural lichenized state (top). Isolated mycobiont (bottom).

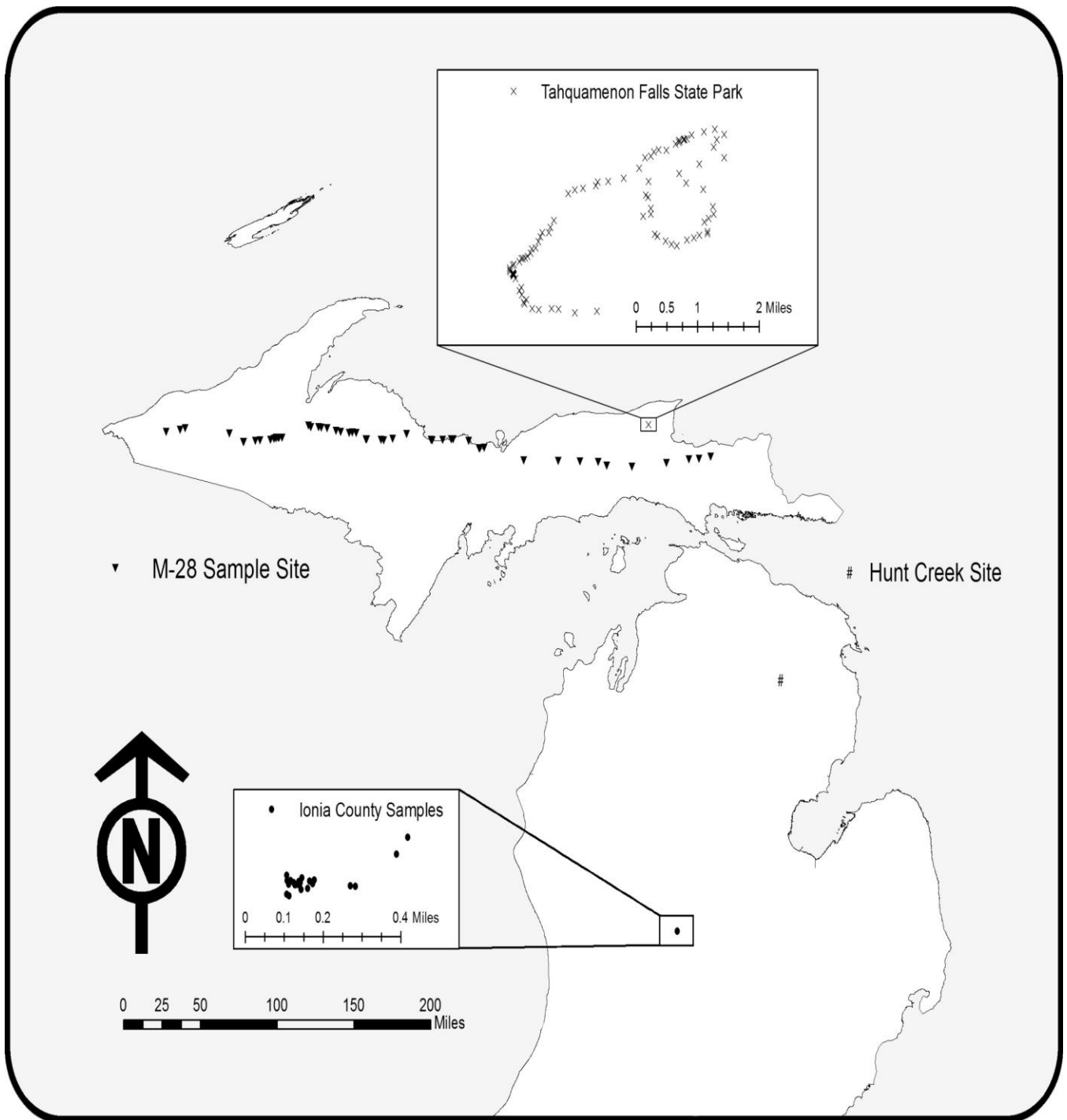


Figure 2 – *C. rangiferina* collection sites.



Benjamin Southwell <bsouthwell@lssu.edu>

CA00128552 ATCC Credit

1 message

ATCC Sales Status <status@atcc.org>
To: "bsouthwell@lssu.edu" <bsouthwell@lssu.edu>

Wed, Mar 23, 2016 at 1:13 PM

Dear Benjamin Southwell,

Thank you for contacting us in regards to your previous ATCC order. After careful review of the order we have created a call record in our system to document the situation. The pertinent order information is listed below for quick reference.

Call Record #:	CA00128552
Sales Order #:	SOJ27717
Purchase Order #:	Southwell
Invoice #:	IVJ28787
Reason for Call:	System charged customer list price instead on non for profit price
Resolution:	ATCC will issue a credit for \$73.75 charged on order SOJ27717 under invoice IVJ28787. This will be processed by our accounting team then refunded back the credit card used for the order.

Please review this information and let us know of any questions or concerns. We thank you for your patience while we process your credit.

Sincerely,

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA

S A L E S O R D E R

Order Number: SOJ27717 Revision: 0
Order Date: 03/15/16 Page: 1
Print Date: 03/18/16

Bill-To: 185018

Ship-To: 00201659

Lake Superior State Universi
650 W Easterday Ave
Sault Saint Marie, MI 49783
USA

Lake Superior State University
1100 Meridian St
Sault Sainte Marie, MI 49783
USA

Attention: Hoornstra, Cheri
Telephone: 906-635-2654
Fax/Telex: 906635-2111

Attention: Kolomyjec, Stephen H
Telephone: 9066352147
Fax/Telex: 9066352266

Salesperson 1:

Credit Terms: 30
Due 30 days from invoice

Purchase Order: Southwell
Ship Via: UPS
DAP: Sault Sainte Marie

Resale:
Remarks:

Ln	Item Number	Planning To Ship	Qty	Open	UM	Price	Extended Price
1	18275	03/21/16	1.0	EA		354.00	354.00
	Cladonia rangiferina						

Non-Taxable: 354.00	Currency: USD	Line Total:	354.00
Taxable: 69.00	0.00%	Discount:	0.00
Tax Date: 03/21/16	Prepaid Shipping 05 :		0.00
Containers: 0.00	Shipping & Handling 20 :		69.00
Line Charges: 0.00	Special Handling 25 :		0.00
	Total Tax:		0.00
	Total:		423.00

ORDER CONFIRMATION

Thank you for your order!

Order Number: SQ268102 has been received and queued for processing.

For quality purposes, requests are reviewed. Additional notifications will be sent when the request is cleared for shipment and when the order ships. Thank you!

Product	ATCC® Number	Qty.	Availability	Customer Specific Price	End User	Permits
Cladonia rangiferina Wiggers (ATCC® 18275™)	18275™	1	Currently Unavailable Contact Sales	\$280.25 \$354.00	Stephen Kolomyjec	N/A

Subtotal: \$280.25
 +\$69.00 Shipping/Handling
 +\$0.00 Sales Tax
 -\$0.00 Discount

Total: \$349.25

Shipping Location

1100 Meridian St
Sault Sainte Marie, MI 49783
USA

*called
3-18-16*

Recipient

Stephen Kolomyjec

Billing Method

Credit Card
PO Number: Southwell

Shipping Preference:

Ship Complete - Ship my order when all items are available (may result in delayed delivery if some items are on backorder).

An order placement confirmation email will be sent to bsouthwell@lssu.edu



USA Scientific
P.O. Box 3565
Ocala, FL 34478, U.S.A
TEL 1-800-LAB-TIPS FAX 352-351-2057
Int'l: 352-237-6288

Bill to account # 9145201000

Bill to address

LAKE SUPERIOR STATE UNIV
ACCTS PAY
650 W EASTERDAY AVENUE
SAULT SAINTE MARIE MI 49783
BSOUTHWELL@LSSU.EDU

Ship to account # 9145201001

Ship to address

LSSU / ATTN: BENJAMINE SOUTHWELL
650 W EASTERDAY AVENUE
SAULT SAINTE MARIE MI 49783

INVOICE

Invoice No. / Date
4801086605 / 09/29/2016
Order No. / Date
2845377 / 09/28/2016
Your Ref. No. / Date
MC0928BS / 09/28/2016
Delivery Note No. / Date
83328015 / 09/28/2016
Sales Rep # and Name
480030 - WENDY ROBERTSON

Terms of payment
Within 30 days Due net
Terms of delivery
FOB ENFIELD
Shipping condition
PP&Add ground
Forwarder & BOL / Tracking number
FEDERAL EXPRESS CORP.
754114471175199
F.E.I. # 59-224457-9
D-U-N-S # 10-6947237

Currency: USD

Item	Material	Description	Quantity	Price	Price Unit	Amount
000010	2396-5000	96-PLACE TUBE RACK*NATURAL*5/PACK	1 UNT	35.00	1 UNT	35.00
	POLYPROPYLENE 96-PL TUBE BESTRACK*NAT COLOR*5/PK* 8 7/16"L X 4 5/8"W X 2"H					
000020	1405-9302	0.5ML SELF-STAND TPE SEAL SCREW CAP TUBE	1 UNT	42.00	1 UNT	42.00
	0.5ML SELF-STANDING TPE SEAL SCREW CAP TUBE* 500/CAPS & 500/TUBES PER BAG=1 UNIT*COLOR CAPS* CAP COLOR GREEN					
000050	1415-9301	1.5ML SELF-STANDING TPE SEAL SCREW CAP				

Remittance by Check:

USA Scientific
Accounts Receivable
P.O. Box 30000
Orlando, FL 32891-8210
Please return remittance copy with payment.

Remittance by ACH/EFT:

Suntrust Bank of North Central Florida
P.O. Box 310
Ocala, FL 34478
Acct. #:0072000163023
ABA#: 061-000-104



USA Scientific
P.O. Box 3565
Ocala, FL 34478, U.S.A
TEL 1-800-LAB-TIPS FAX 352-351-2057
Int'l: 352-237-6288

4801086605 / 09/29/2016

Item	Material Quantity	Description Price	Price Unit	Amount
	1 UNT	35.00	1 UNT	35.00
1.5ML SELF-STDG BASE SCREW CAP* TUBE W/BLUE&WHT TPE RING CAP*CERT RNASE DNASE\& DNA FREE. 500 CAPS & 500 TUBES=1 UNIT				

Items Total	112.00
Freight	11.61
Total	123.61

***** This amount has been charged to your Credit Card *****

Auth. Number	Amount	Transaction Date
092593	123.61	09/29/2016