Michigan Department of Natural Resources
Genetics Program
2023 Program Review
October 1, 2022 – September 31, 2023

Personnel:
Caitlin Ott-Conn: Laboratory Scientist
Anthony Clyne: Student Assistant (October 2022 – present)

Trinity Hinshaw: Student Volunteer (August 2022 – present)
Lorna Musgrave: Student Volunteer (October 2022 – May 2023)
Zach Ziegler: Student Volunteer (October 2022 – present)
Louis Good: Student Volunteer (March 2023 – present)
Cassie Stitzman: Student Volunteer (May 2023 – August 2023)
Emilee Gooch: Student Volunteer (May 2023 – present)
Jacob Morales: Student Volunteer (June 2023 – present)

Summary:
This year has been a year of transition and outreach. While the lab's location has been moved onto the campus of Northern Michigan University and is now housed within the Species Management Unit, it still functions as it has in the past to provide annual genetic data to species specialist programs and utilize collaborations to research new methodologies and conduct analyses on archived samples. The move has allowed for integration within the campus at NMU bringing lots of eager, talented students who have been able to learn about laboratory techniques and directly apply them to DNR projects. The location change has also fostered camaraderie with university professors interested in how genetics are utilized by the Division. Two in-class presentations and one seminar were offered covering topics from job specifications to specific projects and equipment.

This move has also allowed for greater integration and available assistance to the UP regional staff for biological sample requests.

Major projects this year included the preparation of samples from the northern lower peninsula black bear hair snare survey, continued work with the UP close-kin mark recapture research study, and the annual bobcat genetic sexing survey.

We look forward to migrating our archive over to Arctos next year to increase ease of use by external researchers; begin integrating new technologies afforded with the purchase of an Illumina MiniSeq thanks to generously supported funds provided by SCI's Michigan Involvement Committee; and updating and streamlining our archive request process with the help of the Species Management Unit and Biological Social Science Section.

Connectivity/Communications:

The Wildlife Society: Annual National Conference November 2022
Symposium Co-Author: Advances in environmental DNA for wildlife management
Spokane, WA

Museum Archive Protocols November 2022
Dr. Kurt Galbredth, NMU

The Wildlife Society: Annual National Conference November 2022
Presentation Co-Author: Evaluation of close-kin mark–recapture abundance estimates in varied mating systems and sampling strategies Spokane, WA

University Campus Presentations

Conservation Genetics (Michigan Tech) March 2023
Fisheries and Wildlife Seminar (NMU) April 2023
Genetics in Natural Resources (NMU) April 2023

Seminars/Webinars attended:

Molecular evolution in the mountains: Adaptation in the American pika November 2022
Zach Farrand MS Candidate NMU

Use of Environmental DNA to Monitor Population Parameters on DoD Lands October 2022
Strategic Environmental Research and Development Program (SERDP) & Environmental Security Technology Certification Program (ESTCP)

Multiplexed digital PCR techniques to monitor public health threats August 2022
Qiagen

Student Involvement (Northern Michigan University)

Total hours of student volunteer time: 179.5
Total student internships: 3

Citations:

Sévêque, A., Lonsinger, R. C., Waits, L. P., Brzeski, K. E., Ott-Conn, C., Mayhew, S. L., Norton, D. C., Petroelje, T. R. & Morin, D. J. (2022, November 6–10). *Evaluation of close-kin mark–recapture abundance estimates in varied mating systems and sampling strategies* [Conference presentation]. The Wildlife Society's 29th Annual Conference, Spokane, WA, United States.

Fusco, N., Cosentino, B., Gibbs, J., Allen, M., Blumenfeld, A., Boettner, G., Briggs, K., Carlen, E., Collins, M., Dennison, C., DiGiacopo, D., Drapeau Picard, A.P., Edmonson, J., Fisher-Reid, M.C., Fyffe, R., Gallo, T., Grant, A., Harbold, W., Heard, S., Lafferty, D., Lehtinen, R., Marino, S., McDonald, J., Mortelliti, A., Murray, M., Newman, A., Oswald, K., **Ott-Conn, C.**, Richardson, J., Rimbach, R., Salaman, P., Steele, M., Stothart, M., Urban, M., Vandegrift, K., Vanek, J., Vanderluit, S., Vezina, L., Caccone, A. Population genomic structure of a widespread mammal after three centuries of landscape change. In-review. Molecular Ecology.

Current Projects and Collaborations:

Focus	Institution(s)	Lead Genetics PI(s)	Project Title	DNR Involvement	Start Year	Appendix
Black bear	Michigan DNR, Wildlife Genetics International	Caitlin Ott-Conn	Northern Lower hair snare population capture-recapture	Coordination for sample collection, sample and data preparation, laboratory communication, sample shipment, data dispersal	2023	
Black bear	Michigan DNR, Michigan Tech, Mississippi State University, University of Idaho, Oklahoma State University	Kristin Brzeski	Replacement Population Index for Michigan's Upper Peninsula Black Bears	Collect samples, administer collaborations and funding	2021	A
Black bear	USFWS	Hope Draheim	Development of a range-wide forensic genetic database of American black bears (*Ursus americanus*) within the continental United States	Provide DNA for black bear across their range within Michigan	2022	B
White-tailed deer	Michigan DNR, Michigan Tech	Kristin Brzeski	Genetic Implications in Chronic Wasting Disease Management of Michigan White-tailed Deer	Provide genomic dataset and guide objectives and outcomes	2022	C
Marten	University of Wisconsin-Madison	Lydia Druin	Contemporary Genetic Sampling of American Marten within the Upper Peninsula of Michigan, USA	Provide tissue and corresponding registration data	2022	D
Gray wolf	Michigan Tech University	Kristin Brzeski	Molecular Ecology Laboratory Instructional Aide and Gray Wolf Genetic Dataset Development	Selection and preparation of wolf samples and provide virtual guest lectures	2020	E
Eastern gray squirrel	Hobart & William Smith Colleges, State University New York – ESF, Yale University	Brad Cosentino	Gray Squirrel Urban-to-Rural Clines in Melanism	Collection and submission of opportunistic Eastern gray squirrel tissue samples and data	2020	F
River otter	Michigan Tech University	Stacy Cotey	Broadscale Functional Connectivity and *Toxoplasma gondii* prevalence of Northern River Otter in the Upper Peninsula of Michigan	Collection of tongue samples and data	2019	G
White-tailed deer	Colorado State University Prion Research Center	Mark Zabel	Examination of Chronic Wasting Disease Prion Strains from Free-Ranging Cervids	Selection, preparation, and submission of CWD+ white tailed deer tissue samples. Awaiting final publication.	2018	H
White-tailed deer	Michigan DNR, Iowa State University, University of Wisconsin- Milwaukee, Texas A&M University-Kingsville, USGS National Wildlife Health Center, NOAA	Julie Blanchong and Emily Latch	A standardized, high-throughput genetic resource to inform white-tailed deer population and disease management	Principle Investigator. Sample preparation and submission from Michigan white-tailed deer. Collaborate with partnering states.	2018	I
Bobcat	Michigan DNR	Caitlin Ott-Conn	Annual genetic sex assessment of harvested individuals	Collection, preparation, analysis, and data reporting of each harvested individual annually	2011	

Appendix A

Replacement Population Index for Michigan's Upper Peninsula Black Bears

Background:

The DNR's current bear population estimation technique relies on a statistical modeling framework called statistical catch-at-age analysis (SCAA), which provides annual estimates of the bear population in the UP and northern Lower Peninsula (NLP). The SCAA model combines harvest composition and effort data to model changes in the bear population over time, scaling the population abundance estimate with information from periodic independent population estimates. Past research has indicated that without an independent population estimate approximately every 5 years, the UP SCAA model would overestimate the bear population, which would affect the quota-setting process. Since 1990, the DNR has used a mark-recapture technique using the antibiotic tetracycline to calculate an independent population estimate of the UP-bear population. Due to recent changes in federal regulatory guidelines, the DNR can no longer conduct the tetracycline survey. The last UP tetracycline survey was conducted in 2014, making it already over 5 years since an independent population estimate was used to scale the UP SCAA model.

In 2021, the DNR's black bear program initiated a project to test a genetic survey, called close-kin mark-recapture, to replace the discontinued tetracycline survey in the UP. The CKMR technique would allow the DNR to estimate the bear population in the UP based on genetic identification of related individuals in the bear harvest. Using the CKMR technique could save the DNR at least 2,000 hours of staff time per survey year over the tetracycline survey and would provide estimates 1.5 years earlier than with the tetracycline survey. If successful, the DNR could also use the CKMR technique in the northern Lower Peninsula, replacing the current genetic mark-recapture survey used to scale the SCAA model and save an additional 3,000 hours of staff time per survey year.

Objectives:

1) Develop a genetic marker panel to discern relatedness of Michigan UP black bears to the degree needed for CKMR analyses
2) Develop a close-kin mark-recapture (CKMR) model

Progress:

Sévêque, A., Lonsinger, R.C., Waits, L.P., Brzeski, K.E., Komoroske, L.M., **Ott-Conn, C.N.**, Mayhew, S.L., Norton, D.C., Petroelje, T.R., Swenson, J.D. & Morin, D.J. (2023) Sources of bias in applying close-kin mark-recapture to terrestrial game species with different life histories. Ecology – Under review.

Sévêque, A., Lonsinger, R. C., Waits, L. P., Brzeski, K. E., **Ott-Conn, C.**, Mayhew, S. L., Norton, D. C., Petroelje, T. R., Tallon, A. & Morin, D. J. (2023 April) "Evaluating the use of close-kin mark–recapture with lethal samples to estimate the black bear population size in Michigan" [Conference presentation]. Eastern Black Bear workshop. Trego, WI, USA.

Sévêque, A., Lonsinger, R. C., Waits, L. P., Brzeski, K. E., **Ott-Conn, C.**, Mayhew, S. L., Norton, D. C., Petroelje, T. R. & Morin, D. J. (2022 November). *Evaluation of close-kin mark–recapture abundance estimates in varied mating systems and sampling strategies* [Conference presentation]. The Wildlife Society's 29th Annual Conference, Spokane, WA, United States.

Ott-Conn, C., Mayhew, S. (August 2022). *Bear population estimation: close-kin mark recapture* [Public presentation]. Michigan Bear Forum, Sault Saint Marie, MI, United States.

Ott-Conn, C., Brzeski, K. (2021 November). *State and university genetic partnership: Michigan black bear population estimator* [Conference presentation}. The Wildlife Society's 28th Annual Conference, Virtual.

Appendix B

Development of a Range-wide Forensic Genetic Database of American Black Bears (*Ursus americanus*) within the Continental United States

Background:

The National Fish and Wildlife Forensics Laboratory is creating a range wide genetic database for North American black bears within United States. The database will be used for wildlife law enforcement needs including species identification, sex identification, individual identification, relatedness and geographic origin.

The goal is to curate the database for wildlife forensic practitioners to use in casework. Our current sample representation is missing populations from the Great Lakes region and the Missouri/Arkansas populations limiting our ability to successfully determine the origin of bear biological material that comes from this area of the species range.

DNA from both the Lower and Upper Peninsula's of Michigan will be used to account for fine-scale genetic structure and eliminate erroneous assignment due to sample bias.

Objectives:

1) Assess upwards of 50 Michigan black bear genetic samples per bear management unit
2) Provide DNR with resulting data and accompanying analyses
3) Provide DNR or partnering laboratory with training and materials to develop in-house protocols for this analysis when complete

Progress:

All samples have been extracted and are awaiting final plating for shipment.

Appendix C

Genetic Implications in Chronic Wasting Disease Management of Michigan white-tailed deer

Background:

Funding from USGS-APHIS has allowed the opportunity for DNR personnel to collect background population genetic data on white-tailed deer across the state. The goal of this data collection has been to have a dataset for future comparisons for population of origin and relatedness for disease surveillance purposes. By combining this dataset* with data from scat samples collected in the Keweenaw region, an area currently underrepresented in the dataset, the DNR will provide resources for the training of a graduate student while obtaining necessary analyses and downstream tools.

Objectives:

1) Assess relatedness estimates for CWD+ deer to date
2) Provide code relatedness estimates as well as probability of population of origin
3) Provide basic interpretations to tease out potential patterns on the landscape

Progress:

Initial data cleanup and analyses have begun.

Appendix D

Contemporary Genetic Sampling of American Marten within the Upper Peninsula of Michigan, USA

Background:

Historically distributed throughout much of northern North America in coniferous forests, American martens (*Martes americana*) have experienced extirpations throughout North America as a result of forest loss (Gibilisco 1994) and geographic isolation (Krohn 2012). Efforts to restore this species are widespread and American martens are the most reintroduced carnivore in North America, with >50 documented reintroductions (Powell et al. 2012).

In the Great Lakes region, martens were historically distributed throughout the Laurentide forests but were extirpated from much of the region by the 1930s (Mech and Rogers 1977). Reintroduction efforts began in the 1950s in Wisconsin and continued through the 2000s in Wisconsin and Michigan (Williams et al. 2007; Woodford et al. 2013), with mixed success. While Michigan translocated martens from Ontario, Wisconsin serially reintroduced martens in both the Chequamegon and Nicolet National Forests, sourced from Colorado, Ontario, and the remaining marten populations in northeast Minnesota, throughout the latter half of the 20th century (Williams et al. 2007). This history of translocations has resulted in the Great Lakes recovery network: subpopulations of recovering marten subpopulations throughout the region, which appear to exhibit different viabilities and trajectories but possess unknown levels of connectivity (Smith et al. 2021).

While past research has suggested low levels of connectivity throughout the Great Lakes region (Grauer et al. 2017; Grauer et al. 2019; Day et al. 2020), more recent work has revealed populations are likely more connected than previously believed (Smith et al. 2021). This knowledge coupled with recent calls for more thorough investigations into the current state of marten connectivity in the region (Manlick et al. 2017a; Day et al. 2021) has prompted this effort to measure functional connectivity of martens in the recovery network via genetic sampling. The recent recolonization of both Apostle Islands National Lakeshore (Smith et al. 2021) and Isle Royale National Park (Manlick et al. 2018) provides evidence of functional connectivity and dispersal in the Great Lakes recovery network, but a region-wide assessment of connectivity in the recovery network is necessary to further monitor the outcome of reintroductions, especially in Wisconsin martens.

By describing connectivity across the landscape—developed with regionally relevant landscape variables—I hope to identify pathways of marten dispersal throughout the recovery network and uncover potential barriers to connectivity across the landscape, which may shed light on the slowed recovery of martens in Chequamegon National Forest, especially (Manlick et al. 2017a).

Objectives:

Assess contemporary regional genetic connectivity and barriers to dispersal of American marten (*Martes americana*) throughout a recovery network located in the upper Great Lakes states of Michigan, Minnesota, and Wisconsin.

Progress:

Awaiting final publication.

Appendix E

Molecular Ecology Laboratory Instructional Aide and Grey Wolf Genetic Dataset Development

Background:

Gray wolf tissues samples are being provided for use in an undergraduate Conservation Genetics course where students conduct a molecular genetics project from start to finish. This project is designed to be useful for management and conservation science, with class results being reported to the DNR to demonstrate genetic ancestry found in submitted Michigan wolves. The samples will also be utilized to obtain a population-wide survey to better classify the genetic status of Michigan wolves.

There is a continuous debate about the history and genetic origin of Great Lakes wolves, which impacts their conservation and harvest status given the unique genetic make-up of the regional population. The data generated here can inform this debate by identifying contemporary mtDNA haplotypes in the region and nuclear DNA population assignment. Analyses will include a mtDNA neighbor-joining haplotype network using regional gray wolves, coyotes (*Canis latrans*), and Eastern wolves (*Canis lupus lycaon*) as references. We will also conduct Bayesian clustering analyses with program STRUCTURE and run PCA's to compare SNP genotypes.

Objectives:

1) Train MTU students in molecular genetic techniques commonly used in wildlife management programs- **Ongoing**
2) Generate preliminary genetic data for research and management of the regional gray wolf population

Progress:

Brzeski, K. (2023 September) *Wolves, bears, ticks- oh my! Wildlife genetics and biodiversity conservation in the Keweenaw and beyond* [Virtual presentation]. Michigan Tech CFRES 2023 Fall Seminar, Houghton, MI, United States.

Research update to Division personnel (September 2022)

Appendix F

Gray Squirrel Urban-to-Rural Clines in Melanism

Background:

So-called "gray squirrels" and "black squirrels" are two color morphs of the same species: Sciurus carolinensis. Until 150 years ago, the black morph was much more abundant. Yet now the black morph is rare, except in cities. Why has the black morph declined? Why does it linger in cities? Will it return again to the countryside? Will the gray squirrel, and other mammals like it, be able to adapt quickly enough?

Only a tiny genetic difference separates black versus gray morphs. All individuals contain a gene, MC1R, that controls how much dark pigment is added as a squirrel's hairs grow. When a tiny piece of DNA is missing from this gene, it boosts production of dark pigment and makes the fur darker. When a squirrel has a single copy of the gene with the small piece of DNA deleted, or two copies of that altered gene, its fur is mostly black. Technically, the black morph is melanic. The gray morph has two copies of the complete gene. A tiny genetic difference but being black or gray could have many consequences if you are a squirrel!

We will collect data on the abundance of color morphs of eastern gray squirrels along urbanization gradients to test for parallel urban-to-rural clines in melanism. Melanism in eastern gray squirrels is a simple Mendelian trait. Our previous work using opportunistic data suggests clines in melanism are common, but that there's considerable variation in the prevalence of melanism and the shape of urban-to-rural clines among cities. This project will provide greater insight into how city characteristics affect the degree of parallel urban evolution using standardized surveys for squirrel color morphs.

Objectives:

1) Collect data on the abundance of color morphs of eastern gray squirrels along urbanization gradients to test for parallel urban-to-rural clines in melanism-

Results:

Fusco, N., Cosentino, B., Gibbs, J., Allen, M., Blumenfeld, A., Boettner, G., Briggs, K., Carlen, E., Collins, M., Dennison, C., DiGiacopo, D., Drapeau Picard, A.P., Edmonson, J., Fisher-Reid, M.C., Fyffe, R., Gallo, T., Grant, A., Harbold, W., Heard, S., Lafferty, D., Lehtinen, R., Marino, S., McDonald, J., Mortelliti, A., Murray, M., Newman, A., Oswald, K., **Ott-Conn, C.**, Richardson, J., Rimbach, R., Salaman, P., Steele, M., Stothart, M., Urban, M., Vandegrift, K., Vanek, J., Vanderluit, S., Vezina, L., Caccone, A. Population genomic structure of a widespread mammal after three centuries of landscape change. In-review. Molecular Ecology.

Appendix G

Broadscale Functional Connectivity of Northern River Otter in the Upper Peninsula of Michigan

Background:

Connectivity of landscapes is important for the persistence of biological populations and the stability of ecosystems (Fortin *et al.* 2012, Liata *et al.* 2011, Opdam and Wascher 2006, Schick and Lindley 2007). Connectivity, or the ability of organisms to move across the landscape, can be measured structurally through the physical arrangement of habitat patches or functionally by measuring a species behavioral response or dispersal through the physical environment (Baguette et al. 2013, Taylor *et al.* 2006). Connectivity allows for resiliency and recolonization after disturbances (Schick and Lindley 2007). Forested riparian corridors play a vital role in this connectivity (Eros and Grant 2015).

An effective way to measure the functional connectivity of a landscape is through measuring gene flow (Baguette *et al.* 2013). Gene flow is determined by how individuals respond to the structural connectivity of the landscape (Manel *et al.* 2003). Landscape genetics combines this measurement of gene flow with landscape characteristics (Baguette *et al.* 2013). This information may be used for mark-recapture estimates of the population, estimates of offspring per female, and dispersal of individuals. DNA analysis allows for identification of individuals, sex determination, relatedness for analyzing gene flow or barriers to gene flow, and distribution of the population (Schwartz and Monfort 2008).

Population assignment tests or cluster analyses may be used as an indirect method of measuring multiple dispersal events (Broquet and Petit 2009). These types of analyses measure only the influence of effective dispersal on genotype frequencies in the population (gene flow) (Slatkin 1987). In general, populations that have little or no structure have frequent gene flow while populations that show genetic structure have little to no gene flow or dispersal (Cavuela *et al.* 2018, Cope *et al.* 2015). Assignment or cluster methods assume that dispersal is symmetrical between patches, that allele frequencies do not change, and no genetic drift has occurred (Kormann *et al.* 2012). It is also assumed that all source populations have been sampled (Kormann *et al.* 2012). Genetic differentiation is a combination of past and current events and therefore gene flow does not always directly measuring current dispersal events (Fountain *et al.* 2017, Kormann *et al.* 2012).

Objectives:

1. Evaluate which riparian and matrix landscape variables affect functional (genetic) connectivity in otter populations in the Upper Peninsula.
2. Determine if functional connectivity is different for male and female otters.
3. Identify potential barriers to otter dispersal

Results:

Awaiting final publication

Cotey, SR. 2021. Landscape Ecology of North American River Otter (*Lontra canadensis*) in the Upper Peninsula of Michigan. Doctoral dissertation, Michigan Technological University, Houghton, MI.

Cotey SR, Scimeca R, Chang L, Will E, **Ott-Conn C**, Mayer AL, Reichard M. In press. *Toxoplasma gondii* prevalence, partial genotypes, and spatial variation of North American river otter in the Upper Peninsula, Michigan. J. of Wildlife Disease.

Cotey SR, Scimeca R, Chang L, Will E, **Ott-Conn C**, Mayer, AL, and Reichard MV. 2022. (Upcoming) *Toxoplasma gondii* Prevalence in Otters of the Upper Peninsula of Michigan. The Wildlife Society Conference, Spokane, WA.

Cotey SR, Scimeca R, Chang L, Will E, **Ott-Conn C**, and Reichard MV. 2020. *Toxoplasma gondii* prevalence and partial genotypes of North American River Otter (*Lontra canadensis*) in the Upper Peninsula, Michigan. American Association of Veterinary Pathologists Annual Conference, Virtual.

Scimeca R, Cotey S, Chang L, Will E, and Reichard M. 2020. Prevalence of *Sarcosystis spp.* in North American river otters (*Lontra canadensis*) collected in Michigan. American Association of Veterinary Pathologists Annual Conference, Virtual.

Cotey SR. 2019. Keeping track of otters. Ottawa National Forest Seminar Series, Watersmeet, MI.

Chang L, Scimeca RC, Cotey SR, and Reichard MV. 2019. Molecular detection of *Toxoplasma gondii* in North American River Otters (*Lontra canadensis*) from Michigan. National Veterinary Scholars Symposium, Tufts University. North Grafton, MA.

Appendix H

Examination of Chronic Wasting Disease Prion Strains from Free-Ranging Cervids

Background:

The goal of this research project is to begin characterization of CWD strains from across the US to understand strain properties both within and between noncontiguous epizootics. We hypothesize that we will find different strains from distinct outbreaks. Our work will provide insight into new management strategies that may be necessary for distinct prion strains. For example, if we find a strain with enhanced zoonotic potential, new regulations may be required to keep CWD+ meat out of the human food chain. Additionally, different strains may behave differently in infected cervids (temporal differences in shedding, differences in environmental stability, etc.). Understanding the characteristics of unique strains will allow us to assist in new management strategies for CWD outbreaks.

Thus far, we have completed biochemical analyses on the samples from MI and have begun to address our goals of understanding CWD strains circulating in MI. These assays provide an important first look into strain differences from a biochemical perspective.

Results:

Awaiting publication.

Appendix I

A standardized high-throughput genetic resource
to inform white-tailed deer population and disease management

Background:

Genetic data have proven to be a powerful tool for deer management by identifying genetic variation associated with susceptibility to CWD, determining whether CWD-positive deer are of local origin, resolving relationships among CWD-positive animals, and determining how landscape features impact population connectivity and disease spread.

Most genetic research in cervids to date has utilized microsatellite DNA markers, which have been the workhorse of wildlife genetics. Recent technological advancements have facilitated the use of a new type of marker, single-nucleotide polymorphisms (SNPs). SNPs represent a single-base change in the DNA sequence and can be genotyped much more efficiently than microsatellites. SNP data, unlike microsatellites, are easily shared among laboratories and can facilitate the creation of custom, high-throughput marker panels to run many hundreds or thousands of individuals using hundreds to many tens of thousands of SNPs.

Objectives:

1. Develop high (600,000) and medium-density (60,000) SNP panels to enable high-resolution genetic analyses- IN FINAL STAGES
2. Develop a low-density (6,000), low-cost SNP panel for population assignment, parentage analysis, and prion gene genotyping- SAMPLES SUBMITTED
3. Assess low-density panel utilizing CWD positive samples from partnering states
4. Produce training materials and a shared resource for managers

Final Report for state granted funds:

A standardized high-throughput genetic resource to inform white-tailed deer population and disease management: A continuation study

Final Report

Date Issued:
29 September 2023

Submitted to:
Wildlife Disease Initiative Funding Committee

Prepared by:
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Daniel Walsh – USGS National Wildlife Health Center
Wesley Larson – NOAA Alaska Fisheries Science Center Genetic Program

Introduction:

Genetic data have proven to be a powerful tool for deer management by identifying genetic variation associated with susceptibility to CWD, determining whether CWD-positive deer are of local origin, resolving relationships among CWD-positive animals, and determining how landscape features impact population connectivity and disease spread.

Recent technological advancements have facilitated the development of tools using genetic markers coined single nucleotide polymorphisms (SNPs). SNPs can be genotyped efficiently, are able to handle low-quality DNA, can be formatted into high-throughput genotyping panels, and produce data that are easily shared among laboratories and require no complex bioinformatics skills.

With complementary funding, in part, through the Wildlife Disease Initiative, the Association of Fish and Wildlife Agencies, and partnerships with 17 states, we have developed 3 different genomic SNP panels to address top priority questions relevant to white-tailed deer management.

Objectives:

1. Collect and evaluate detailed genomic variation in CWD-positive and CWD-non detected deer from areas in which CWD is found in Michigan using the white-tailed deer GT-seq SNP panel.
2. Analyze the resulting data to quantify SNP variation associated with CWD status. This information could provide a foundation for collating a suite of genetic markers that inform individual CWD risk.
3. Provide a thorough report of genomic characteristics of white-tailed deer in Michigan's current CWD-positive counties.

EXECUTIVE SUMMARY

We report the achievements of our research project focused on leveraging genomic data for white-tailed deer management in Michigan, particularly in relation to chronic wasting disease. We optimized a GT-seq panel to survey genetic variation at 575 SNPs, and generated a high-quality SNP dataset for 413 white-tailed deer samples from throughout Michigan, including both CWD-positive and CWD-non detected. We identified SNPs potentially associated with CWD status, but these data require further validation given that CWD status covaries with other variables in the dataset. Genetic diversity data revealed structure between deer from the Upper and Lower Peninsulas, with a low but significant level of genetic differentiation (F_{ST} = 0.0019). These accomplishments offer valuable insights for white-tailed deer management, specifically in CWD-affected areas of Michigan.

ACCOMPLISHMENTS

Objective 1

- Collected 413 samples from Michigan white-tailed deer (217 CWD-positive and 196 CWD-non detected) (Appendix 1, Figure 1).
- Optimized our GT-seq panel to include a) SNPs that accurately quantify genome-wide variation and b) SNPs that have been associated with CWD in prior studies (Appendix 1, Figure A1).
- Generated high-quality genotype data from Michigan white-tailed deer samples. The optimized GT-seq panel includes 508 loci containing 575 SNPs; 90% of samples at 94% of loci on the GT-seq panel passed quality filters (Figure 2). Across the 508 loci, the average primer-probe read count per sample per locus was 99.1 (i.e., an average of 99 useable, on-target sequencing reads per locus). We generated GT-seq genotypes for the 217 CWD-positive deer. To this dataset we added 196 CWD-non detected deer samples that we genotyped previously using the high-density (OvSNP600) and medium-density (OvSNP60) panels, subsampling these larger panels to extract genotypes at the same set of SNPs that are on the GT-seq panel.

Objective 2

- Found 21 SNPs from the GT-seq panel associated with CWD status in Michigan white-tailed deer (Figure 3). Correlations with other variables (geography, panel type) means that these likely contain false positives and we need to analyze further and interpret with caution.
- Assessed 30 SNPs that have previously been associated with CWD status in captive herds; only 1 of these was retained in our final combined dataset once we aligned it to the white-tailed deer genome. That locus was not found to be associated with CWD status in Michigan white-tailed deer.
- Characterized overall SNP differentiation between CWD-positive and CWD-non detected deer in Michigan (Figure 4).

Objective 3

- Generated basic genetic diversity statistics for Michigan's white-tailed deer using data from the GT-seq panel (Table 1).
- Characterized population genetic structure in Michigan's white-tailed deer using data from the GT-seq panel (Figure 4). The level of genetic differentiation between the Upper and Lower Peninsula was $F_{ST} = 0.019$ (SE = 0.001).

MODIFICATIONS TO ORIGINAL WORK PLAN

No modifications have been made to the original structure of this work. We did have some impact on timing due to laboratory closures and restrictions associated with the Covid19 pandemic. We have utilized this time to secure additional partners to expand the impact and reach of this work. We also experienced some staffing changes during the course of the project but were able to replace staff to complete the original objectives.

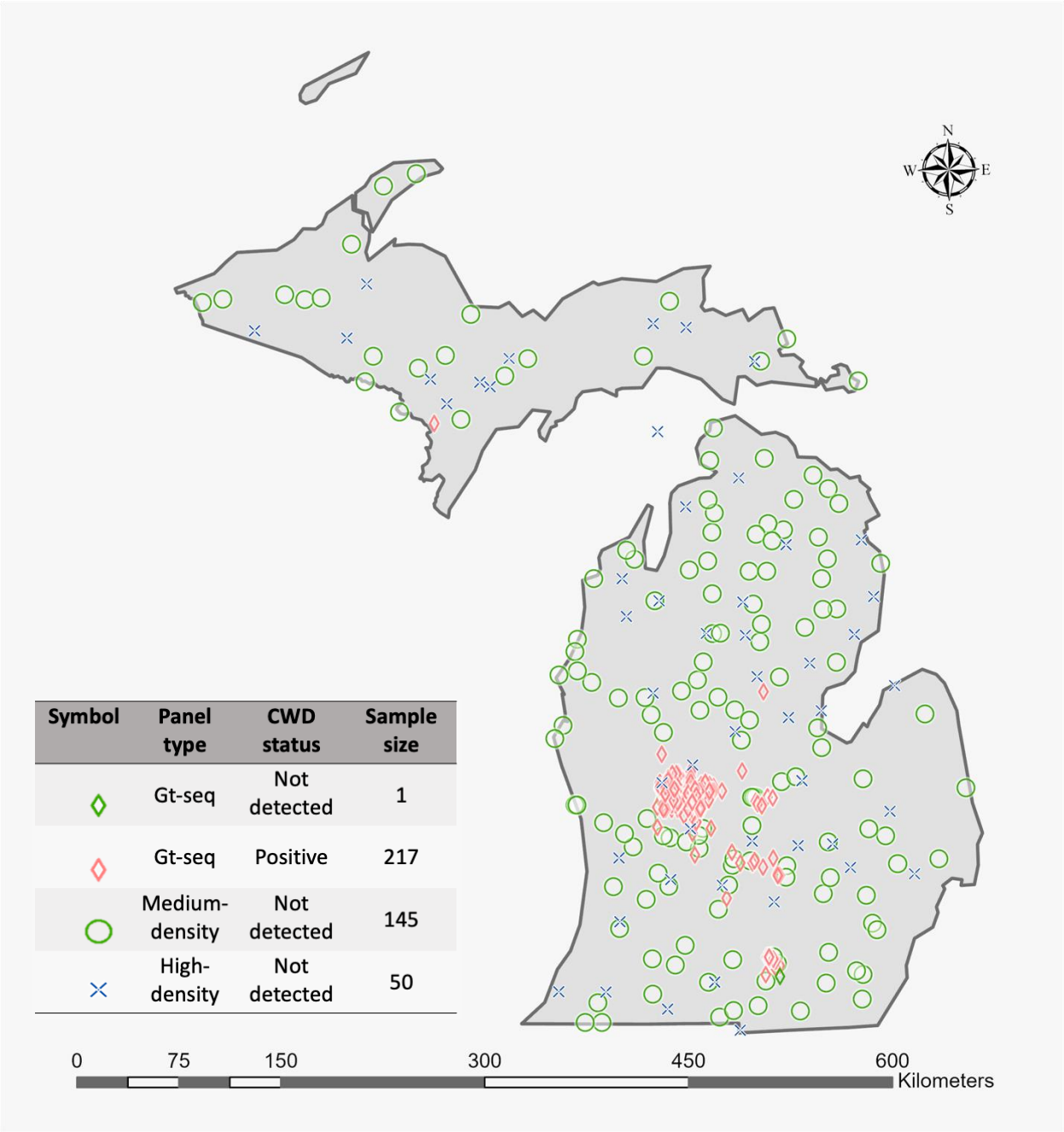
OUTREACH AND IMPACTS

- Provided genotype data, maps, and associated metadata for 413 Michigan white-tailed deer to the Michigan Department of Natural Resources
- Gave a presentation on our work to develop the GT-seq panel at The Wildlife Society's Annual Conference in Spokane, WA in November 2022
- Established a website (https://storymaps.arcgis.com/stories/9794d395588b45d7a055e86bf42d602b) which describes our three different SNP panels, shows the samples that have been analyzed on each, and assists collaborating agencies with accessing these resources. We also include information about what arrays are and how to purchase them, list labs that can assist with genotyping, and provide a video from a webinar we held to orient partner agencies to our first two SNP panels.

Table 1. Genetic diversity data for Michigan white-tailed deer. The number of alleles (Na), number of effective alleles (Ne), information index (I), observed heterozygosity (Ho), unbiased expected heterozygosity (uHe), and fixation index (F) are provided for the total Michigan population, the Lower Peninsula, and the Upper Peninsula. Means and standard errors (SE) are provided for each statistic. Data were generated using GenAlEx software.

Pop	n		Na	Ne	I	Ho	uHe	F
LP	359	Mean	1.998	1.547	0.497	0.303	0.328	0.076
		SE	0.002	0.014	0.008	0.006	0.006	0.007
UP	34	Mean	1.979	1.553	0.498	0.320	0.335	0.037
		SE	0.007	0.014	0.008	0.007	0.006	0.010
Total	393	Mean	1.988	1.550	0.498	0.311	0.332	0.056
		SE	0.003	0.010	0.006	0.005	0.004	0.006

Figure 1. White-tailed deer sample locations. Symbols indicate the panel on which samples were genotyped (high-density OvSNP600 panel, medium-density OvSNP60 panel, GT-seq panel). Red diamonds are samples from CWD-positive white-tailed deer; all other samples were tested for CWD but it was not detected.



Symbol	Panel type	CWD status	Sample size
◊	Gt-seq	Not detected	1
◊	Gt-seq	Positive	217
○	Medium-density	Not detected	145
×	High-density	Not detected	50

Figure 2. Diagram showing filtering steps taken for the Michigan white-tailed deer GT-seq dataset (left) and for the medium-density and high-density panel datasets that were previously generated as part of the original panel development (right). In brief, the GT-seq filtering included removal of low-quality white-tailed deer samples (those that yielded < 80% genotypes; n=19 removed). To generate a larger dataset with CWD-positive and CWD-non detected samples, we extracted the homologous GT-seq SNPs from the medium-density (OvSNP60) and high-density (OvSNP600) panel data that we had collected previously to generate a combined dataset of 393 samples genotyped at 478 SNPs. In this combined dataset, we also removed SNPs that were genotyped in < 70% of samples (n=6 SNPs removed). The final combined dataset contained 393 Michigan white-tailed deer genotyped at 472 SNPs with an overall genotyping rate of 97%.

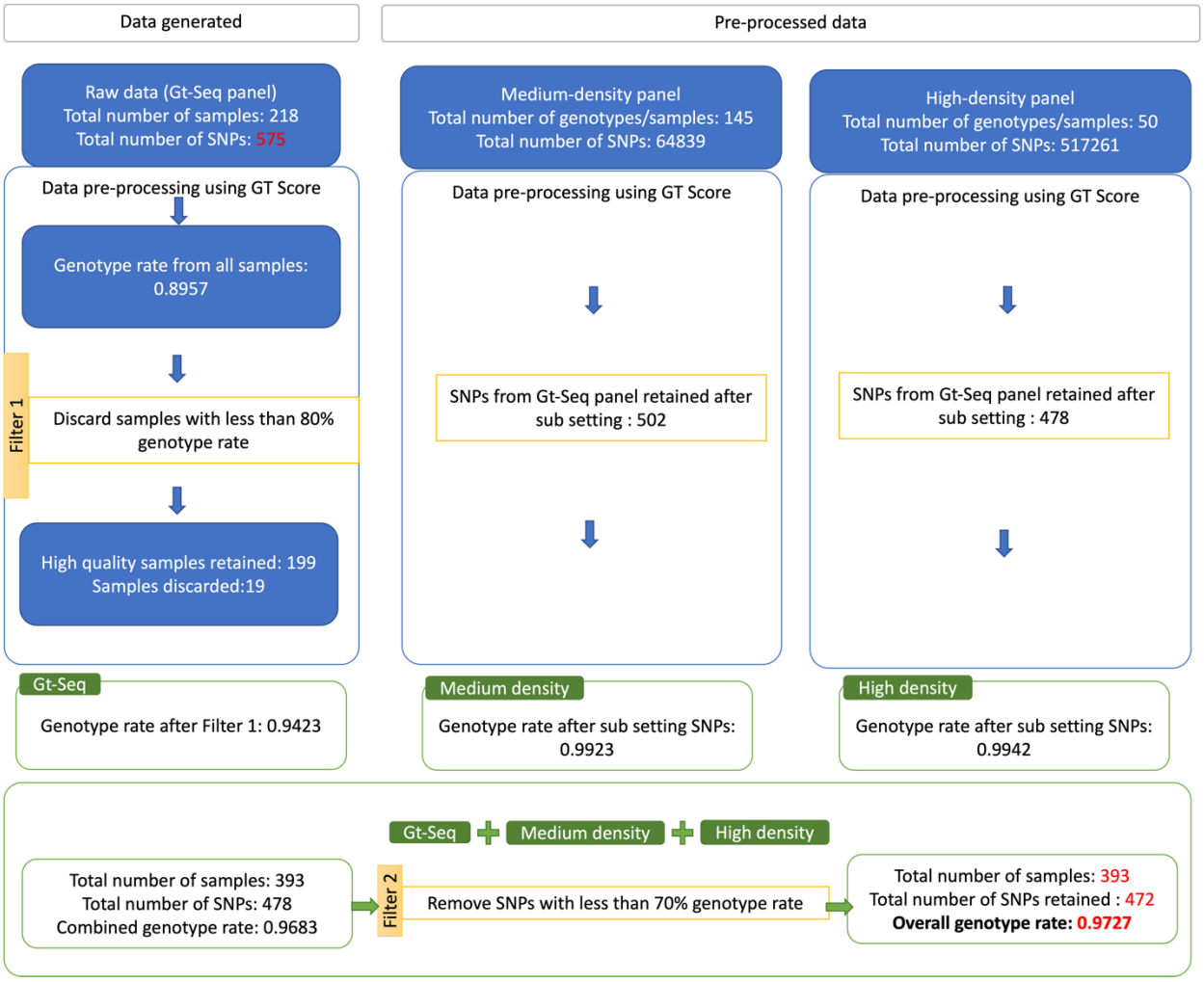
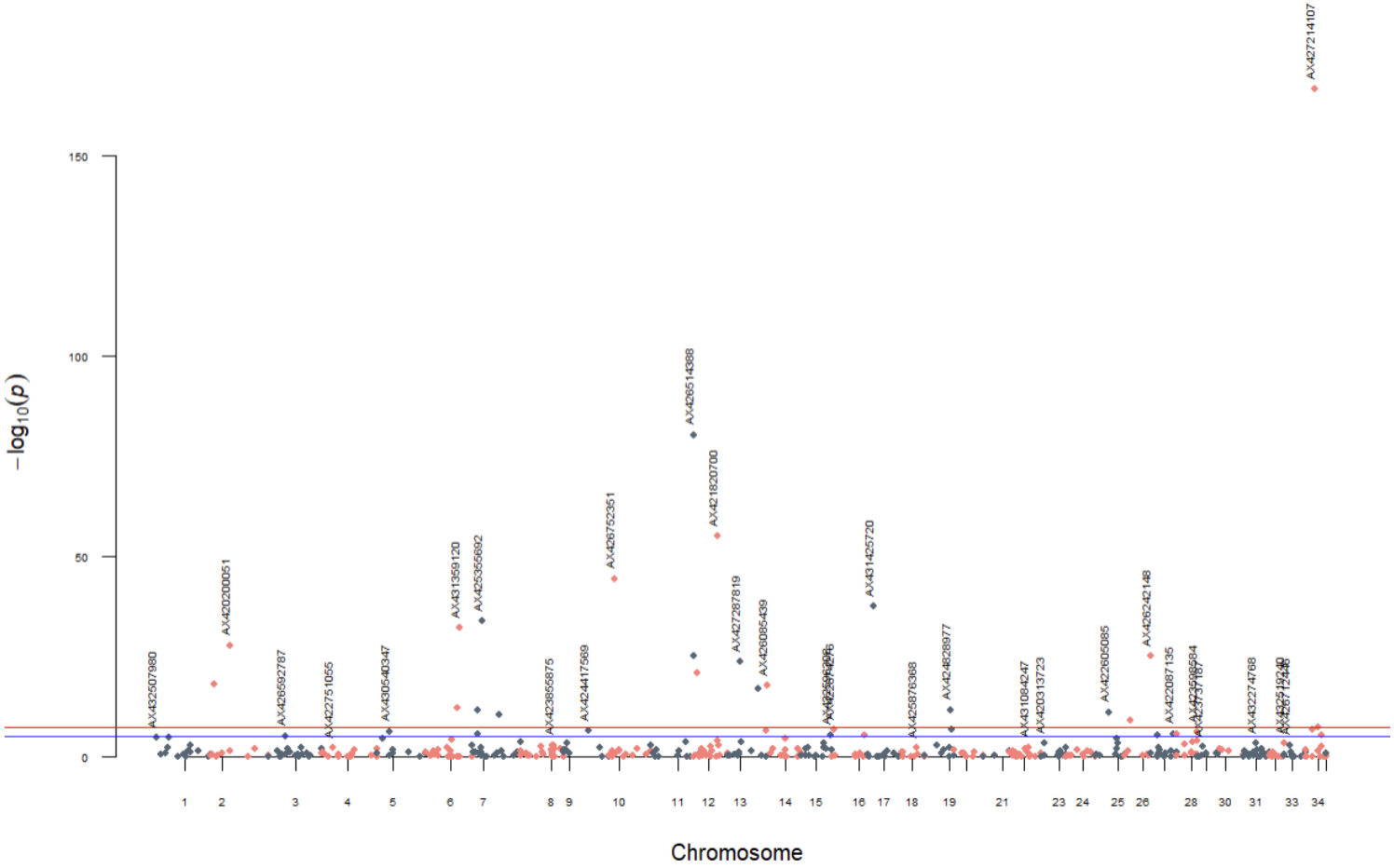


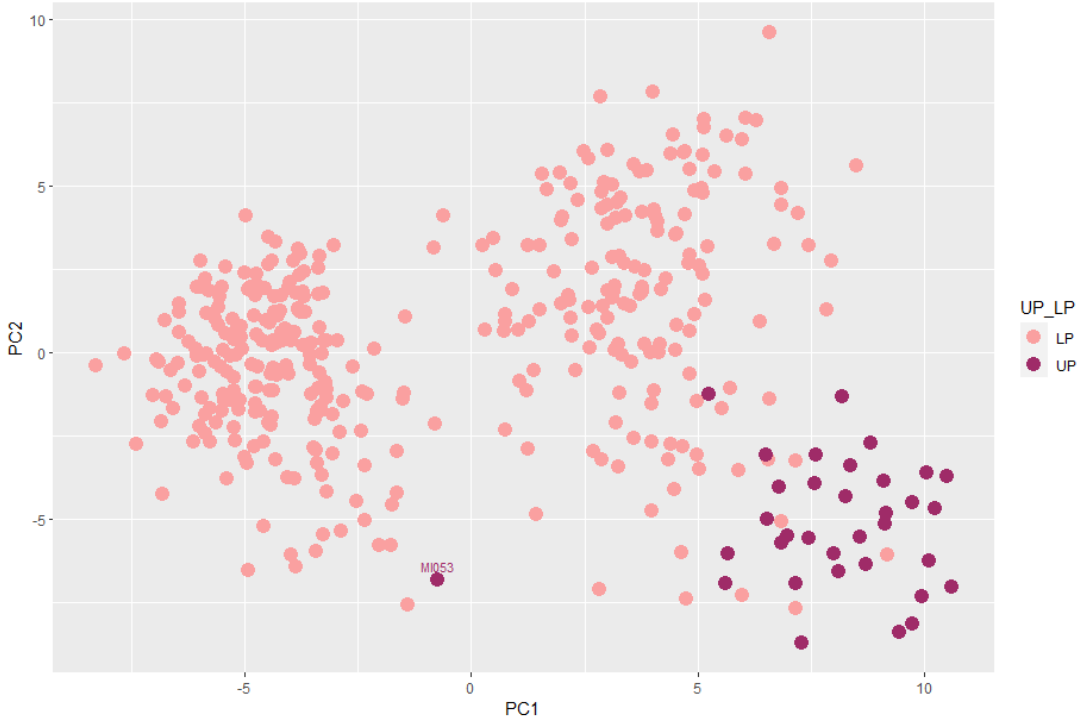
Figure 3. Manhattan plot showing SNPs associated with CWD status. Each SNP is ordered by its chromosome position along the X axis; Y axis values are the association statistical significance plotted as -$\log_{10}$ of the p-value. These data are based on aligning against the white-tailed deer reference genome, and thus has only 433 of the 472 genotyped SNPs represented. The red (-$\log_{10}$(5e-8)) and blue (-$\log_{10}$(1e-5)) lines indicate standard cutoffs for significance that are typically used in the analysis of human genetic data. Using a preliminary chi-squared test and false discovery rate correction, we identified 21 SNPs in our combined dataset that were associated with CWD status in this dataset (above the red line). Because CWD status also correlates with geography and with panel type in this dataset it is likely that some of these are false positives. Indeed, some of these significant SNPs are also ones that contribute most to the geographic structure in the PCA analysis. We need to work with these data more to try to disentangle these factors; we are currently collecting data from other states that may also help us interpret these findings.



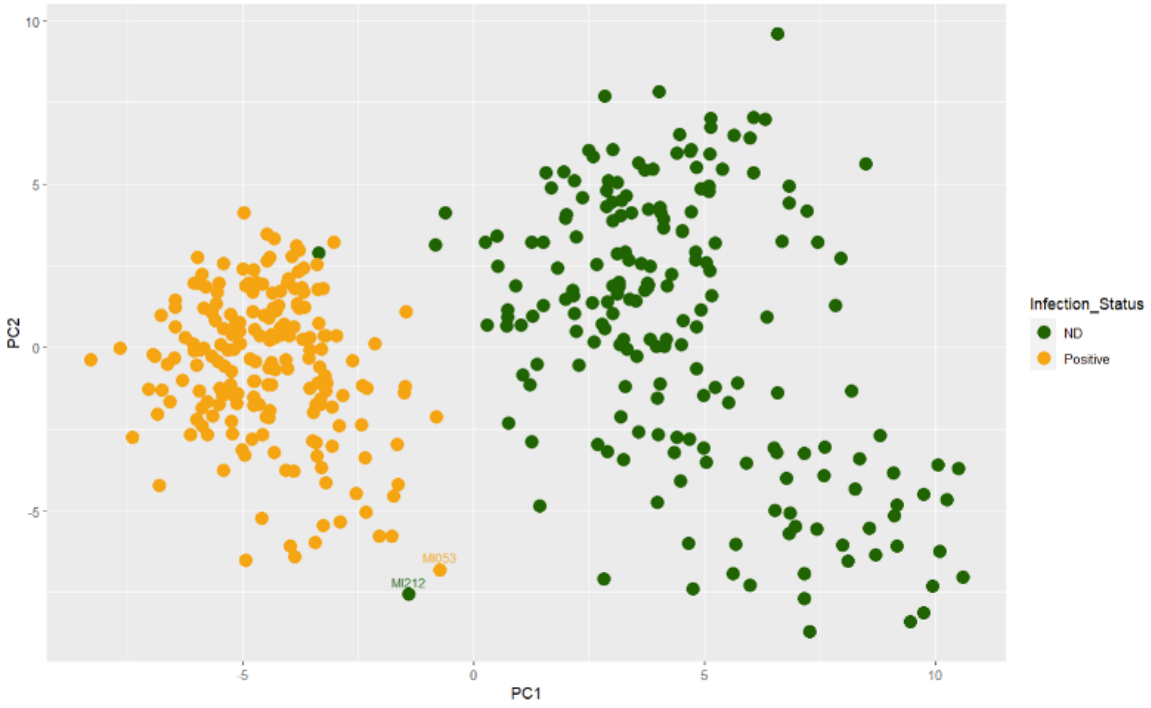
A

Figure 4. Principal component analysis (PCA) of white-tailed deer in Michigan using the combined dataset of 393 white-tailed deer genotyped at 472 SNPs. PCAs are color-coded by A) geographic location (Upper and Lower Peninsulas of Michigan), B) infection status (CWD-positive and CWD-non detected), and C) panel type (GT-seq, medium-density, high-density). Though we do not anticipate any differences in genotyping between panels, all samples submitted for the high- and medium-density panels were CWD-non detected (ThermoFisher requirement), and most of the GT-seq samples were CWD positive, thus it is difficult to disentangle the effects of infection status (B) from panel type (C). Further, because the high- and medium-density samples were collected from across the entire state, whereas the GT-seq samples were collected from CWD-positive areas primarily in the lower peninsula, it is difficult to disentangle the effects of infection status (B) from geographic location (A). However, it does appear that the combined dataset does show genetic differentiation between white-tailed deer from the Upper and Lower Peninsulas, though these groups are not completely discrete (A). It also seems that the GT-seq panel captures genetic differences between CWD-positive and CWD-non detected deer (B). This relationship requires further interpretation, primarily because CWD-positive samples were concentrated primarily in one geographic area (where CWD occurs) and because those samples were run only on one panel type (C). One exception is the sample MI212 (labeled in B) which was CWD-non detected but sampled near other CWD-positive samples and genotyped on the GT-seq panel. Sample MI053 is an interesting case, because it was sampled in the Upper Peninsula and is CWD-positive. That sample is denoted in (A), and does group with other CWD-positive samples, and is found in the lower portion of the PCA where UP samples are clustered. These results are promising for CWD management in Michigan white-tailed deer.

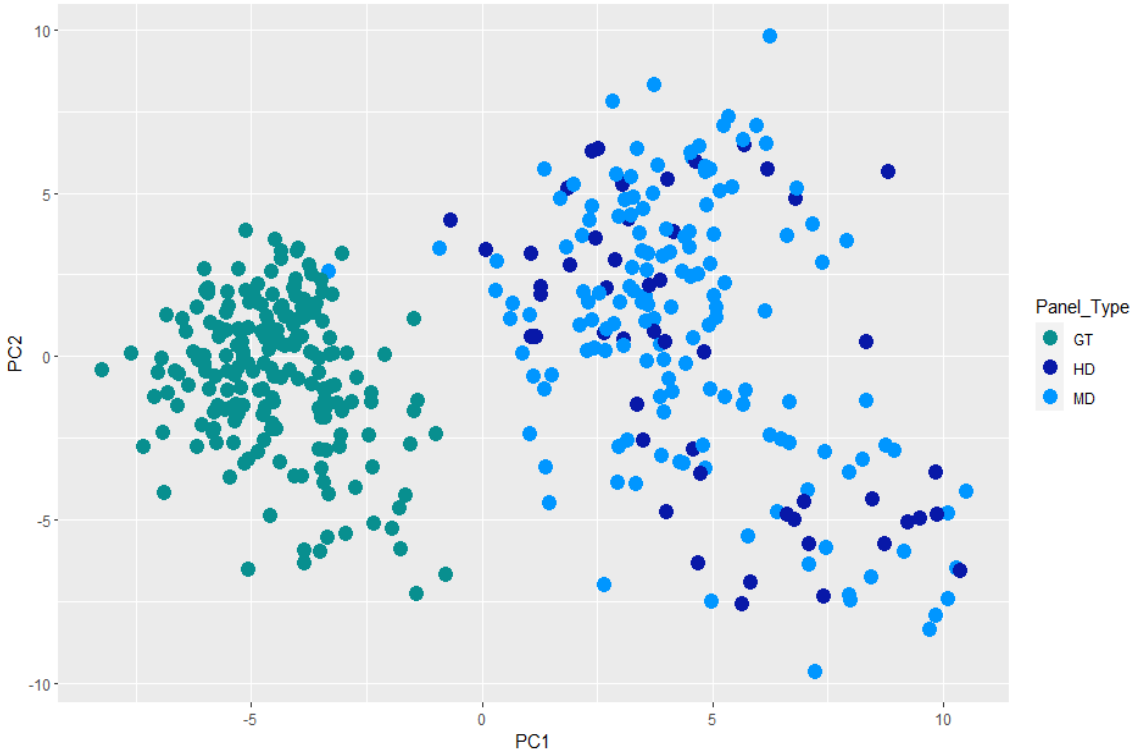
A) Geographic location



B) Infection status



C) Panel type

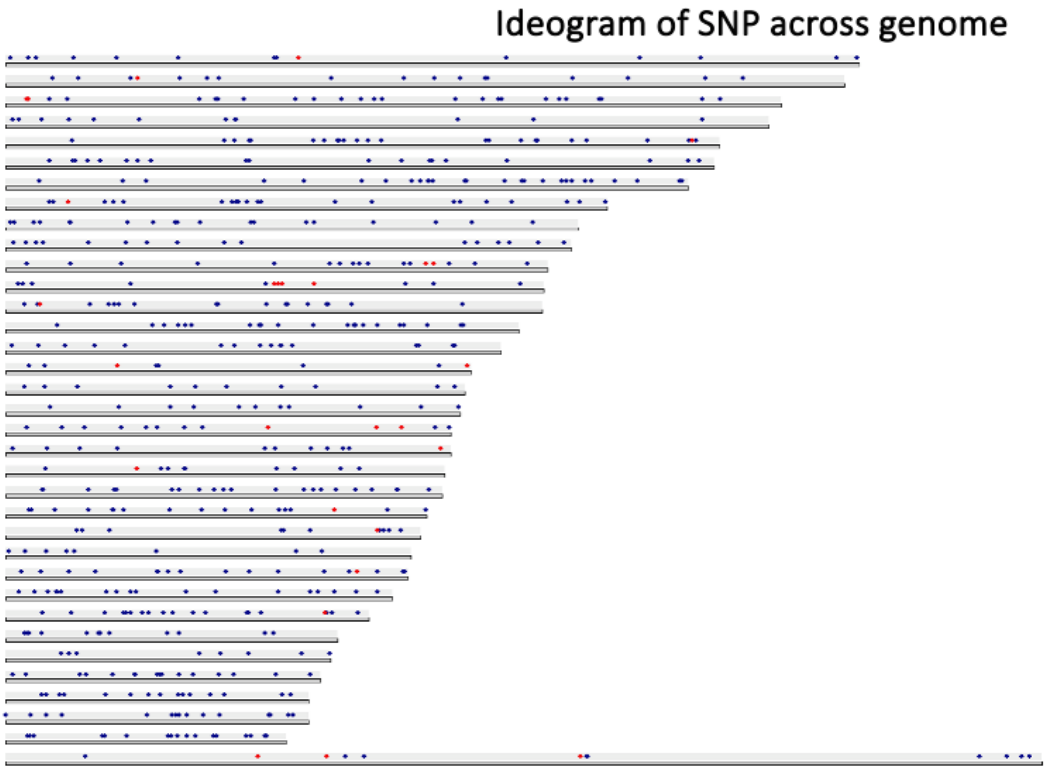


Appendix 1. Sample database with metadata and genotypes. Two files are provided as Excel (.xls) files. One file contains all of the GT-seq genotypes (just prior to Filter 1 in Figure 2; 575 SNPs genotyped in 218 white-tailed deer), and the other file contains the combined SNP dataset of 472 SNPs genotyped in 393 white-tailed deer.

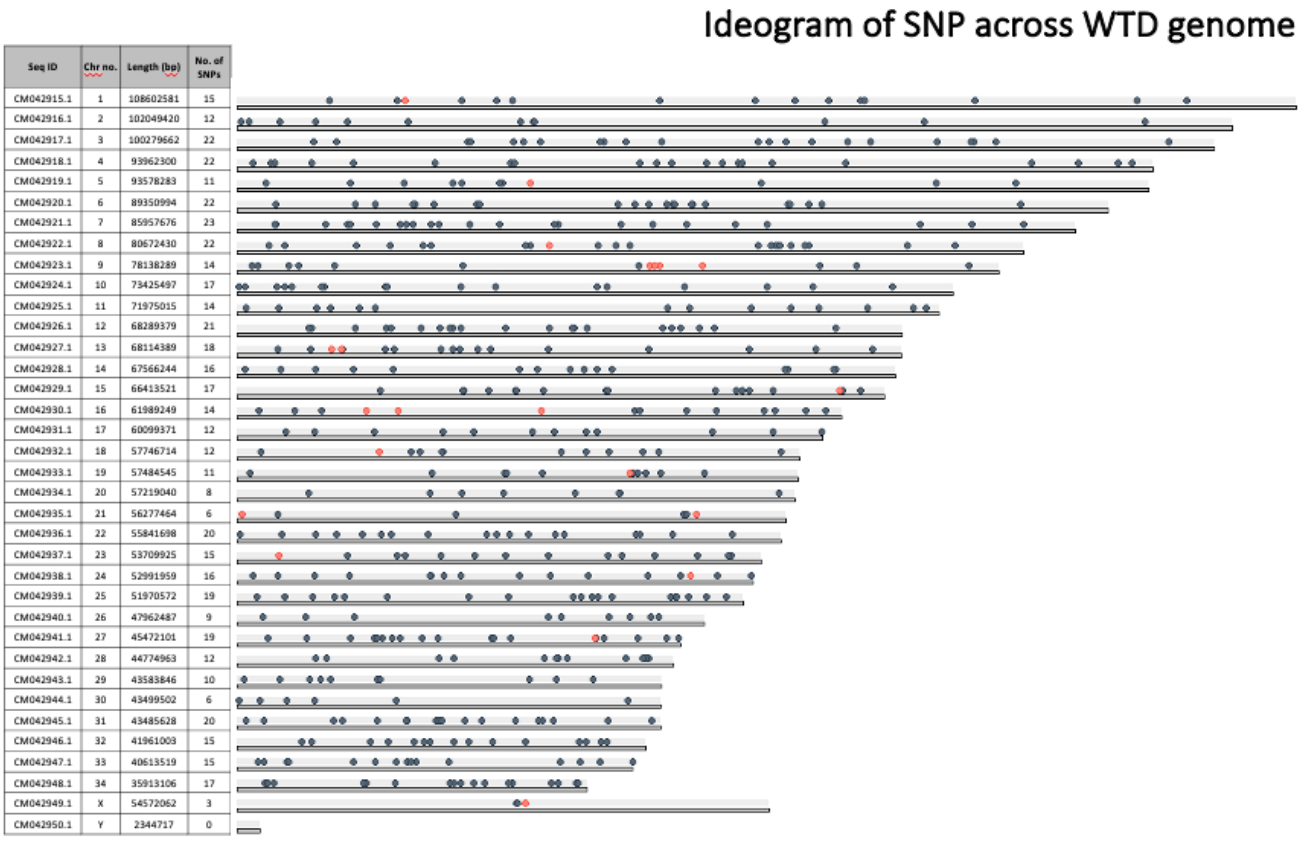
Figure A1. Ideograms showing the distribution of SNPs across chromosomes. The GT-seq panel was developed using the white-tailed deer genome of scaffold-level assembly. In (A), we aligned the reference sequences of SNPs in the GT-seq panel against the mule deer genome because it has chromosome-level assembly and thus we can better see how the SNPs are distributed across chromosomes. The total number of aligned SNPs is 555 of the 578 total SNPs in the GT-seq panel. In (B) we aligned the reference sequences of SNPs in the GT-seq panel against the white-tailed deer genome; despite lower quality it is the same species so potentially more useful for comparison. The total number of aligned SNPs is 525 of the 578 total SNPs in the GT-seq panel. In both plots, red dots indicate SNPs that had been identified previously as being associated with CWD in captive deer herds. Both images show that the SNPs on the GT-seq panel are well distributed across the genome.

(A) Aligned against mule deer genome

Sequence ID	Chr no.	Chromosome length	No. of SNPs
CM037420.1	1	114823825	14
CM037421.1	2	112787451	17
CM037422.1	3	104294071	26
CM037423.1	4	102606451	12
CM037424.1	5	95956063	25
CM037425.1	6	95313685	20
CM037426.1	7	91763312	27
CM037427.1	8	80885226	23
CM037428.1	9	77029860	19
CM037429.1	10	76003156	16
CM037430.1	11	72885977	17
CM037431.1	12	72371423	12
CM037432.1	13	72140960	18
CM037433.1	14	68976353	21
CM037434.1	15	66648629	16
CM037435.1	16	62597288	8
CM037436.1	17	61808552	11
CM037437.1	18	61089879	11
CM037438.1	19	59874755	13
CM037439.1	20	59825373	11
CM037440.1	21	59026025	10
CM037441.1	22	58711282	21
CM037442.1	23	56626343	18
CM037443.1	24	55759230	11
CM037444.1	25	54486009	8
CM037445.1	26	54056770	17
CM037446.1	27	51957095	18
CM037447.1	28	48843394	19
CM037448.1	29	44605452	12
CM037449.1	30	43703396	8
CM037450.1	31	42387623	17
CM037451.1	32	40772476	15
CM037452.1	33	40741728	15
CM037453.1	34	37701695	17
CM037454.1	35 (X chr)	139398775	12



(B) Aligned against white-tailed deer genome



Seq ID	Chr no.	Length (bp)	No. of SNPs
CM042915.1	1	108602581	15
CM042916.1	2	102049420	12
CM042917.1	3	100279662	22
CM042918.1	4	93962300	22
CM042919.1	5	93578283	11
CM042920.1	6	89350994	22
CM042921.1	7	85957676	23
CM042922.1	8	80672430	22
CM042923.1	9	78138289	14
CM042924.1	10	73425497	17
CM042925.1	11	71975015	14
CM042926.1	12	68289379	21
CM042927.1	13	68114389	18
CM042928.1	14	67566244	16
CM042929.1	15	66413521	17
CM042930.1	16	61989249	14
CM042931.1	17	60099371	12
CM042932.1	18	57746714	12
CM042933.1	19	57484545	11
CM042934.1	20	57219040	8
CM042935.1	21	56277464	6
CM042936.1	22	55841698	20
CM042937.1	23	53709925	15
CM042938.1	24	52991959	16
CM042939.1	25	51970572	19
CM042940.1	26	47962487	9
CM042941.1	27	45472101	19
CM042942.1	28	44774963	12
CM042943.1	29	43583846	10
CM042944.1	30	43499502	6
CM042945.1	31	43485628	20
CM042946.1	32	41961003	15
CM042947.1	33	40613519	15
CM042948.1	34	35913106	17
CM042949.1	X	54572062	3
CM042950.1	Y	2344717	0