As I read my way through the half shelf of books about infectious diseases at my high school library, I found Randall Packard's *The Making of a Tropical Disease: A Short History of Malaria,* which opens with an epidemic in northern Russia. The epidemic, he explained, was driven by intersecting drought and socioeconomic crises. This was the wackiest thing I'd ever heard: I promptly told every kid within earshot on the bus about *malaria in Russia? Because of drought?* The ecology of mosquito-borne diseases had me hooked, though I'd already decided that I wanted to study viruses, enticed by the sophisticated infections they carried out with such tiny genomes. I was thrilled to learn that vector-borne virus ecology was no less wacky. With that, my lifelong love of the complexity of vector-borne viral disease systems was born.

In the decade since, my pursuit of the complex drivers of vector-borne viral disease problems has led me across disciplines, institutions, and into assembling a nascent systems biology framework to anticipate the ways interacting complexities shape virus transmission. I've found my most dedicated advocates, exciting projects, useful statistical tools, and informative first principles in evolutionary biology. The dynamic adaptive processes that shape insects, viruses, and their interactions with each other can drastically change the magnitude and range of vector-borne disease problems, and understanding those processes can help us prevent – or at least prepare for – new problems. *My ongoing doctoral study of* Aedes triseriatus *molecular and evolutionary genetics in western North Carolina will be foundational to my intended career as the principal investigator (PI) of a research group that uses evolutionary genetics to understand and interrupt complex vector-borne viral disease transmission systems.*

Intellectual merit: I began studying arthropod-borne (arbo) viruses as a Biology major and Center for Undergraduate Research Opportunities Honors Scholar at the University of Georgia in the fall of 2015. Working in a mosquito and wildlife testing service, I saw firsthand the resource scarcity that limits arbovirus control efforts in the U.S. Still, I loved working on so many diverse, ecologically-nuanced disease systems, and served as a Vice President of our Wildlife Disease Association chapter for three years. During this time, I led a project on an unusual virus we'd isolated from a white-tailed deer, and *discovered a novel recombinant of two Cache Valley orthobunyavirus lineages.* This ignited my career-long love of orthobunyaviruses, a ubiquitous, understudied genus of human and animal pathogens that frequently and dramatically recombine with each other. I appreciated the unique perspectives on arboviral diseases that I cultivated by studying such 'unusual' systems, relative to the predominant ones in arbovirology. For my undergraduate thesis, I conducted a comprehensive literature review on Cache Valley and closely related viruses. This project received UGA's top prize for undergraduate literature review. *I also actively pursued opportunities for cross-disciplinary collaboration and training:* I built expertise in virus evolutionary theory to help design and interpret a machine learning model of emergent virus transmissibility between humans, resulting in my first publication [1], and completed summer internships in arbovirus pathogenesis and bacteria-phage coevolution. The second internship, with Dr. Michael Baym, taught me new ways to think about microbial evolution. Whenever I spot a suspiciously fast adaptive process, I still ask: *what baseline adaptations and variation did selection have to start with?*

After graduation, I worked as a lab technician for several years: *I knew I needed to build expertise in cutting-edge molecular techniques to ask incisive questions about the evolution and ecology of temperate arboviral diseases.* In 2019, I joined a research group at Yale University developing bacteriophage therapies for multi-drug resistant pathogens, an exciting evolutionary problem that taught me clinical respiratory sample handling practices. This proved essential when, midway through the year, I took a temporary volunteer posting on Yale's COVID-19 pandemic response, which turned into a multi-year position with Dr. Nathan Grubaugh on SARS-CoV-2 (SC2) and dengue virus (DENV) surveillance and genomics. In addition to contributing to groundbreaking papers on SC2 diagnostics,

genomic epidemiology, and pathogenesis, I co-led papers demonstrating SC2 RNA stability in untreated saliva, used to support the deployment of affordable mail-in testing [2], and – in collaboration with Dr. Baym's group – applying transcriptomics-based approaches to estimate variant abundance from wastewater sequencing, aiming to support genomic surveillance across sequencing capacity levels [3]. The pandemic response was challenging, but intensely rewarding: *I received invaluable training in high-throughput molecular biology techniques and the diverse ways they could inform public health decision-making.* As SC2 work wound down, I eagerly returned to the lab's previous investigations of DENV evolution: I sequenced and built interactive NextStrain phylogeographic interfaces for a library of historical dengue virus isolates, with the ultimate goal of assessing their restriction heterogeneity in *Aedes* mosquitoes carrying *Wolbachia* endosymbionts. Background reading for this project introduced me to the nuanced roles that vector genetics and distribution can play in shaping arbovirus disease incidence: when I compared my viral phylogenies with those for mosquitoes, I realized that the introduction of *Aedes aegypti* to south Asia corresponded to the independent divergence of all four DENV serotypes' endemic genotypes. *The introduction of a domesticated vector had driven the domestication of a virus four times in parallel.* Following this, I spent a year learning human immune profiling methods, and gained a new appreciation of the selective environments that shape viral evolutionary trajectories.

In the fall of 2023, I began a Ph.D. in the Biological and Biomedical Sciences Program (BBSP) at the University of North Carolina at Chapel Hill to continue my training as a professional academic scientist. I completed my first two rotations in virology labs participating in a nascent research program on La Crosse orthobunyavirus (LACV), a persistent cause of disabling pediatric encephalitis in Appalachia. I conducted an extensive literature review on the transmission system and built my own working relationship with the program's local expert, Dr. Brian Byrd, a medical entomologist and eco-epidemiologist at Western Carolina University (WCU). My readings and Byrd lab meetings pointed me towards the primary vector, *Aedes triseriatus,* as a uniquely targetable component of these transmission systems. Unlike many temperate arboviruses, LACV persists for years in incredibly specific locations – down to the household level – because it can be efficiently transmitted by infected female mosquitoes to their eggs, though very few species' eggs can survive hosting these infections during the harsh winter months. Thanks to my earlier work, *I knew that basic science research into vector biology and population genetics could elucidate viral reservoir formation, maintenance, and mobility.* I sought out a third rotation that could support this work and train me in these research methods. I developed these ideas into a thesis project while rotating with Dr. Daniel Matute, and joined his lab and the Curriculum in Genetics and Molecular Biology at the end of my first year. Working in collaboration with Dr. Byrd, we are producing the first full genome for *Ae. triseriatus*, studying the molecular basis of winter diapause programs that limit fitness costs of viral infection, and preparing to sequence material from 100 sampling sites for the first year of an unprecedented longitudinal study of population structure and dispersal.

Looking back, I've learned to appreciate my career's successes and setbacks alike: by creatively adapting to challenges so often, I was exposed to many different nuanced systems, and learned diverse methods to interrogate specific, actionable aspects of them. In always re-orienting myself towards new facets of the complexity that I first fell in love with, I grew into the complex scientist that my systems deserved. I learned patience, humor, and how to let the world tell me about itself, rather than trying to make it fit my expectations. After years of pandemic response left me feeling conflicted over doing science that wasn't immediately translational, Dr. Byrd's exemplary career in both 'basic' and 'applied' science helped me remember: *when 'basic' science nurtures the joy that motivates us and the insights that let us work more effectively, nothing could be more essential to cultivate and defend.*

Broader Impacts: When designing vector-borne disease interventions in biodiversity hotspots like southern Appalachia, particularly for container mosquitoes that lay eggs in small, protected larval habitats, population genetics can inform how broadly to target interventions, and how well targeted interventions like *Wolbachia* or gene drives might spread within mosquito populations. As mosquitoes can transmit LACV sexually and to offspring, gene flow could serve as a proxy for viral reservoir mobility: comparative population structure of vectors and viruses can assess the viability of this approach. For our longitudinal study, Dr. Byrd recruited local collaborators at public health departments in county and tribal governments to assist with collection. Drawing on his decades of experience in community outreach and policy development in the region, we will convey relevant findings to these collaborators. With Dr. Byrd's guidance, I am designing a genomic epidemiology summer course for students at WCU, a primarily undergraduate regional university, that I will lead while in the area for fieldwork. By exploring diverse case studies, and teaching students to generate their own interactive phylogeographic analyses in NextStrain, I aim to help students explore ways to use modern molecular and statistical evolutionary biology tools to understand (and mitigate suffering in) the world around them.

Mentorship: As a queer woman in STEM, I had to navigate adverse training environments from the earliest stages of my scientific career. I used social media to build my own professional networks, and was diligently mentored by the female graduate students and technicians I worked with. Fortunately, I've found more supportive environments since, but have prioritized continuing this training tradition: *fostering a sense of belonging is essential to maintaining and supporting diversity in scientific workforces.* The Matute and Byrd labs are both training hubs for diverse cohorts of undergraduate and post-baccalaureate researchers, including work-study students. I've been thrilled to have the chance to share lessons I was once taught, on everything from molecular biology to project management, and to help them identify their own paths through science. I am now pursuing mentorship training through BBSP that will equip me to supervise undergraduate researchers in a more formal capacity.

I also serve as a first-year group peer mentor for BBSP, and am training for further leadership in this position. As a first-year student, I was surprised by how much I enjoyed brainstorming optimal lab and program fits with my cohort mates. I've found continuing this with the new BBSP cohort very rewarding.

Future goals: *My research history reflects my ability to tackle complex problems, synthesize innovative research questions, and quickly master new skills as needed.* In finding my own path, I built expertise in 'unusual' arbovirus-vector systems and in sophisticated molecular and analytical toolkits to understand and intervene in the outbreaks they cause. This will serve me well as an academic PI in an era of rapid global change and accelerating disease emergence. I am already helping a diverse next generation of scientists find their way along easier paths through research than the one I took; I look forward to continuing to make these paths even safer as I gain more training and power to do so, and to guiding a new generation of talented scientists through them. *With the support of the NSF GRFP, I will be able to prioritize building a strong foundation in the evolutionary biology principles that shape dipteran disease vector populations and their ability to transmit human and animal pathogens.* My graduate research will integrate and apply these insights to advance knowledge across both fundamental and translational domains. I am so excited to spend my career exploring new facets of complexity in arbovirus disease systems: it brings me such delight to know that, even if I diligently study them for the rest of my life, their unexplored complexities will still outlive me.

References: (1) Walker *et al.* 2018 *PLoS ONE.* (2) Ott *et al.* 2021 *Emerg Infect Dis.* (3) Baaijens *et al.* 2022 *Genome Biol.*

Investigating molecular features of diapause in *Aedes triseriatus*

Background: *Aedes* mosquitoes are globally invasive vectors of debilitating and deadly viral diseases, including dengue, chikungunya, and yellow fever. While *Ae. aegypti* spread throughout the global tropics over several centuries, multiple temperate *Aedes* – including *Ae. albopictus,* a vector of dengue and chikungunya viruses – have spread globally over the last 50 years. The persistence of newly introduced *Aedes* in temperate regions depends on their ability to survive the winter. This is mediated by **diapause,** a heritable program for developmental arrest initiated in advance of adverse conditions that lasts for a fixed period of time [1]. Most temperate *Aedes* diapause as fully-developed embryos within their eggs in response to day length cues, though the 'prediapause' life stage that perceives and responds to these cues differs between species. For some, the embryo initiates diapause in its generation ('embryonic prediapause'); for others, the adult mother initiates diapause in the subsequent generation of offspring ('maternal prediapause') [2]. Although the rapid U.S. invasion of *Ae. albopictus* prompted extensive molecular study of its maternal prediapause programs [3], embryonic prediapause has not received the same attention, despite its necessity for the winter survival of several potentially invasive disease vectors.

Aedes triseriatus, a classical model system for phenotypic studies of embryonic prediapause, is the primary vector and winter reservoir of La Crosse orthobunyavirus (LACV), the leading cause of pediatric arboviral encephalitis in the United States. Persistence in diapausing eggs is essential to LACV survival: during winter months, the virus is *only* found in mosquito eggs. When adults emerge from these eggs in the spring, they are systemically infected with LACV and can immediately transmit it to their offspring and to vertebrates, including humans [4]. Diapausing *Ae. triseriatus* populations transmit LACV to offspring more efficiently than non-diapausing populations do [5]. Although maternal prediapausing *Aedes*, including *Ae. albopictus,* can transmit LACV to their eggs, they do not overwinter infection efficiently [2,4]. Thus, *efficient transseasonal persistence of LACV is strongly correlated with embryonic prediapause.* This may be conditioned by transcript- or pathway-specific mechanisms; both can be explored through comparative analysis of transcriptomic data. To identify differentiating features of maternal and embryonic prediapause programs, I will generate a highly contiguous genome assembly for *Ae. triseriatus*, and transcriptomic profiles of diapause induction, maintenance, and exit.

AIM 1: Long-read whole genome sequencing of colony and wild-caught *Ae. triseriatus.*

A diapausing *Ae. triseriatus* colony has been maintained by our collaborator, Dr. Brian Byrd, for several years. PacBio HiFi long-read whole-genome sequencing of males and females from the colony is currently being used to generate long-read draft genome assemblies. The *Ae. triseriatus* genome is estimated to be 300-500 megabases longer than *Ae. albopictus*' [6]. *This suggests the hypothesis that diapause differences may result from copy-number variation in diapause-relevant genes.* I will assess support for this and alternative hypotheses by measuring copy-number variation in addition to assessing coding and regulatory differences in regions implicated in diapause processes and cold tolerance in other insects. To ensure that identified patterns are not artifacts of lab colony maintenance, I am performing whole-genome sequencing of wild-caught *Ae. triseriatus* males and females that I gathered from western North Carolina in May 2024. The Matute lab has previously assembled *Aedes* genomes from HiFi data.

AIM 2: mRNA expression profiling of diapause induction, maintenance, and exit in *Ae. triseriatus*.

Methods for pooled egg RNA-Seq are adapted from those outlined by Poelchau *et al*. for diapausing *Ae. albopictus* transcriptomics studies [7]. Briefly, I will gather 3 biological replicates of ≥1,000 *Ae. triseriatus* eggs per time point on egg papers and allow them to embryonate for 2 weeks. I will cut papers in half and split them between diapausing and non-diapausing treatments. I will use standard methods for *Ae. triseriatus* diapause induction [8] after confirming their timing in the colony population. I will conduct

treatments at 21ºC unless otherwise specified to prevent confounding cold temperature quiescence or metabolic suppression. At each time point in both sub-aims, I will hatch-stimulate a subset of eggs to assess diapause prevalence; I will homogenize the rest in Trizol, extract RNA, and submit it to UNC's High Throughput Sequencing Facility for mRNA library preparation and Illumina sequencing. I will conduct gene ontology and pathway analysis within and across species. I have extensive experience extracting low-abundance RNA for downstream sequencing and analyzing these datasets.

Aim 2A: Diapause induction: The molecular transition between quiescence, a state of developmental arrest that can be terminated immediately in response to favorable conditions, and true diapause, which cannot, has not been studied in embryonic prediapause [1]. The two states are indistinguishable outside of hatch response in *Ae. triseriatus.* I will establish a time course of diapause induction, collecting matched time points at 0, 1, 3, and 5 days. *Ae. albopictus* diapause preparation and induction datasets were generated using maternal tissue and are therefore not direct parallels [3], but broad pathway trends will still be informative. *I hypothesize that fewer genes involved in lipid sequestration and hydrocarbon lengthening will be differentially expressed in* Ae. triseriatus *diapause preparation, as they do not produce structurally distinct diapausing eggs* [8].

Aim 2B: Diapause maintenance and exit: *Aedes* eggs typically exit the diapause stage and transition to quiescence gradually after some period of chilling, preparing them to emerge once warm temperatures resume and hatch conditions are met [1]. Following diapause induction or control treatment, I will transfer all samples to 4ºC to begin the diapause termination process. Collection time points were chosen to parallel published data for *Ae. albopictus* [3], with an additional time point to account for longer diapause in *Ae. triseriatus* [9]. At 10, 20, 40, and 60 days of 4ºC treatment, I will return eggs to 21ºC for 7 days to allow metabolic activity to normalize before sample collection [9]. Abundance of specific transcripts known to prime LACV mRNA translation will be assessed across species and treatments, as this may condition fitness costs of infection [10]. *I hypothesize that some distinct pathways between species will be homologous to known cold tolerance processes in other insects, as temperate populations of the ancestrally tropical* Ae. albopictus *evolved cold tolerance* de novo *through still-elusive mechanisms* [3].

Intellectual Merit: The findings of these projects will be a vital first step in my study of *Ae. triseriatus* adaptive processes and their complex repercussions for virus transmission. Aim 1's genome assemblies will be a valuable resource for *Aedes* research: none of the few *Aedes* genomes currently available are from species native to the Americas. Aim 2's time courses will be the second produced for any *Aedes* (and the third for any mosquito), and can inform winter survival and invasion potential of disease vectors and dipterans more broadly. All data will promptly be made publicly available, and compatibility with *Ae. albopictus* datasets will be ensured.

Broader Impacts: Drawing on my unique cross-disciplinary background, I have built a research partnership, mentorship team, and set of projects that synthesize the strengths of both labs and support my training goals. Dr. Matute's expertise in dipteran development and evolution and Dr. Byrd's expertise in *Ae. triseriatus* biology will provide complementary training in research methods and translational approaches, which I will be able to explore more fully as an NSF fellow. I will use Aim 1's assemblies to design exome capture probes to improve scalability of my population genetics studies in North Carolina, which can inform vector control strategies. I will train undergraduate researchers at both collaborating institutions in these methods and supervise their independent management of follow-up projects.

References: (1) Denlinger and Armbruster 2014 *Annu Rev Entomol.* (2) Bova *et al*. 2019 *Insects.* (3) Armbruster 2016 *J Med Entomol.* (4) Day *et al*. 2023 *J Med Entomol.* (5) Woodring *et al*. 1998 *Am J Trop Med Hyg.* (6) Rao and Rai 1987 *Heredity.* (7) Poelchau *et al*. 2014 *J Vis Exp.* (8) Shroyer and Craig 1980 *Ann Entomol Soc Am.* (9) Shroyer and Craig 1983 *J Med Entomol.* (10) Dobie *et al*. 1997 *J Virol.*