

Evolutionary genetics of host-microbe interactions

I am an integrative evolutionary geneticist, working at the nexus of host-symbiont coevolution and speciation. I use approaches from genomics, genetics, physiology, and chemistry combined with intense sampling of natural populations to evaluate interactions between host flies in the *Drosophila* genus and endosymbiotic *Wolbachia* bacteria that reside within host cells. As an Assistant Professor at the University of XXX, my students and I will use cutting edge approaches to gain a general understanding of the evolutionary history of *Wolbachia* across diverse host clades. This work will take us from evaluations of genomic variation in host and *Wolbachia* genomes, to intracellular analysis of *Wolbachia* density and proliferation, and to population surveys at field sites I have established on a number of continents. This integrative approach will enable my lab at the University of XXX to rigorously determine how and why *Wolbachia* became the most pervasive symbiont on the planet.

Coevolution of hosts and endosymbiotic Wolbachia bacteria

Wolbachia alphaproteobacteria infect the cells of most arthropods, and are transmitted vertically by host females. *Wolbachia* became famous for their manipulation of host reproduction that includes male killing and cytoplasmic incompatibility (CI); the latter reduces the number of offspring an uninfected female produces when paired with an infected male. More recently *Wolbachia* from *Drosophila* have shown promise as a bio-control of vector-borne disease by suppressing virus transmission in mosquitoes (www.eliminatedengue.com). Whole cities are being transformed by releasing and establishing *Wolbachia* transinfected mosquitoes in local populations, and in these areas, the occurrence of dengue fever has been greatly reduced. Despite the success of this applied work, we do not understand the origin and maintenance of *Wolbachia* in their natural host systems.

Theory shows that the spread of CI-inducing *Wolbachia* depends primarily on the proportion of uninfected ova produced by infected females (μ), the hatch rate of uninfected eggs fertilized by infected males (H), and the fecundity—or other components of fitness—of infected females relative to uninfected females (F). CI-inducing infections that generate $H < F(1 - \mu) < 1$ lead to “bistable” dynamics with stable equilibria at 0 and at a higher frequency denoted ps , where $0.50 < ps \leq 1$. Simply stated, many infected males are needed before CI can drive *Wolbachia* infections to higher frequencies. These conditions apply to the *Wolbachia* transinfected mosquitoes currently being released, but seem not to apply to many natural *Wolbachia* infections in *Drosophila* species. Indeed, many natural *Wolbachia* infections do not manipulate host reproduction, and even when they do, we do not understand how *Wolbachia* affect host fitness in a way that enables infections to spread from low frequencies (corresponding to $F(1 - \mu) > 1$). This is a general question that my research seeks to answer: how and why do *Wolbachia* originate and spread, and how and why are they maintained? To answer these questions, I am using integrative approaches to connect genomic variation, to intracellular variation in *Wolbachia* density, to phenotypes that affect host fitness, and ultimately to variation in *Wolbachia* infection frequencies within host populations. And in systems where *Wolbachia* have become obligate symbionts, I am evaluating the genetics of reproductive isolation among sister host species.

Research program over the next 5 years

i) *Coevolution and diversification of host-Wolbachia associations*

A large focus of my research program is understanding the origin and maintenance of *Wolbachia* within host populations. As an Assistant Professor, I will continue this interest, while also expanding into new systems as my lab grows. As an example of what I have already discovered, within the hybridizing *D. yakuba* complex—*D. yakuba*, *D. teissieri*, and *D. santomea*—*Wolbachia* infection frequencies vary through time (15 years) and space (between west African islands) (Figure 1). It is my conjecture that the fitness effects of *Wolbachia* on hosts are a function of both abiotic and genetic backgrounds. Indeed, I have recently discovered that only certain crosses generate CI in *D. teissieri*, and that the same *Wolbachia* variant in different genetic backgrounds produces anywhere from no CI to a 25% reduction in egg hatch (Figure 1).

I will combine these analyses of *Wolbachia* phenotypes with phylogenetic analyses to evaluate the phylogeographic history of *Wolbachia*-host associations and coevolution. With Drs. Michael Turelli and Ary Hoffmann, I have obtained and constructed a phylogeny of approximately 40 species from the *montium* subgroup (Figure

2): approximately 80 widespread species in Southeast Asia, Africa, and Australia comprise this clade. In a research effort that I am coordinating, we have identified 22 *montium* species thus far that are infected with *Wolbachia*, five of which form a clade that is concordant with their *Wolbachia*. This pattern suggests cladogenic transmission of *Wolbachia* through evolutionary time. As an Assistant Professor at the University of XXX, I will use these phylogenetic and phylogenomic approaches to disentangle the evolutionary history of *Wolbachia* in a variety of host clades.

ii) Genomic diversity of hosts and their *Wolbachia*

A second focus of my research is on identifying and characterizing genomic variation that underlies variation in *Wolbachia* phenotypes and variation in infection frequencies within host populations. Variation in *Wolbachia* phenotypes could be controlled by variation in either *Wolbachia* or host genomes, or from interactions between genomes. My lab will use genomics and classical genetics to identify and map such variants. For example, I have identified systems where the strength of CI depends on both host and *Wolbachia* genomes—I will map nuclear modifiers of CI and other *Wolbachia* phenotypes using a combination of approaches available in *Drosophila*.

My work generates whole genome data sets, but I have also had success at identifying genomic reads within sequenced host samples. For example, in collaboration with Dr. Daniel Matute at UNC Chapel Hill I already have whole genomes for 60 *yakuba* clade individuals, their mitochondria, and the *Wolbachia* of infected host individuals. Our results show that *Wolbachia* variation partitions along species boundaries despite regular hybridization (Figure 1). I am currently testing for concordance among *Wolbachia*, host mitochondrial, and host nuclear genomes. With collaborators (see above), I am using similar data to evaluate sequence variants from the *montium* subgroup, and at the University of XXX my students and I will use similar approaches to expand into new systems as the lab grows. Finally, the density of *Wolbachia* cells can also vary greatly within host individuals indicating a need for characterizing infections specific to bacterial cell densities within hosts. I currently characterize this variation using qRT-PCR, but I am optimizing methods for sequencing very low titer infections. As an Assistant Professor I will continue to generate genomic data, and leverage existing data to elucidate genomic variation underlying *Wolbachia* phenotypes.

iii) Local adaptation and speciation of *Wolbachia* infected hosts

Another main focus of my research is evaluating adaptation of *Drosophila* hosts to their local environments and on understanding reproductive isolation between sister species pairs. I have recently developed many new systems within the *montium* subgroup, and established their phylogenetic relationships (Figure 2). As an Assistant Professor, my students and I will use these resources to better understand the coevolution of *Wolbachia* with their hosts. Sister species pairs and their *Wolbachia* will be evaluated, and when relevant, my students and I will sample natural populations to evaluate variation in *Wolbachia* infection frequencies in nature, to evaluate host differentiation in relevant phenotypes, and to evaluate reproductive isolation in hybridizing species pairs.

I am particularly excited about the *willistoni* group of flies that are part of the New World radiation of the *Sophophora* subgenus in South and Central America—some flies in this group have obligate relationships with their *Wolbachia*. I have obtained lines of all available species, and in collaboration with Dr. Matute, I will soon sample these species from Colombia and other areas of South America. We have already begun hybridization experiments using the existing lines, and are submitting the species we have in hand for genomic sequencing. With the exception of the *D. paulistorum* semispecies—that have recently been evaluated by Dr. Wolfgang Miller’s group—this group of flies has gone mostly under explored since Dr. Theodosius Dobzhansky’s early work during the mid 20th century. As an Assistant Professor I will develop this group as a model for understanding reproductive isolation in species that may have obligate relationships with their microbes with emphasis on intense sampling of natural populations of each species. Finally, in August of 2015 I collected a transect of a number of species from northeastern Namibia, through Zambia, to the border with Malawi, Africa. This is one of the largest collections of *melanogaster* subgroup species in existence, and is immediately available for my students at the University of XXX to exploit.

Teaching Statement

I seek to convey my interest and enthusiasm for evolutionary biology to the next generation of students and scientists, and specifically to underrepresented groups. During my graduate training I had the opportunity to teach a number of courses with a number of educators that employed different techniques in the classroom. I

also served as Head Teaching Assistant for introductory biology labs for one semester, which enabled me to design new laboratories and to learn how to effectively manage a group of instructors. My partner Rae Cooper is an educator tasked with implementing novel teaching techniques in Sacramento Public Schools. She serves as an excellent resource for me, and together we collaborate to bring science to underprivileged children in Sacramento, CA public schools. I would be excited to teach courses at the intersection of evolution, genetics, and host-symbiont coevolution as this overlaps nicely with my own research interests. I would also be happy to teach microbiology and general biology courses to undergraduate students at the University of XXX.

Figures

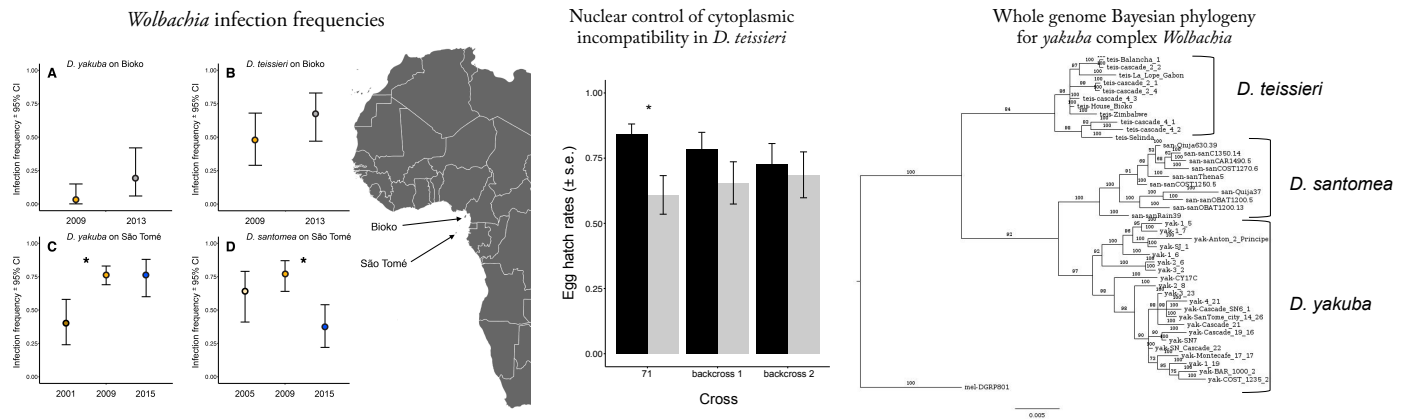


Figure 1: Left panel - *Wolbachia* infection frequencies on west African islands through time. Middle panel - A particular cross of *D. teissieri* (71) generates relatively strong CI. This effect disappears when CI inducing *Wolbachia* are backcrossed into other *D. teissieri* backgrounds (bc1 and bc2). This demonstrates host control of *Wolbachia* phenotypes. Black bars are for compatible and grey bars are for the incompatible reciprocal crosses. Right panel - Bayesian phylogeny of *Wolbachia* in the *yakuba* complex showing that variants partition along species boundaries. I am working on similar analyses for the mitochondrial genomes and looking for whether both genomes show evidence of concordant cladogenic transmission.

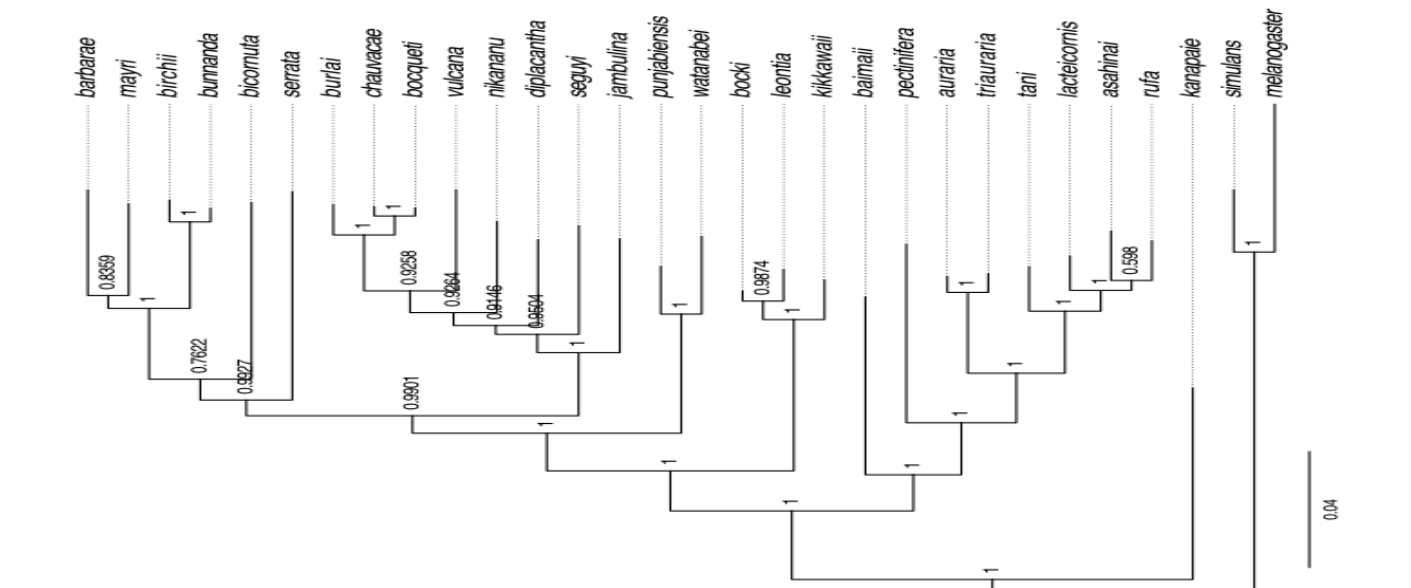


Figure 2: Bayesian phylogeny of the *montium* subgroup of *Drosophila* generated using concatenated sequence from ten loci.