BIOGRAPHICAL SKETCH

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NAME: Joseph J. Gillespie, Ph.D.

eRA COMMONS USER NAME (credential, e.g., agency login): JOEGILLESPIE

POSITION TITLE: Assistant Professor of Microbiology and Immunology

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Widener University, Chester, PA	BS	01/1998	Biology
University of Delaware, Newark, DE	MS	01/2001	Entomology/Applied Ecology
Texas A&M University, College Station, TX	Ph.D.	12/2005	Entomology
Virginia Bioinformatics Institute (Virginia Tech), Blacksburg, VA	Post-Doctoral	06/2013	Bioinformatics/ Microbiology

A. Personal Statement

My research program predominantly focuses on the biology of obligate intracellular bacteria, especially rickettsial species. Specifically, I have an intense interest in bacterial coevolution with their eukaryotic hosts. My work is heavily rooted in evolutionary biology, as I was trained in the fields of systematics and phylogenetics. I have always strived to incorporate empiricism into the analysis of molecular sequence data, believing that computational methods involving 'human input from the biologist' are far superior to purely automated methods. During my postdoctoral studies, I applied the advances I made in systematic theory and methodology to the fields of bioinformatics and comparative genomics. While collaborating with many researchers on a diverse range of organisms (e.g. mammals, many different groups of arthropods, trypanosomes), my work has largely focused on arthropod-borne rickettsial pathogens. Initially, applying phylogenetics to the analysis of *Rickettsia* genomes, I led a seminal report on the reclassification of pathogenic *Rickettsia* species (Gillespie *et al*., 2007), with several subsequent studies identifying numerous rickettsial pathogenicity factors, and importantly, supporting our reclassification scheme. I led another seminal study that elucidated across all genera of Rickettsiales the composition of an enigmatic type IV secretion system (Gillespie *et al*., 2010), which has subsequently been shown in several studies to translocate virulence factors into host cells. Finally, a growing familiarity with rickettsial genomes allowed for the description of a large, dynamic mobilome for *Rickettsia* species, identifying integrative conjugative elements as the vehicles for seeding *Rickettsia* genomes with many of the factors underlying obligate intracellular biology and pathogenesis (Gillespie *et al*., 2012). This research ultimately steered my interest in the evolution of reproductive parasitism, a new niche for me to explore some untapped aspects of this fascinating biology (Gillespie *et al.*, 2018)!

As of May 2020, I have published 56 peer-reviewed articles and seven book chapters and have collaborated extensively with dozens of laboratories around the world (35 h-index, Google Scholar). My research has three focal areas: **1) *Rickettsia* pathogenesis:** I primarily utilize bioinformatics to identify virulence factors (or other interesting molecules), which are subsequently characterized in the laboratory. Protein structural biology (stemming from my previous training in RNA structure) is incorporated this work; **2) Flea genomics:** I lead a collaboration for sequencing the cat flea genome/transcriptome and characterizing novel *Wolbachia* strains for their potential in pathogen biocontrol; **3) *Rickettsia* metabolomics:** Utilizing comparative genomics and metabolomics to identify host metabolites that are imported by pathogenic *Rickettsia* species. Currently, I am a co-Principal Investigator on an NIH award to characterize rickettsial sugar acquisition from their hosts (R21AI146773-01). I am actively seeking additional extramural funding for all the research projects that I lead.

1. **Gillespie, J.J.**, Beier, M.S., Rahman, M.S., Ammerman, N.C., Purkayastha, A., Shallom, J.M., Sobral, B.S., Azad, A.F. **(2007)** Plasmids and rickettsial evolution: insight from *Rickettsia felis*. *PLoS ONE* **2**: e266. **PMC1800911**
2. **Gillespie, J.J.**, Brayton, K.A., Williams, K.P., Quevedo Diaz, M.A., Brown, W.C., Azad, A.F. & Sobral, B.W. **(2010)** Phylogenomics reveals a diverse Rickettsiales type IV secretion system. *Infection and Immunity* **78**: 1809-1823. **PMC2863512**
3. **Gillespie, J.J.**, Joardar, V., Williams, K.P., Driscoll, T., Hostetler, J.B., Nordberg, E.K., Shukla, M., Walenz, B., Hill, C.A., Nene, V.M., Azad, A.F., Sobral, B.W. & Caler, E. **(2012)** A *Rickettsia* genome overrun by mobile genetic elements provides insight into the acquisition of genes characteristic of an obligate intracellular lifestyle. *Journal of Bacteriology* **194**: 376-394. **PMC3256634**
4. **Gillespie, J.J.**, Driscoll, T.P., Verhoeve, V.I., Rahman, M.S., Macaluso, K.R., Azad, A.F. **(2018)** A tangled web: origins of reproductive parasitism. *Genome Biology and Evolution* **10**: 2292-2309. **PMC6133264**

B. Positions and Honors

Positions and Employment

2006-2008	Postdoctoral Fellow, Virginia Bioinformatics Institute (VA Tech), Blacksburg, VA
2006-2013	Visiting Scientist, Department of Microbiology and Immunology, University of Maryland, School of Medicine, Baltimore, MD
2008-2010	Research Associate, Virginia Bioinformatics Institute (VA Tech), Blacksburg, VA
2010-2013	Senior Research Associate, Virginia Bioinformatics Institute (VA Tech), Blacksburg, VA
2013-2018	Assistant Professor (non-tenure track), University Department of Microbiology and Immunology, University of Maryland, School of Medicine, Baltimore, MD
2018-	Assistant Professor (tenure track), University Department of Microbiology and Immunology, University of Maryland, School of Medicine, Baltimore, MD

Professional Memberships

1998-2005	Member, Entomological Society of America
2000-	Member, American Association for the Advancement of Science
2000-	Member, Society for Molecular Biology and Evolution
2000-2003	Member, Society for the Study of Evolution
2000-	Member, Society of Systematic Biologists
2004-2005	Member, European Society for Evolutionary Biology
2006-	Member, American Society for Microbiology
2006-	Member, American Society of Rickettsiology
2009-2016	Editorial board, Society of Systematic Biologists

Other Experience

2001-	*Ad hoc* reviewer for 60 scientific journals (too numerous to list; I now review incessantly)
2013-	On a yearly basis, I provide lectures on intracellular bacteria (GPLS 710: Principles of Microbial Pathogenesis), bacterial secretion systems (GPLS 710: Advanced Microbial Pathogenesis), and research ethics (CIPP907: Responsible Conduct of Research). I also participate in HDID medical student laboratories on Koch's postulates and parasitology. I regularly judge at the annual Graduate Research Conference, M&I Graduate Research Competition, and Medical Student Research Day.
2016-	University of Maryland School of Medicine Council.
2017-	Editorial Board, *PeerJ*

C. Contributions to Science

1. During my graduate studies (1998-2005) I developed and honed my expertise in molecular phylogenetics and RNA structure prediction. Under the tutelage of two prominent workers in Systematic Entomology, Dr. Douglas Tallamy (Behavioral Ecologist, University of Delaware) and Dr. Anthony Cognato (Insect Taxonomist formerly at Texas A&M University, currently at Michigan State University), I learned the principles of organism-based systematic biology, with a heavy focus on integrating all aspects of an organism's system (e.g., ecology, behavior, phylogeny, etc.) into its classification and biological description. I have always had a deep interest

in molecular biology and biochemistry, particularly structural biology, and during my graduate work I studied devotedly under direct tutelage of two prominent molecular phylogeneticists, Dr. Karl Kjer (Rutgers University) and Dr. Rodney Honeycutt (Texas A&M University, currently at Pepperdine University), as well as one of the world's leading authorities on RNA structure (Dr. Robin Gutell, University of Texas). The work from my graduate studies resulted in the publication of 19 peer-reviewed articles and four book chapters, much of which stemmed from utilizing my strengths in comparative sequence analysis to contribute to studies on a variety of different organisms, as well as advancements in methods in molecular phylogeny reconstruction. Particularly notable are works that demonstrated the efficacy of incorporating structural and evolutionary criteria into multiple sequence alignment and phylogeny estimation (e.g., Gillespie, 2004), and also studies on prediction of novel RNA structures (e.g., Gillespie *et al*., 2005; Gillespie *et al*., 2006). Importantly, during this time period I learned that I could contribute to many diverse projects, despite being agnostic to the central organism, by way of applying my expertise in molecular sequence analysis. I also learned that my skillsets were valuable to large genome sequencing projects, such as that for the Honey bee, wherein I contributed to the discovery of novel retrotransposable elements within *Apis mellifera* ribosomal RNA gene regions (Honey Bee Genome Sequencing Consortium, 2006).

a. **Gillespie, J.J. (2004)** Characterizing regions of ambiguous alignment caused by the expansion and contraction of hairpin-stem loops in ribosomal RNA molecules. *Molecular Phylogenetics and Evolution* **33**: 936-943. **PMID: 15522814**
b. **Gillespie, J.J.**, McKenna, C.H., Yoder, M.J., Gutell, R.R., Johnston, J.S., Kathirithamby, J. & Cognato, A.I. **(2005)** Assessing the odd secondary structural properties of nuclear small subunit ribosomal RNA sequences (18S) of the twisted-wing parasites (Insecta: Strepsiptera). *Insect Molecular Biology* **14**: 625-643. **PMID: 16313563**
c. **Gillespie, J.J.**, Johnston, J.S., Cannone, J.J. & Gutell, R.R. **(2006)** Characteristics of the nuclear (18S, 5.8S, 28S and 5S) and mitochondrial (12S and 16S) rRNA genes of *Apis mellifera* (Insecta: Hymenoptera): structure, organization, and retrotransposable elements. *Insect Molecular Biology* **15**: 657-686. **PMC2048585**
d. Honey Bee Genome Sequencing Consortium. **(2006)** Insights into social insects from the genome of the honeybee *Apis mellifera*. *Nature* **443**: 931-949. **PMC2048586**

2. As a Postdoctoral Fellow and Research Associate (2006-2013) under the supervision of Dr. Bruno Sobral (former director of the Virginia Bioinformatics Institute at Virginia Tech) I applied my skillsets to the NIAID-funded PathoSystems Resource Integration Center (PATRIC), mostly by improving genome annotation protocols and instilling more practical comparative genomics tools for the biologist (Gillespie *et al*., 2011). During this time, I was also trained in Rickettsiology by Dr. Abdu Azad (University of Maryland, School of Medicine), gaining valuable experience in the laboratory working on rickettsial pathogenesis. Aside from seminal reports on the reclassification of pathogenic *Rickettsia* species, comparative phylogenomics analyses of *Rickettsia* spp., and illumination of substantial lateral gene transfer shaping *Rickettsia* genomes (see section A above), I contributed to various research projects in Dr. Azad's group that focused on *Rickettsia* pathogenicity. Most noteworthy were the identification of a novel translocation pathway for a secreted *Rickettsia* effector (Kaur *et al*., 2012) and characterization of the *Rickettsia typhi* surface proteome (Sears *et al*., 2012). Throughout this time, I also developed a keen interest in bacterial protein secretion systems, exemplified by a report on the atypical type IV secretion system carried by *Rickettsia* spp. (Gillespie *et al*., 2009). Collectively, I published 18 peer-reviewed articles and two book chapters during this time, works focused on either *Rickettsia* pathogenesis and/or advancements in bioinformatics/comparative genomics.

a. **Gillespie, J.J.**, Ammerman, N.C., Dreher-Lesnick, S., Rahman, M.S., Worley, M.J., Setubal, J.C., Sobral, B.S. & Azad, A.F. **(2009)** An Anomalous Type IV Secretion System in *Rickettsia* is Evolutionarily Conserved. *PLoS ONE* **4**: e4833. **PMC2653234**
b. **Gillespie, J.J.**, Wattam, A.R., Cammer, S.A., Gabbard, J., Shukla, M.P., Dalay, O., Driscoll, T.P., Hix, D., Mane, S.P., Mao, C., Nordberg, E.K., Scott, M., Schulman, J.R., Snyder, E.E., Sullivan, D.E., Wang, C., Warren, A., Williams, K.P., Xue, T., Yoo, H.S., Zhang, C., Zhang, Y., Will, R., Kenyon, R.W., Sobral, B.W. **(2011)** PATRIC: The Comprehensive Bacterial Bioinformatics Resource with a Focus on Human Pathogenic Species. *Infection and Immunity* **79**: 4286-4298. **PMC3257917**

c. Kaur, S., Rahman MS, Ammerman, N.C, Vasudevan, P., Beier-Sexton, M., Ceraul, S.M., **Gillespie, J.J.** & Azad, A.F. **(2012)** TolC-dependent secretion of an ankyrin repeat-containing protein of *Rickettsia typhi*. *Journal of Bacteriology* **194**: 4920-4932. **PMC3430354**
d. Sears, K.T., Ceraul, S.M., **Gillespie, J.J.**, Ammerman, N.C, Rahman M.S. & Azad, A.F. **(2012)** Surface proteome analysis and initial characterization of Surface cell antigen (Sca) or autotransporters family of *Rickettsia typhi*. *PLoS Pathogens* **8**: e1002856. **PMC3415449**

3. In July of 2013, I joined the University of Maryland, School of Medicine as a faculty member of the Department of Microbiology and Immunology, electing to focus my career more exclusively on rickettsial pathogenesis. During this time, I published seven peer-reviewed articles and one book chapter, and also have received continued training from Dr. Azad and his research group in classical microbiology, as well as laboratory approaches tailored to Rickettsiology (including BSL-3 certification). Throughout this period, I led projects on *Rickettsia* comparative genomics, with the main goal of these studies to elucidate genome-specific mechanisms of virulence (e.g., Gillespie *et al*., 2014). I have also continued utilizing bioinformatics and comparative genomics to identify lineage specific virulence factors for *R. typhi* (Rahman *et al*., 2013), as well as better understand the host response pathways in arthropods that vector rickettsial pathogens (Choy *et al*., 2013). My growing expertise in *Rickettsia* protein secretion culminated in an extensive review (Gillespie *et al*., 2015), and during this period I began applying for extramural funding to support my research on rickettsiae.

a. Rahman, M.S., **Gillespie, J.J.**, Kaur, S., Sears, K.T., Ceraul, S.M., Beier-Sexton, M. & Azad, A.F. **(2013)** *Rickettsia typhi* possesses phospholipase A2 enzymes that are involved in infection of host cells. *PLoS Pathogens* **9**: e1003399. **PMC3688537**
b. Choy, A., Severo, M.S., Sun, R., Girke, T., **Gillespie, J.J.**, Pedra, J.H.F. **(2013)** Decoding the ubiquitin-mediated pathway of arthropod disease vectors. *PLoS ONE* **8**: e78077. **PMC3804464**
c. **Gillespie, J.J.**, Driscoll, T., Verhoeve, V.I., Utsuki, T., Husseneder, C., Chouljenko, V.N., Azad, A.F. & Macaluso, K.R. **(2014)** Genomic diversification in strains of *Rickettsia felis* isolated from different arthropods. *Genome Biology and Evolution* **7**: 35-56. **PMC4316617**
d. **Gillespie, J.J.**, Kaur, S.J., Rahman, M.S., Rennoll-Bankert, K., Sears, K.T., Azad, A.F. **(2015)** Secretome of obligate intracellular *Rickettsia*. *FEMS Microbiology Reviews* **39**: 47-80. **PMC4344940**

4. Since 2015, I have primarily continued work on identifying and characterizing rickettsial secretion systems and their substrates. One effector, the Arf-GEF RalF, is the first *Rickettsia* protein demonstrated to interact with the *rvh* T4SS substrate recognition particle (RvhD4), and was also shown to be critical for *R. typhi* invasion of host cells (Rennoll-Bankert *et al*., 2015). We recently showed that RalF is the first bacterial effector to directly activate Arf6, a process that initiates alterations in phosphoinositol metabolism critical for rickettsial entry (Rennoll-Bankert *et al*., 2016). Work on other identified *rvh* effectors is on-going. I have also continued to characterize the *rvh* machine itself, working with a collaborative team to solve the structure of one of its duplicate components (Gillespie *et al*., 2015). Over half of the *rvh* components have recently been approved by NIAID for structure determination in collaboration with the Seattle Structural Genomics Center for Infectious Disease. A recent study has further characterized the complexity underpinned by *rvh* gene family expansion, and also identified an insertion within the T4SS pore that might prove to be a promising drug target. Many studies to further dissect out the intricacies of the *rvh* T4SS are underway. In support of this work, my colleague at UMB (Dr. M. Sayeed Rahman) and myself have been awarded an NIH grant to decipher the structure and function of the *rvh* T4SS (R21 AI126108).

a. Rennoll-Bankert, K.E., Rahman, M.S., **Gillespie, J.J.**, Guillotte, M.L., Kaur, S.J., Lehman, S.S., Beier-Sexton, M. & Azad, A.F. **(2015)** Which Way In? The RalF Arf-GEF Orchestrates *Rickettsia* Host Cell Invasion. *PLoS Pathogens* **11**: e1005115. **PMC4546372**
b. Rennoll-Bankert, K.E., Rahman, M.S., Guillotte, M.L., Lehman, S.S., Beier-Sexton, M., **Gillespie, J.J.**, & Azad, A.F. **(2016)** RalF-Mediated Activation of Arf6 Controls *Rickettsia typhi* Invasion by Co-Opting Phosphoinositol Metabolism. *Infection and Immunity* **84**: 3496-3506. **PMC5116726**
c. **Gillespie, J.J.**, Phan, I.Q., Scheib, H., Subramanian, S., Edwards, T.E., Lehman, S.S., Piitulainen, H., Rahman, M.S., Rennoll-Bankert, K.E., Staker, B.L., Taira, S., Stacy, R., Myler, P.J., Azad, A.F. & Pulliainen, A.T. **(2015)** Structural Insight into How Bacteria Prevent Interference between Multiple Divergent Type IV Secretion Systems. *MBio* **6**: e01867-15. **PMC4676284**

d. **Gillespie, J.J.**, Phan, I.Q., Driscoll, T., Guillotte, M.L., Lehman, S.S., Rennoll-Bankert, K.E., Subramanian, S., Beier-Sexton, M., Myler, P.J., Rahman, M.S., Azad, A.F. **(2016)** The *Rickettsia* type IV secretion system: unrealized complexity mired by gene family expansion. *Pathogens and Disease* **74**: ftw058 [Editor's Choice]. **PMID: 27307105**

Complete List of Published Work in MyBibliography
https://www.ncbi.nlm.nih.gov/myncbi/browse/collection/46907614/?sort=date&direction=descending

D. Research Support

Ongoing Research Support

NIH/NIAID, R21AI146773-01 (PI: Gillespie/Rhaman) Co-Pi 07/01/2019 to 06/30/2011
Rickettsia cell envelope glycoconjugates are derived from the host cell amino sugar biosynthesis pathway
Goal: Determine if *Rickettsia typhi* uniquely utilizes host-derived N-acetylglucosamine 1-P (NAG-1-P) for biosynthesis of cell envelope glycoconjugates (Rickettsiae lack glycolysis, yet they make cell wall sugars).

NIH/NIAID, R01AI017828 (PI: Azad), Key Personnel 09/01/1982 to 05/31/2018
Murine Typhus: Vector Biology and Transmission
Goal: determine the degree of interplay between *R. typhi* in fleas and mammalian hosts. Proposed experiments will elucidate mechanisms of rickettsial infection in flea gut epithelia and mammalian cell lines.

NIH/NIAID, R01AI126853-01 (PI: Azad) Key Personnel 07/30/2016 to 06/30/2021
Rickettsia-host interface and multiple paths to invasion
Goal: investigate the roles of secreted proteins by non-Spotted Fever Group species during host cell infection. This information will provide more efficacious vaccine and therapeutic interventions for rickettsial diseases.

NIH/NIAID, R01 AI116523-01AI (PI: Pedra) Key Personnel 12/01/2015 to 11/30/2020
Ubiquitylation and Rickettsial Colonization of a Tick Vector
Goals: (1) investigate the tick IMD signaling pathway during *A. phagocytophilum* (*Ap*) infection; (2) examine interactions between the E2 conjugating enzyme Bendless and E3 ubiquitin ligase XIAP during *Ap* tick colonization; and (3) characterize XIAP-derived substrates during *Ap* tick colonization.

Completed Research Support

NIH/NIAID, R21AI26108-01 (PI: Gillespie/Rhaman) Co-Pi 07/01/2016 to 06/30/2019
Characterizing gene family expansion in an atypical bacterial secretion system
Goal: characterize the Rickettsiales *vir* homolog (*rvh*) type IV secretion system (T4SS) from the agent of murine typhus (*Rickettsia typhi*). The *rvh* T4SS is anomalous compared to other bacterial T4SSs, and our work aims to understand the odd gene family expansion that encodes four dynamic components of the secretion machine.

NIH/NIAID, R01AI43006 (PI: Azad), Key Personnel 03/01/1998 to 06/30/2015
Interspecific Competition Between Rickettsiae in Ticks
Goal: This proposal specifically addresses transovarial interference of the human pathogen, *Rickettsia rickettsii*, in the tick vector transovarially infected with nonpathogenic rickettsiae.