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MORRIS M. ATTA
Deputy to the Chairperson

State of Hawaii
DEPARTMENT OF AGRICULTURE
1428 South King Street
Honolulu, Hawaii 96814-2512
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June 8, 2021

To: Advisory Committee on Plants and Animals

From: Floyd Reed, Ph.D., University of Hawaii at Mānoa
Matthew Medeiros, Ph.D., University of Hawaii at Mānoa

Through: Christopher Kishimoto
Entomologist
Plant Quarantine Branch
Hawaii Department of Agriculture

Subject: Request to: (1) Determine if the Establishment of the Southern House Mosquito, *Culex quinquefasciatus*, a Vector of Avian Influenza in Hawaii, Constitutes an Ecological Disaster;

(2) Allow the Importation of the Southern House Mosquito, *Culex quinquefasciatus*, an Unlisted Insect, Inoculated with a Foreign *Wolbachia* Bacteria Species, by Special Permit, for Laboratory, Field-Release, and Area-Wide Mosquito Suppression Research, by the University of Hawaii at Mānoa;

(3) Establish Special Permit Conditions for the Importation of the Southern House Mosquito, *Culex quinquefasciatus*, an Unlisted Insect, Inoculated with a Foreign *Wolbachia* Bacteria Species, by Special Permit, for Laboratory, Field-Release, and Area-Wide Mosquito Suppression Research, by the University of Hawaii at Mānoa;

(4) Determine the Probable Impact on the Environment if the Southern House Mosquito, *Culex quinquefasciatus*, an Unlisted Insect, Inoculated with a Foreign *Wolbachia* Bacteria Species, is Accidently Released; and

(5) Are the proposed permit conditions sufficient to ensure that the Southern House Mosquito, *Culex quinquefasciatus*, an Unlisted Insect, Inoculated with a Foreign *Wolbachia* Bacteria Species, presents probably minimal or no significant effect on the environment.

I. Summary Description of the Request

PQB NOTES: *The Plant Quarantine Branch (PQB) submittal for requests for import or possession permits, as revised, distinguishes information provided by the applicant from procedural information and advisory comment and evaluation presented by PQB. With the exception of PQB notes, hereafter "PQB NOTES," the text shown below in Section II from page 2 through page 6 of the submittal was taken directly from Drs. Reed and Medeiros' application and subsequent written communications provided by the applicants. For instance, the statements on pages 18 through 22 regarding effects on the environment are the applicant's statements in response to standard PQB questions and are not PQB's statements. This approach for PQB submittals aims for greater applicant participation in presenting import requests in order to move these requests to the Board of Agriculture (Board) more quickly, while distinguishing applicant provided information from PQB information. The portion of the submittal prepared by PQB, including the environmental assessment, Advisory Subcommittee review, and proposed permit conditions, are identified as Sections III, IV, and V of the submittal, which start at pages 25, 26, and 36 respectively.*

We have a request to review the following:

COMMODITY: 2,500 southern house mosquitoes, *Culex quinquefasciatus*. Mixed sex, eggs and larvae.

SHIPPERS:

1) Robert Harrell II**,** University of Maryland Institute for Bioscience and Biotechnology Research, Insect Transformation Facility**,** 9600 Gudelsky Dr., Rockville, Maryland 20850

2) Zhiyong Xi, Michigan State University, Department of Microbiology and Molecular Genetics, Giltner Hall, 293 Farm Lane, Room 314M, East Lansing, Michigan 48824

3) Stephen Dobson, University of Kentucky, Department of Entomology, S-307D Ag Science Ctr N, Lexington Kentucky 40546-0091

IMPORTERS:

1) Floyd Reed**,** University of Hawaii at Mānoa (UHM)**,** 2538 McCarthy Mall, Edmondson Hall 216**,** Honolulu, Hawaii 96822**,** Ph: (808) 956-6489

2) Matthew Medeiros, University of Hawaii at Mānoa, 1993 East-West Road, Honolulu, Hawaii 96822 Ph: (808) 956-8187

CATEGORY: Southern house mosquitoes, *C. quinquefasciatus,* is an unlisted animal. Chapter 4-71, Hawaii Administrative Rules, allows importation of unlisted animals into Hawaii for the purpose of remediating medical emergencies or ecological disasters, or conducting scientific research that is not detrimental to agriculture, the environment, or humans by special permit on a case-by-case basis.

PQB NOTES: *The importation of an unlisted animal is prohibited except under special permit purposes determined appropriate by the Board (i.e., remediating medical emergencies or ecological disasters, or conducting scientific research that is not detrimental to agriculture, the environment, or humans). For example, an unlisted fish or bird may only be imported for purposes such as remediating a medical emergency or ecological disaster, or conducting scientific research that is not detrimental to agriculture, the environment, or humans.*

II. Information Provided by the Applicants in Support of the Application

OBJECTIVE: To conduct scientific research and mass rearing of *C. quinquefasciatus* in controlled laboratory settings at the University of Hawaii at Mānoa, and to enable field applications of *C. quinquefasciatus* inoculated with bacteria (*Wolbachia* spp.) to suppress mosquito numbers and [attempt to] prevent an ecological disaster.

PROJECT: This is an application for a permit to conduct laboratory and field release research using imported southern house mosquitoes, *C. quinquefasciatus* (Diptera: Culicidae), that have been inoculated with bacteria (*Wolbachia* spp.) not native to the mosquito's current internal fauna. The presence of the foreign bacteria within the mosquito's reproductive system will render the inoculated imported mosquitoes unable to successfully mate with their own species in the islands, a term called cytoplasmic incompatibility. Cytoplasmic incompatibility has been used with much success in other parts of the world to reduce mosquito populations and thus reduce transmission of mosquito-vectored diseases to humans. We propose to use cytoplasmic incompatibility to reduce current populations of *C. quinquefasciatus*, the vector of avian malaria, a disease which has devastated Hawaii's native bird populations, causing an ecological disaster which threatens the extinction of Hawaii's native bird species.

Mosquitoes are not native to Hawaii, and they negatively affect multiple sectors that are important to the State, from human health to conservation to tourism and economics. On January 17, 2017, the Hawaii Invasive Species Council, an inter-departmental collaboration comprised of the Departments of Land and Natural Resources (DLNR), Agriculture (HDOA), Health (HDOH), Transportation (HDOT),

Business, Economic Development & Tourism (HDBEDT), and the University of Hawaii (UH) passed resolution 17-2, specifically pertaining to mosquitoes. Resolution 17-2, entitled, "Supporting Evaluation and Implementation of Technologies for Landscape-Scale Control of Mosquitoes, With a Focus on Mitigating Both Human and Wildlife Health Risks," recognizes that mosquitoes in the State of Hawaii are non-native and an important pest species to control. The resolution encourages researchers and management agencies to pursue research that can benefit human and wildlife health, as well as the economy by controlling and/or preventing the establishment of mosquito populations. (https://dlnr.hawaii.gov/hisc/files/2013/02/HISC-Reso-17-2-signed.pdf).

C. quinquefasciatus is an invasive, disease-spreading mosquito found throughout Hawaii. The species was introduced accidentally to Hawaii in the early 1800s. *C. quinquefasciatus* is a known vector of zoonotic diseases such as West Nile virus and lymphatic filariasis. In Hawaii, *C. quinquefasciatus* is also the primary vector of avian pox and of *Plasmodium relictum*, a parasite responsible for avian malaria (van Riper *et al*. 1986). These two diseases were major factors in the extinction of more than half of Hawaii's endemic honeycreepers and continue to be the leading driver in the ongoing extinction risk of most of the remaining species (reviewed in Atkinson and LaPointe 2009; Atkinson *et al.* 2014). Several extant bird species, such as the ʻiʻiwi (*Drepanis coccinea*), ʻapapane (*Himatione sanguinea*), ʻakohekohe (*Palmeria dolei*), Kauaʻi ʻamakihi (*Chlorodrepanis stejnegeri*), ʻakiapolaʻau (*Hemignathus wilsoni*), ʻanianiau (*Magumma parva*), ʻakekeʻe (*Loxops caeruleirostris*), Hawaii ʻakepa (*Loxops coccineus*), ʻakikiki (*Oreomystis bairdi*), palila (*Loxioides bailleui*), and kiwikiu (*Pseudonestor xanthophrys*) are now restricted to upper elevations where temperatures are too cold for the mosquito and plasmodium parasite to persist (Atkinson *et al.* 1995). However, climate modeling predicts that the disease distribution will spread to higher elevations in the Hawaiian Islands and, therefore, contribute to continued loss of avian diversity (Fortini *et al.* 2015; Liao *et al.* 2017). Indeed, at least two native honeycreeper species have experienced sudden, severe declines in the last decade, and are predicted to become extinct in the wild in the next 5-10 years if mosquito-borne diseases are not mitigated. Efforts to address these diseases through utilization of traditional vector control methods (e.g., pesticides) are inadequate at a landscape scale, and may be problematic for other nontarget protected species (e.g., endangered Hawaiian *Drosophila*, US Fish and Wildlife Service (USFWS) 2006). Current efforts to control mosquito-vectored disease outbreaks are limited to reducing mosquito breeding site locations and applications of various larvicides and adulticides when

travel imported cases of human arboviral diseases are identified by the HDOH.

On September 6-7, 2016, local, national, and international experts gathered in Hawaii to discuss how to mitigate mosquito-borne diseases, including avian malaria and avian pox. The strategy deemed most favorable in terms of its effectiveness, technical readiness, and safety was *Wolbachia*-based cytoplasmic incompatibility. Cytoplasmic incompatibility results from the presence of a bacterium, *Wolbachia*, in the cells of the mosquito. Many arthropod species, including several native species here in Hawaii, naturally contain diverse strains of *Wolbachia* (Bennett *et al*. 2012; Hoffmann *et al.* 2015). *Wolbachia* are a type of arthropod endosymbiont that do not occur in humans or other vertebrates. Approximately 50% of insect species naturally have *Wolbachia* (Weinert *et al*. 2015), although many of these insects can survive without *Wolbachia (*e.g., Hamm *et al*. 2014*)*. The largest effect of *Wolbachia* is on mating compatibility between individual insects that carry the bacteria. However, there are secondary effects that are being studied by many labs. These include altered host insect lifespan and reduced vector competence (see Hoffmann *et al*. 2015 for review).

In nature, *Wolbachia* are passed from females to their offspring. Different strains of *Wolbachia* have also been introduced into insects in laboratories. If a male mosquito with one type of *Wolbachia* mates with a female mosquito that has a different strain of *Wolbachia* the resulting offspring can be inviable and not develop into mosquito larvae because of a mismatch of cellular signals (loss of the male parental chromosomes, Callaini *et al*. 1997; Tram and Sullivan 2002; see also Hamm *et al*. 2014) originating from *Wolbachia*. If sufficient numbers, on the order to 10 times the wild population size (Dame *et al.* 2009), of male mosquitoes of a different *Wolbachia* type are released, wild females are more likely to mate with males of a different *Wolbachia* type and are predicted to have far fewer viable offspring. With subsequent releases, this process can significantly suppress the wild population numbers of mosquitoes over the following generations over a geographic area (Laven 1967; Sinkins *et al*. 1995; Dobson *et al.* 2001; Blagrove *et al.* 2012). *Wolbachia* male-based insect control programs have been highly successful for reducing local mosquito populations around the world (e.g., Atyame *et al.* 2015; Atyame *et al.* 2016; Mains *et al.* 2016; Waltz 2016), and this approach has received U.S. federal, state, and local approvals of field trials in California, Florida, and Kentucky (Waltz 2016). *Wolbachia* cannot be spread by the released males, because *Wolbachia* are only passed from mother to offspring. It is also worth noting that male mosquitoes do not bite or vector disease. [As discussed below, only males would be released.] Note that in addition to *Wolbachia* strategies for mosquito population

suppression, other *Wolbachia*-based strategies have also been used to alter (rather than suppress) insect populations (*e.g.,* O'Neill *et al.* 2018).

In order to generate the mosquitoes with a different *Wolbachia* type, the naturally-occurring *Wolbachia* strain is cleared from the mosquitoes using the antibiotic tetracycline. Then *Wolbachia* can be harvested from cells of another insect species (this can be another mosquito or a non-mosquito species) and introduced into the cleared mosquitoes via microinjection. Another method to establish new *Wolbachia* strains is to mate a *Wolbachia*-carrying female insect to males that have been cleared of their naturally-occurring *Wolbachia* via antibiotic. Because *Wolbachia* are maternally inherited (described above), this cross results in all of the offspring inheriting whichever *Wolbachia* strain is contained in the female parent.

Purpose of application

In this application, we seek to import *C. quinquefasciatus* that have already had their endogenous *Wolbachia* replaced with a different strain, or which naturally carry *Wolbachia* strains that are not already found here. Imported strains will be those that are reported to be incompatible with strains currently present in local *C. quinquefasciatus*. When male *C. quinquefasciatus* carrying the imported strain mate with female *C. quinquefasciatus* carrying the local strain, the eggs should not hatch, and mosquito population suppression should result. This import permit application has been developed in conjunction with staff at the HDOH, DLNR, and the USFWS. This application is being submitted in response to Resolution 17-2, so that we are better able to pursue research that can benefit human and wildlife health, as well as the economy, by controlling mosquito populations (https://dlnr.hawaii.gov/hisc/files/2013/02/HISC-Reso-17-2-signed.pdf).

In the case of certain shippers, we seek to re-import mosquitoes which have been physically collected from the Hawaiian Islands (*e.g.,* Michigan State University, United States Department of Agriculture (USDA)-Animal Plant and Health Inspection Service (APHIS)-Veterinary Services (VS) collection permit 16-3). In these cases, the naturally-occurring *Wolbachia* will be cleared, and the new strain of *Wolbachia* introduced in the laboratory via microinjection and/or crossing with female mosquitoes that carry a different *Wolbachia*.

PROCEDURE: We would like to import three strains, two of which already exist here. These are:

- *Wolbachia albopictus A* (*wAlbA*) imported in *C. quinquefasciatus*. In Hawaii, this strain already exists in *Aedes albopictus*.
- *Wolbachia albopictus B* (*wAlbB*) imported in *C. quinquefasciatus*. In Hawaii, this strain already exists in *Aedes albopictus*.
- *Wolbachia wPip4* imported in *C. quinquefasciatus*. This strain does not currently exist in Hawaii. It naturally exists in parts of Europe, Asia, the Middle East, and Africa, and is bidirectionally incompatible with strain *wPip5*. Strain *wPip5* is the most common strain in *C. quinquefasciatus* in Hawaii (Atkinson, C. T., W. Watcher-Weatherwax, and D. A. LaPointe. (2016) Genetic diversity of *Wolbachia* endosymbionts in *C. quinquefasciatus* from Hawaii, Midway Atoll and American Samoa. Technical Report HCSU-074).

Once imported, we will rear the imported mosquitoes to the maximum capacity of our facilities. Male mosquitoes with one or more of the imported strains (*wAlbA / wAlbB / wPip4*) could then be used for incompatible crosses to females that carry *wPip5.* The attached letter from the DLNR describes how there is an ecological disaster occurring (*i.e.* Hawaii's native birds going extinct). The imported mosquito[e]s are intended for release (only males are intended for release) to mitigate this disaster. Based on the prior use of this technology in California, Florida, and Kentucky, we do not expect releases of these male mosquitoes to have a negative impact on agriculture, the environment, or public health and safety.

PQB NOTES: *In addition to this request, the applicants have submitted a request to import the aforementioned species of unlisted Wolbachia bacteria. The import request for the Wolbachia species was submitted to the PQB Advisory Subcommittee on Bacteria for review and recommendation. The Advisory Subcommittee on Bacteria unanimously deemed these Wolbachia species to be low risk, and recommended approval of the import request via a letter of authorization. Hawaii Administrative Rules §4-71A-25(b) states: "An unlisted microorganism that is determined by the department to be a low risk microorganism may be allowed import by a letter of authorization issued by the Chief without advisory committee review or board approval."*

DISCUSSION:

1. <u>Persons Responsible</u>:

1) Floyd A. Reed, UHM, 2538 McCarthy Mall, Edmondson Hall 216, Honolulu, Hawaii 96822, (808) 956-6489.

2) Matthew Medeiros, University of Hawaii at Mānoa, 1993 East-West Road, Honolulu, Hawaii 96822 Ph: (808) 956-8187

2. Safeguard Facility and Practices:

Safeguards: All mosquitoes for import have been lab reared for at least three (3) generations. This will mitigate the risks of infections of microorganisms and parasites to the mosquitoes – thus lowering the risk of the mosquitoes accidentally introducing a new parasite or pathogen that may attack other insects to Hawaii. To eliminate the risk of escape during transport, only eggs and/or larvae will be imported. In order for these mosquitoes to acquire and vector a disease, an adult female must blood feed from a disease-infected vertebrate, and the pathogen must survive in the mosquito and be injected into another vertebrate during a subsequent blood feeding. These mosquitoes will be reared from eggs/larvae to adults under laboratory conditions with no opportunity for wild blood feeding. We use commercially available bovine blood (Lampire Biological Laboratories) that has been screened for pathogens prior to shipment. This dramatically minimizes the possibility of infection. We also intend to release only male *C. quinquefasciatu*s into the environment. Male mosquitoes do not feed on blood and, therefore, cannot vector a disease. To achieve this goal, lab reared pupae will be passed through a mesh to screen out the larger female pupae while allowing the smaller male pupae through for collection and field release. Additional mechanisms for sex separation may also be used.

We do plan for the mosquitoes to carry *Wolbachia* bacterium. *Wolbachia* is an obligate endosymbiont and cannot survive outside of the host invertebrate. *Wolbachia* strains already exist in Hawaii in a range of invertebrates in the wild, including mosquitoes (Bennett *et al*. 2012). The presence of *Wolbachia* endosymbionts is the normal state for 40% to 60% of arthropods and does not represent an unusual or pathogenic bacterial infection (Stouthamer *et al*. 1999; Zug and Hammerstein 2012). *Wolbachia* are not capable of infecting human cells.

We will use DNA isolation and sequencing to confirm the identity of the *Wolbachia* strain present in the mosquitoes. We have already used these techniques to identify some strains already present in Hawaii.

In addition to Hawaii's import requirements, the shipper and/or receiver will obtain the following permit: USDA-APHIS-VS permit VS16-6A (Mar 95): US Veterinary Permit for Importation and Transportation of Controlled Materials and Organisms and Vectors. One of our shippers, Michigan State University, has already applied for a USDA-APHIS-VS permit VS16-6A permit.

Facilities: All imported mosquitoes and will be kept at UHM, 2538 McCarthy Mall, Edmondson Hall 401, Honolulu, Hawaii 96822.

A dedicated mosquito 250 ft^2 insectary is located within a locked Biosafety Level 2 (BSL-2 is defined by the CDC, Richmond and McKinney 1999, for work with agents associated with human diseases (i.e., pathogenic or infectious organisms) that pose a moderate health hazard) biological research laboratory within Edmondson

Hall on the UHM campus. This facility is subject to institutional biosafety committee review annually. Edmondson Hall is locked outside of standard business hours and is patrolled by campus security. The research lab is locked at all times, 24 hours a day, and is only accessible by approved individuals that are conducting research in the lab and have completed biosafety training. Within this space is a single door access insectary (ASL-2, Scott 2005) that is prominently posted as restricted access with only the people working directly with the mosquitoes named on the sign (Floyd Reed, Matthew Medeiros, and their lab personnel). This room has an electric "bug zapper" that attracts and kills any possible escaped mosquitoes. Larvae are kept within covered trays, and adult mosquitoes are kept within cages. See Figures 1 - 4.

The cages and trays are [the] primary containment. The insectary room is a secondary level of containment with a door, drop cloth, and electric adult mosquito attractant and killer. The locked lab with no openings (door or window) directly to the outdoors are a third level of containment.

Laboratory floor plan - 401-404 entire, basic

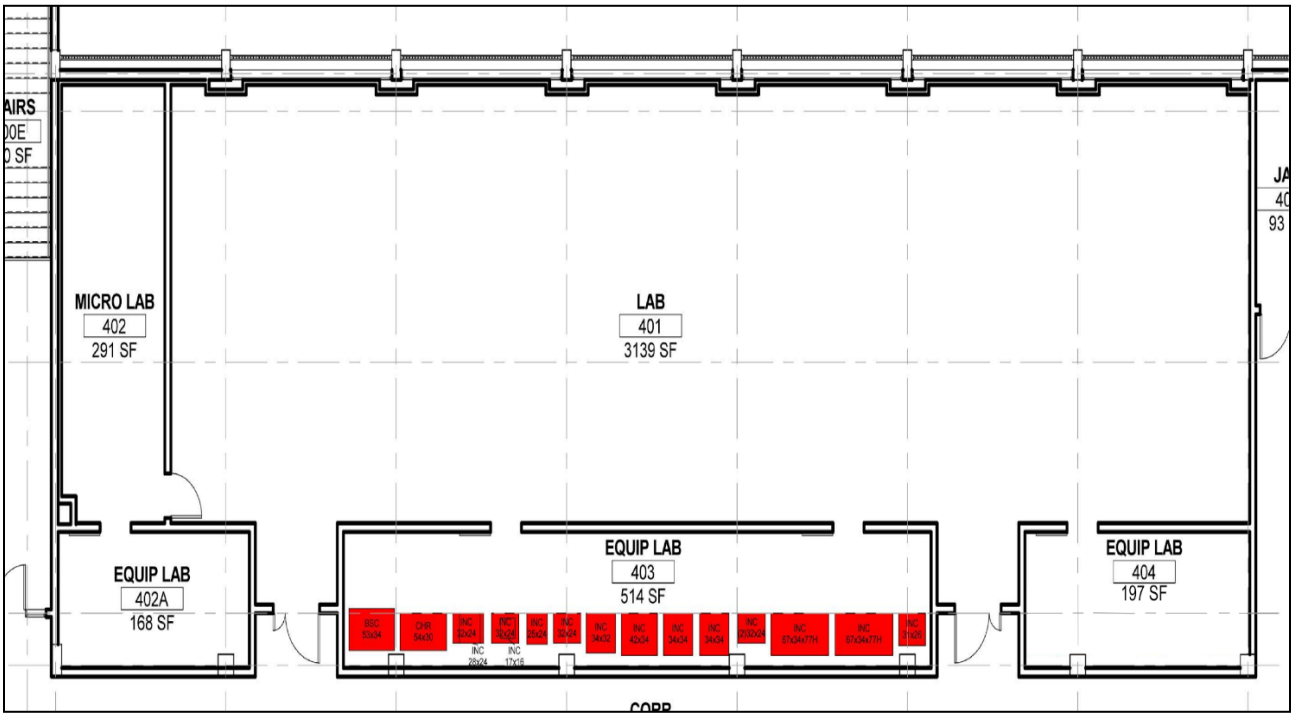


Figure 1: Floor plan (part A) for the UHM laboratory facilities described in this application.

Laboratory floor plan - 401-404, Reed lab space

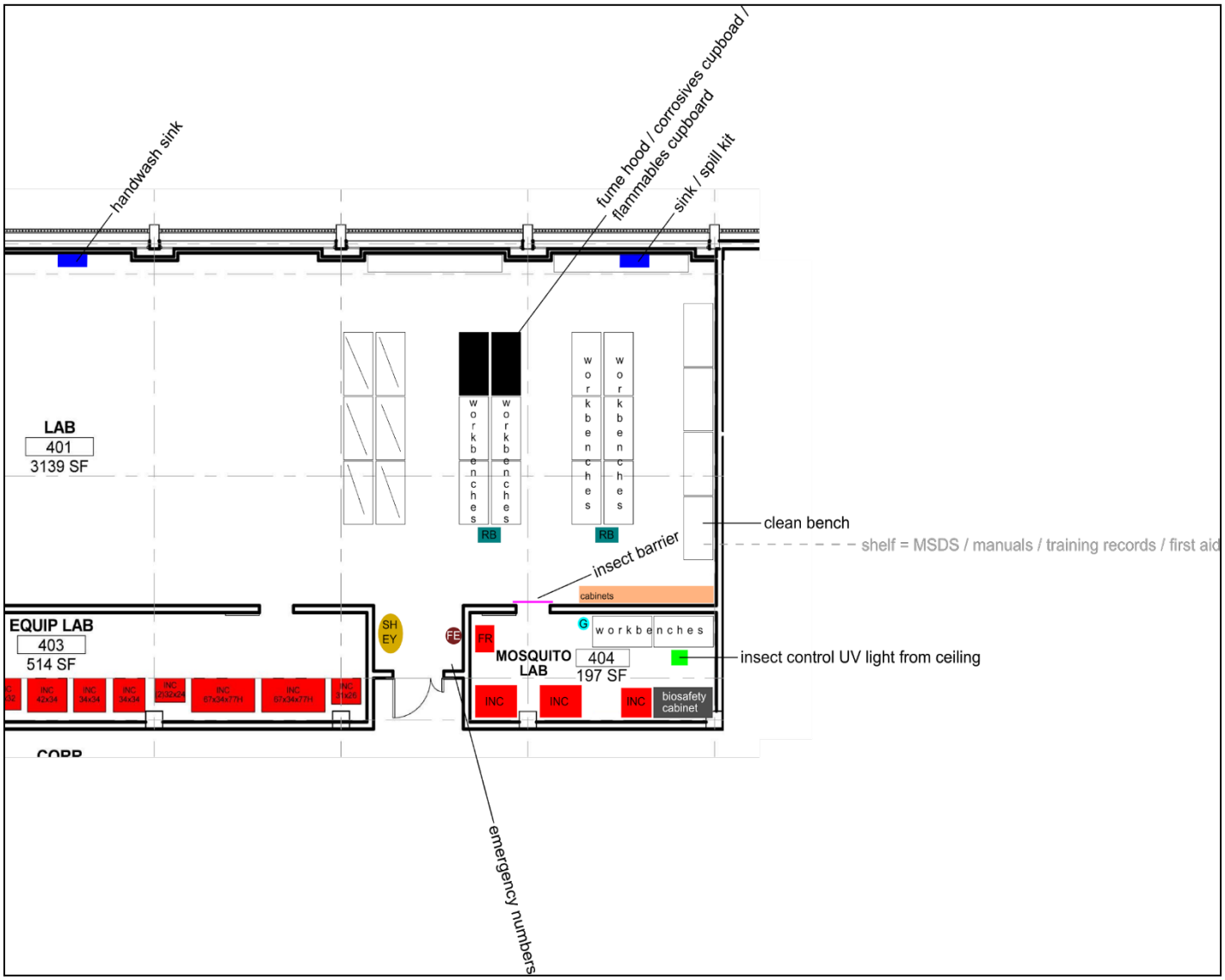


Figure 2: Floor plan (part B) for the UHM laboratory facilities described in this application.

Figure 3. Locked entrance to the 401 lab within Edmondson Hall.

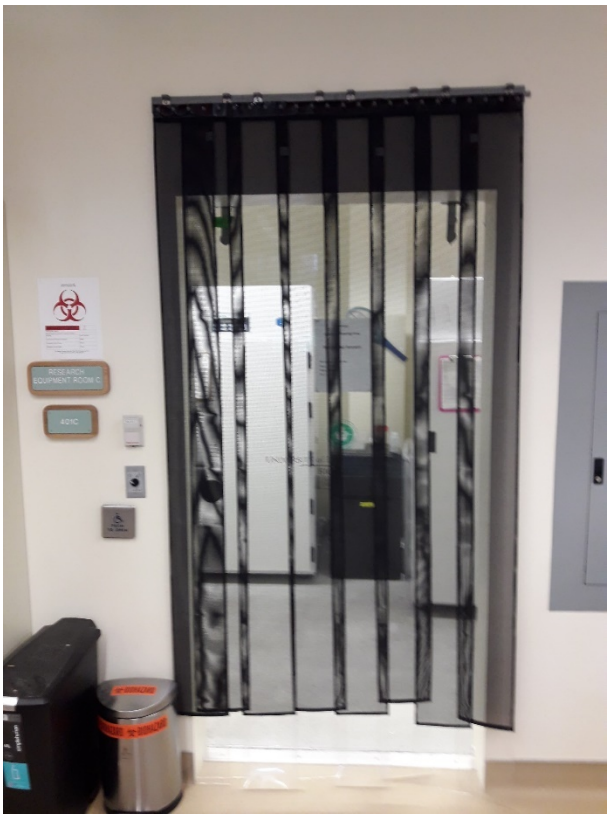


Figure 4. Entrance to the mosquito lab within Edm. 401.
This is a sliding glass door behind a “strip door” curtain.

University of Hawaii Biosafety
The Institutional Biosafety Committee (IBC) was created in accordance with National Institutes of Health (NIH) Guidelines for Research Involving Recombinant DNA Molecules. Additional areas of oversight include federal requirements regulated by the

- NIH Office of Science Policy, now "Program on Biosecurity and Biosafety Policy" (PBBP)
- NIH PBBP Dual Use Research of Concern (DURC)
- CDC/USDA Select Agent Program
- Hawaii State requirements that include Hawaii Department of Agriculture importation regulations.

Additional details can be found at: **https://www.hawaii.edu/researchcompliance/ibc.**

The IBC is comprised of university faculty and staff and representatives from the community. The committee is responsible for reviewing all research and instructional-use activities involving recombinant DNA activity, infectious agents, and toxins. All members are appointed by the vice president for research and serve a three-year term.

Institutional Biosafety Committee Chair: Eric Ako

Designated Institutional Official: Leonard R. Gouveia, Jr.

Members

Member Designation	Number of Members	Area of Expertise
Institutional Member	1	Animal Medicine
Non-institutional Member	1	Animal Medicine
University Scientist	1	Cell Biology
Institutional Member	2	Environmental Issues
University Scientist	1	Genetics
University Scientist	3	Infectious Disease

Non-institutional Member	1	Infectious Disease
University Scientist	1	Microbiology
University Scientist	3	Molecular Biology
University Scientist	1	Plant Pathology
Institutional Member	2	Plant Sciences
Non-institutional Member	1	Plant Sciences
University Scientist	2	Reproductive Biology
Designated NIH IBC Biosafety Representative	1	
Ex Officio	1	

Additional details can be found at: **https://www.hawaii.edu/researchcompliance/ibc.**

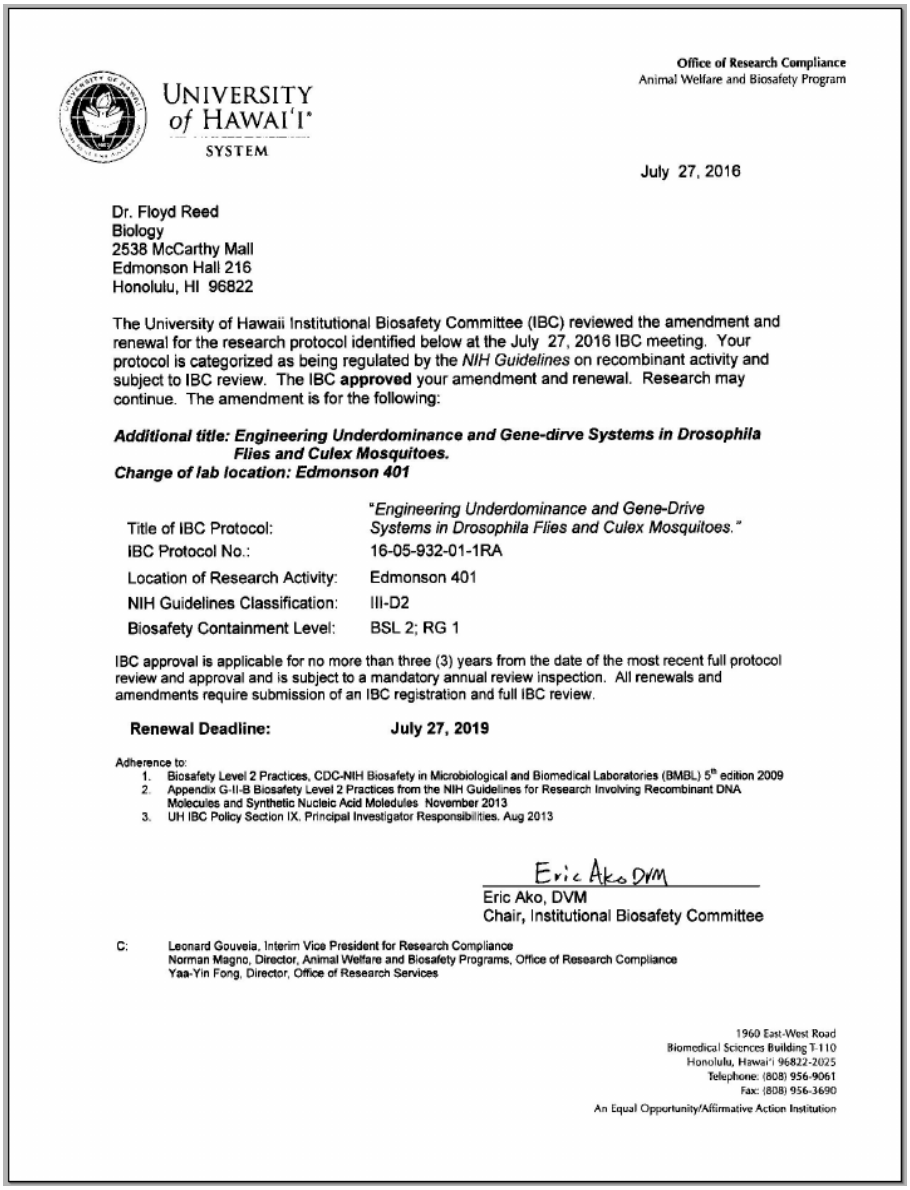
University research involving recombinant DNA must comply with National Institutes of Health (NIH) Guidelines. Failure to comply risks suspension, limitation, or termination of financial assistance of NIH projects or all NIH funding for recombinant DNA research projects (NIH Guidelines Section I-D). Visit the NIH Office of Biotechnology Activities, now PBBP, for more information on these guidelines. Lab inspections for compliance are scheduled on an annual basis.

Observations of non-compliance: Any purported or observed non-compliance will be investigated and documented in an inspection report. Each violation will be evaluated on a case-by-case basis and classified as either “major” or “minor” and subject to the following IBC actions:

Major Violations: The Principle Investigator (PI) will be notified to immediately cease and desist all research activities and a full investigation will be conducted. During such time, grant funding may be withheld. The PI’s Department Chair and Dean will also receive copies of the notification. The IBC will inform all parties when research activates may recommence. Situation details, investigation findings and corrective

actions will be reported to all appropriate agencies no later than 30 days from the initial inspection report.

Minor Violations: First infraction/notice – The PI will receive a copy of the report which serves as the first notification of the violation. The report will contain the recommended corrective action and a deadline for completion. The lab will be required to undergo a follow-up inspection to verify that the violations were corrected. The PI and any lab personnel involved in the non-compliance will also have to complete a re-training session with the Animal Welfare and Biosafety Program (AWBP) Training Specialist. The Reed Laboratory is certified as compliant by the IBC (See Figs. 13-16).



UNIVERSITY of HAWAI'I SYSTEM

Office of Research Compliance
Animal Welfare and Biosafety Program

July 27, 2016

Dr. Floyd Reed
Biology
2538 McCarthy Mall
Edmonson Hall 216
Honolulu, HI 96822

The University of Hawaii Institutional Biosafety Committee (IBC) reviewed the amendment and renewal for the research protocol identified below at the July 27, 2016 IBC meeting. Your protocol is categorized as being regulated by the *NIH Guidelines* on recombinant activity and subject to IBC review. The IBC **approved** your amendment and renewal. Research may continue. The amendment is for the following:

Additional title: Engineering Underdominance and Gene-dirve Systems in Drosophila Flies and Culex Mosquitoes.
Change of lab location: Edmonson 401

Title of IBC Protocol:	*"Engineering Underdominance and Gene-Drive Systems in Drosophila Flies and Culex Mosquitoes."*
IBC Protocol No.:	16-05-932-01-1RA
Location of Research Activity:	Edmonson 401
NIH Guidelines Classification:	III-D2
Biosafety Containment Level:	BSL 2; RG 1

IBC approval is applicable for no more than three (3) years from the date of the most recent full protocol review and approval and is subject to a mandatory annual review inspection. All renewals and amendments require submission of an IBC registration and full IBC review.

Renewal Deadline: **July 27, 2019**

Adherence to:
1. Biosafety Level 2 Practices, CDC-NIH Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th edition 2009
2. Appendix G-II-B Biosafety Level 2 Practices from the NIH Guidelines for Research Involving Recombinant DNA Molecules and Synthetic Nucleic Acid Moledules November 2013
3. UH IBC Policy Section IX. Principal Investigator Responsibilities. Aug 2013

Eric Ako DVM
Eric Ako, DVM
Chair, Institutional Biosafety Committee

C: Leonard Gouveia, Interim Vice President for Research Compliance
Norman Magno, Director, Animal Welfare and Biosafety Programs, Office of Research Compliance
Yaa-Yin Fong, Director, Office of Research Services

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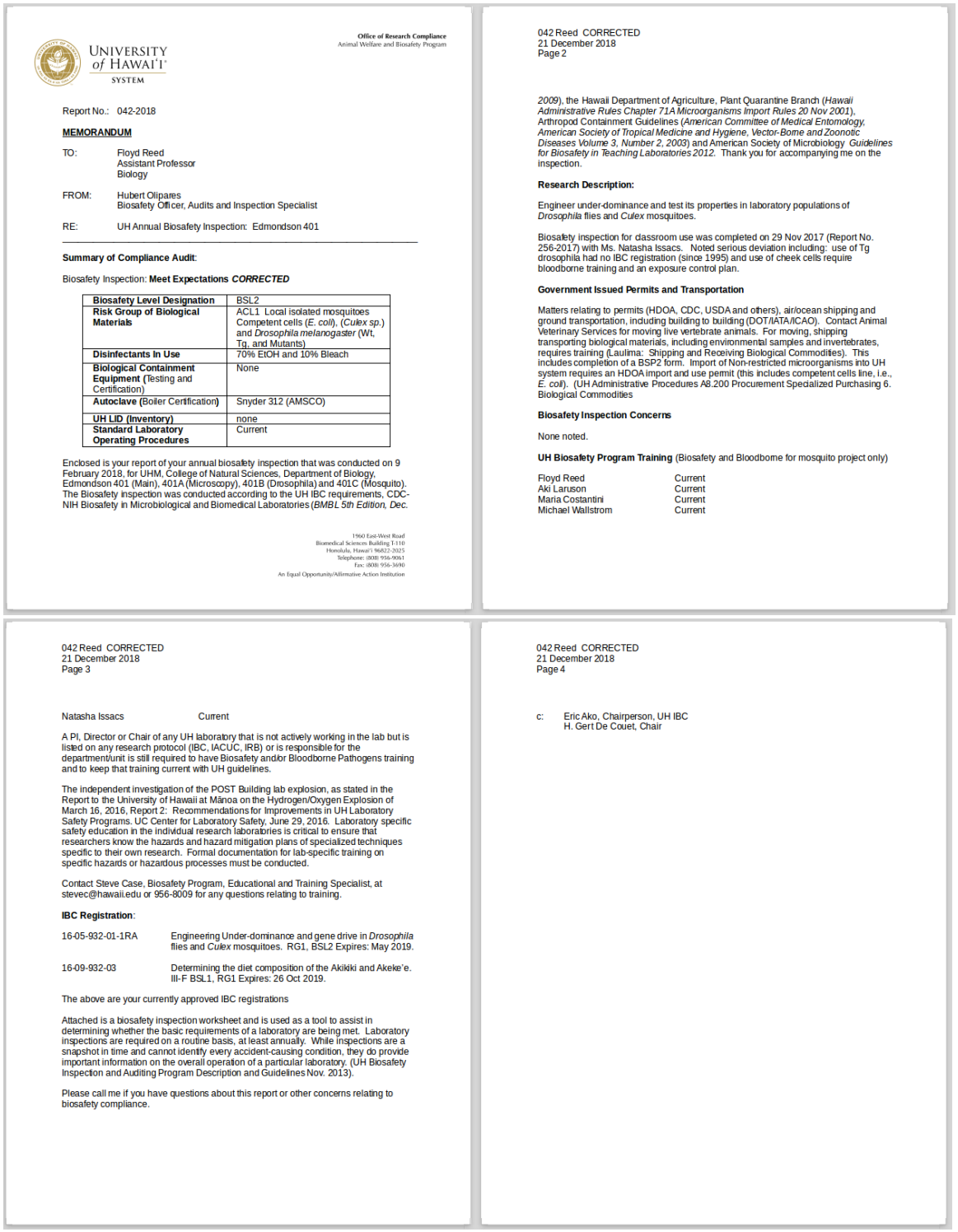
Figure 13: Copy of renewal approval letter for Reed's research to control *C. quinquefasciatus*



UNIVERSITY of HAWAI'I SYSTEM

Office of Research Compliance
Animal Welfare and Biosafety Program

Report No.: 042-2018

MEMORANDUM

TO: Floyd Reed
Assistant Professor
Biology

FROM: Hubert Olipares
Biosafety Officer, Audits and Inspection Specialist

RE: UH Annual Biosafety Inspection: Edmondson 401

Summary of Compliance Audit:

Biosafety Inspection: **Meet Expectations *CORRECTED***

Biosafety Level Designation	BSL2
Risk Group of Biological Materials	ACL1 Local isolated mosquitoes Competent cells (*E. coli*), (*Culex sp.*) and *Drosophila melanogaster* (Wt, Tg, and Mutants)
Disinfectants In Use	70% EtOH and 10% Bleach
Biological Containment Equipment (Testing and Certification)	None
Autoclave (Boiler Certification)	Snyder 312 (AMSCO)
UH LID (Inventory)	none
Standard Laboratory Operating Procedures	Current

Enclosed is your report of your annual biosafety inspection that was conducted on 9 February 2018, for UHM, College of Natural Sciences, Department of Biology, Edmondson 401 (Main), 401A (Microscopy), 401B (Drosophila) and 401C (Mosquito). The Biosafety inspection was conducted according to the UH IBC requirements, CDC-NIH Biosafety in Microbiological and Biomedical Laboratories (*BMBL 5th Edition, Dec.*

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042 Reed CORRECTED
21 December 2018
Page 2

2009), the Hawaii Department of Agriculture, Plant Quarantine Branch (*Hawaii Administrative Rules Chapter 71A Microorganisms Import Rules 20 Nov 2001*), Arthropod Containment Guidelines (*American Committee of Medical Entomology, American Society of Tropical Medicine and Hygiene, Vector-Borne and Zoonotic Diseases Volume 3, Number 2, 2003*) and American Society of Microbiology *Guidelines for Biosafety in Teaching Laboratories 2012*. Thank you for accompanying me on the inspection.

Research Description:

Engineer under-dominance and test its properties in laboratory populations of *Drosophila* flies and *Culex* mosquitoes.

Biosafety inspection for classroom use was completed on 29 Nov 2017 (Report No. 256-2017) with Ms. Natasha Issacs. Noted serious deviation including: use of Tg drosophila had no IBC registration (since 1995) and use of cheek cells require bloodborne training and an exposure control plan.

Government Issued Permits and Transportation

Matters relating to permits (HDOA, CDC, USDA and others), air/ocean shipping and ground transportation, including building to building (DOT/IATA/ICAO). Contact Animal Veterinary Services for moving live vertebrate animals. For moving, shipping transporting biological materials, including environmental samples and invertebrates, requires training (Laulima: Shipping and Receiving Biological Commodities). This includes completion of a BSP2 form. Import of Non-restricted microorganisms into UH system requires an HDOA import and use permit (this includes competent cells line, i.e., *E. coli*). (UH Administrative Procedures A8.200 Procurement Specialized Purchasing 6. Biological Commodities

Biosafety Inspection Concerns

None noted.

UH Biosafety Program Training (Biosafety and Bloodborne for mosquito project only)

Floyd Reed	Current
Aki Laruson	Current
Maria Costantini	Current
Michael Wallstrom	Current

042 Reed CORRECTED
21 December 2018
Page 3

Natasha Issacs Current

A PI, Director or Chair of any UH laboratory that is not actively working in the lab but is listed on any research protocol (IBC, IACUC, IRB) or is responsible for the department/unit is still required to have Biosafety and/or Bloodborne Pathogens training and to keep that training current with UH guidelines.

The independent investigation of the POST Building lab explosion, as stated in the Report to the University of Hawaii at Mānoa on the Hydrogen/Oxygen Explosion of March 16, 2016, Report 2: Recommendations for Improvements in UH Laboratory Safety Programs. UC Center for Laboratory Safety, June 29, 2016. Laboratory specific safety education in the individual research laboratories is critical to ensure that researchers know the hazards and hazard mitigation plans of specialized techniques specific to their own research. Formal documentation for lab-specific training on specific hazards or hazardous processes must be conducted.

Contact Steve Case, Biosafety Program, Educational and Training Specialist, at stevec@hawaii.edu or 956-8009 for any questions relating to training.

IBC Registration:

16-05-932-01-1RA Engineering Under-dominance and gene drive in *Drosophila* flies and *Culex* mosquitoes. RG1, BSL2 Expires: May 2019.

16-09-932-03 Determining the diet composition of the Akikiki and Akeke'e. III-F BSL1, RG1 Expires: 26 Oct 2019.

The above are your currently approved IBC registrations

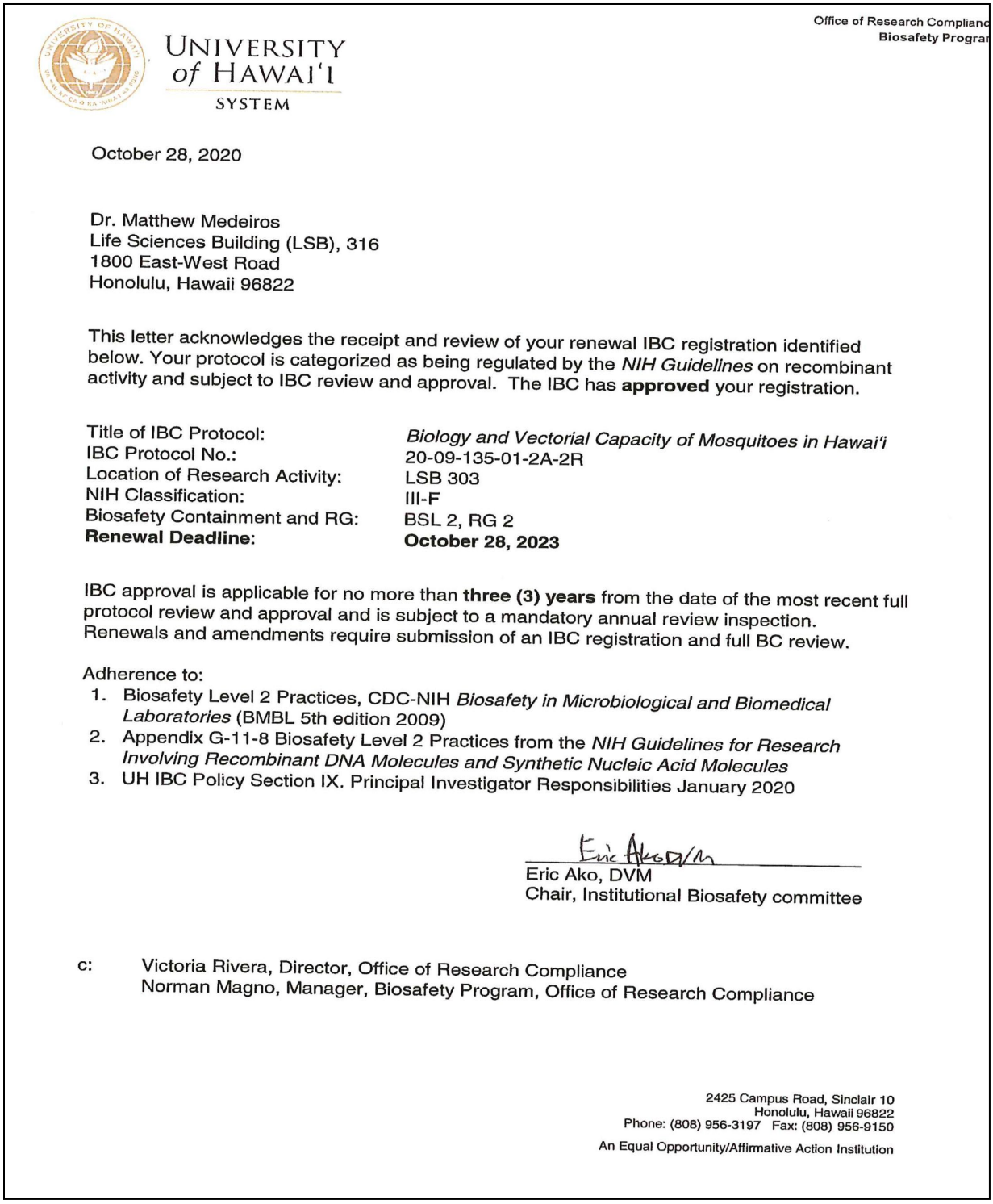
Attached is a biosafety inspection worksheet and is used as a tool to assist in determining whether the basic requirements of a laboratory are being met. Laboratory inspections are required on a routine basis, at least annually. While inspections are a snapshot in time and cannot identify every accident-causing condition, they do provide important information on the overall operation of a particular laboratory. (UH Biosafety Inspection and Auditing Program Description and Guidelines Nov. 2013).

Please call me if you have questions about this report or other concerns relating to biosafety compliance.

042 Reed CORRECTED
21 December 2018
Page 4

c: Eric Ako, Chairperson, UH IBC
H. Gert De Couet, Chair

Figure 14: Copy of most recent inspection approval letter for Reed. Most recent inspection date was 9 February 2018. Approval letter is incorrectly dated 21 December 2018.



UNIVERSITY of HAWAI'I SYSTEM

Office of Research Compliance
Biosafety Program

October 28, 2020

Dr. Matthew Medeiros
Life Sciences Building (LSB), 316
1800 East-West Road
Honolulu, Hawaii 96822

This letter acknowledges the receipt and review of your renewal IBC registration identified below. Your protocol is categorized as being regulated by the *NIH Guidelines* on recombinant activity and subject to IBC review and approval. The IBC has **approved** your registration.

Title of IBC Protocol:	*Biology and Vectorial Capacity of Mosquitoes in Hawai'i*
IBC Protocol No.:	20-09-135-01-2A-2R
Location of Research Activity:	LSB 303
NIH Classification:	III-F
Biosafety Containment and RG:	BSL 2, RG 2
Renewal Deadline:	**October 28, 2023**

IBC approval is applicable for no more than **three (3) years** from the date of the most recent full protocol review and approval and is subject to a mandatory annual review inspection. Renewals and amendments require submission of an IBC registration and full BC review.

Adherence to:
1. Biosafety Level 2 Practices, CDC-NIH *Biosafety in Microbiological and Biomedical Laboratories* (BMBL 5th edition 2009)
2. Appendix G-11-8 Biosafety Level 2 Practices from the *NIH Guidelines for Research Involving Recombinant DNA Molecules and Synthetic Nucleic Acid Molecules*
3. UH IBC Policy Section IX. Principal Investigator Responsibilities January 2020

Eric Ako, DVM
Chair, Institutional Biosafety committee

c: Victoria Rivera, Director, Office of Research Compliance
Norman Magno, Manager, Biosafety Program, Office of Research Compliance

2425 Campus Road, Sinclair 10
Honolulu, Hawaii 96822
Phone: (808) 956-3197 Fax: (808) 956-9150
An Equal Opportunity/Affirmative Action Institution

Figure 16: Copy of renewal approval letter for Medeiros research to control *Culex quinquefasciatus*

3. Method of Disposition:

Mosquitoes are killed by freezing at -20° C for 24 hours, and then all biological waste is autoclaved prior to disposal. Shipping substrate will also be autoclaved prior to disposal.

4. Abstract of the Organism:

C. quinquefasciatus is a sexually reproducing species. Minimum generation times are approximately three weeks. A single fertilized female can lay over 100 eggs (*C. quinquefasciatus* in the form of egg rafts) so there is a tremendous and rapid potential growth rate. Mature adults are up to approximately a centimeter in length and can live for a few months. Mosquito life cycles are well understood for most species, including all those established in Hawaii (e.g., Kauffman *et al*. 2017).

Larvae feed on organic material found in pools of water. Both adult males and females feed on water that contains carbohydrates (water with sap or nectar). Only mature females seek out and feed on vertebrate blood prior to egg laying. *C. quinquefasciatus* in Hawaii appears to prefer avian blood sources but will also feed on mammalian blood including humans. Some species of mosquitoes can be considered generalists and can obtain blood meals from a variety of hosts, including reptiles such as skinks (Mendenhall *et al*. 2012).

C. quinquefasciatus is thought to have originated in Southeast Asia but has spread worldwide in tropical and temperate climates (there is uncertainty about the precise geographic origin of *C. quinquefasciatu*s, which has long been distributed over a broad geographic range (Fonseca *et al.* 2000; 2006). Today, this species is established in Asia, Africa, Europe, North America, South America and Oceania including Hawaii. Specifically, in Hawaii, this species is widespread on all the main islands.

The optimal temperature range for development and growth of this species is approximately 20° C to 29° C (Rueda *et al*.1990; Delatte *et al*. 2009). This species does not tolerate temperatures above 35° C and can withstand limited exposure to temperatures near freezing (eggs are more tolerant of freezing than the adults).

This species relies on pools of still water with organic material for the growth of larvae. Only adult females bite, as they require blood meals from vertebrate hosts to develop their eggs (e.g., Kamgang *et al.* 2012; Takken and Verhulst 2013).

This species has the potential to vector important human pathogens (Gratz 2004; Effler *et al*. 2005; Arensburger *et al.* 2010; Bonizzoni *et al*. 2013). It is also the primary vector of avian malaria and avian pox which are major factors in the

decline and extinction of many Hawaiian forest bird species (Warner 1968; van Riper *et al*. 1986; Atkinson *et al*. 1995)

5. Potential Impact to the Environment:

C. quinquefasciatus is already widely established in the wild on all of the main islands in Hawaii. An additional five other "biting" non-native mosquito species have also become established: *Aedes. albopictus* (Asian tiger mosquito) *Ae. aegypti* (Yellow fever mosquito), *Ae. japonicus* (Rock pool mosquito), *Wyeomyia mitchellii* (Bromeliad mosquito), and *Ae. vexans* (Inland floodwater mosquito*).*

C. quinquefasciatus also carries a strain of *Wolbachia* in the wild here in Hawaii. *Wolbachia* are not infectious to humans and are vertically transmitted through the eggs from one generation to another (Werren *et al*. 2008). The *Wolbachia* bacteria are obligate endosymbionts and can only survive inside the insect host's cytoplasm. A mosquito transinfected with a different strain of *Wolbachia* that results in cytoplasmic incompatibility (e.g., *C. quinquefasciatus* carrying the *Ae. albopictus* strain *Wolbachia*) would not be able to successfully reproduce with a wild mosquito. Therefore, if individual mosquitoes did become temporarily established, then they will quickly die off over the following generations because of cytoplasmic incompatibility with wild mosquitoes of the same species (with which they would be expected to encounter and mate).

If both sexes of transinfected mosquito were to be accidentally released, they are unlikely to maintain a breeding population of a transinfected mosquito. *Wolbachia* invasions into populations require a critical threshold frequency of infection that needs to be overcome before a novel *Wolbachia* infection can spread into a population. The *Wolbachia* infection rate must exceed 20-45% before it can spread/establish (Hoffmann *et al.* 2011; Barton and Turelli 2011; Jiggins 2017). This is evident in large scale releases such as in Cairns Australia, where millions of transinfected mosquitoes (both sexes) with *Wolbachia* are released into the environment to control disease transmission, yet they do not easily reach fixation in the wild. If transinfected mosquitoes were to become established, the establishment is likely to be spatially localized due to incompatibility with neighboring mosquito populations.

Potential impacts of this introduction

Pros: Importation of *C. quinquefasciatus* will allow the study of *Wolbachia*-based control strategies for mosquitoes that are widespread in Hawaii and which have negative impacts to humans, wildlife, and pets. This research could be a valuable future resource for mosquito management applications, including preventing the extinction of native forest birds and preventing human disease outbreaks. This would have a wide range of positive effects on human health, conservation, the economy, and tourism in Hawaii.

Cons: It is hard to imagine any negative effects since the species is already established in Hawaii. Importing these organisms will not have any foreseeable beneficial effect to the organisms already in Hawaii. The introduction of, for example, increased genetic variation within the mosquito species will be minimized by crossing the lines to mosquitoes originating from Hawaii.

Potential environmental, economic and societal impacts of pathogens, parasites or other contaminants that may accompany this introduction

The presence of unintended accompanying microbiota is minimized by the sterile laboratory-rearing conditions used. These mosquitoes have been maintained for many generations in the lab environment and have not had the opportunity to obtain pathogens from the wild from blood feeding. The presence of intended microbiota, the *Wolbachia*, potentially has very positive effects on the environment—via population suppression of mosquitoes that vector avian pathogens—societal—the suppression of human disease vectored by mosquitoes—and economic—potential increased tourism and lessened disease burden—effects.

Potential for this organism to become established in Hawaii should it escape confinement

This mosquito species is already well-established in Hawaii, as are many different strains of *Wolbachia*. Because of cytoplasmic incompatibility, the escape of mosquitoes carrying a new *Wolbachia* strain is not expected to be stable over the following generations. Outcrossing to locally established mosquitoes will result in cytoplasmic incompatibility and the failure of offspring to develop. There is an extensive body of literature surrounding this mosquito species, its impact upon Hawaii, and *Wolbachia*-mediated cytoplasmic incompatibility. This is not meant to be an exhaustive literature review, rather this provides a broad foundation of relevant points.

6. References:

General information about *C. quinquefasciatus*:

Arensburger, P., Megy, K., Waterhouse, R. M., Abrudan, J., Amedeo, P., Antelo, B., ... & Campbell, C. L. (2010). Sequencing of *C. quinquefasciatus* establishes a platform for mosquito comparative genomics. *Science*, *330*(6000), 86-88.

Rueda, L. M., Patel, K. J., Axtell, R. C., & Stinner, R. E. (1990). Temperature-dependent development and survival rates of *C. quinquefasciatus* and *Aedes aegypti* (Diptera: Culicidae). *Journal of Medical Entomology*, *27*(5), 892-898.

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Warner, R. E. (1968). The role of introduced diseases in the extinction of the endemic Hawaiian avifauna. *The Condor*, *70*(2), 101-120.

Information about *Wolbachia,* with a focus on cytoplasmic incompatibility and the *C.* mosquito species discussed in this permit:

Atyame, C. M., Cattel, J., Lebon, C., Flores, O., Dehecq, J.-S., Weill, M., Gouagna, L. C. & Tortosa, P. (2015) Wolbachia-based population control strategy targeting *C. quinquefasciatus* mosquitoes proves efficient under semi-field conditions. *PLoS ONE* 10, e0119288.

Atyame, C. M., Labbé, P., Lebon, C., Weill, M., Moretti, R., Marini, F., Gouagna, L. C., Calvitti, M. & Tortosa, P. (2016) Comparison of irradiation and Wolbachia based approaches for sterile-male strategies targeting *Aedes albopictus*. *PLoS ONE* 11, e0146834.

Barton, N. H., & Turelli, M. (2011). Spatial waves of advance with bistable dynamics: cytoplasmic and genetic analogues of Allee effects. The American Naturalist, 178(3), E48-E75.

Blagrove, M. S., Arias-Goeta, C., Failloux, A. B., & Sinkins, S. P. (2012). Wolbachia strain wMel induces cytoplasmic incompatibility and blocks dengue transmission in *Aedes albopictus*. *Proceedings of the National Academy of Sciences*, *109*(1), 255-260.

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Stouthamer, R., Breeuwer, J. A., & Hurst, G. D. (1999). *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annual Reviews in Microbiology*, *53*(1), 71-102.

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Zug, R., & Hammerstein, P. (2012). Still a host of hosts for Wolbachia: analysis of recent data suggests that 40% of terrestrial arthropod species are infected. *PLoS ONE*, *7*(6), e38544.

Wolbachia in Hawaii:

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Anonymous. (2017) To Restore a Mosquito-Free Hawaii. Summary Report of the Workshop to Formulate Strategic Solutions for a "Mosquito-Free Hawaii". Available at: http://www.cpcfoundation.org/uploads/7/6/2/6/76260637/report_on_mosquito_free_workshop.pdf.

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III. Environmental Assessment (EA):

Pursuant to a May 2008 Hawai'i Intermediate Court of Appeals decision ('Ohana Pale Ke Ao v. Board of Agriculture, 118 Haw. 247 (Haw. App. 2008), the Department of Agriculture's (Department's) import permit process is subject to the requirements of the Hawai'i Environmental Protection Act, Chapter 343, Hawai'i Revised Statutes (HRS). Under this decision, the requirement for an EA as a condition of the import permit or related authorization applies in those circumstances where the underlying permit activity for the importation initiates a "program or project" and where the use of state or county funds or state or county lands is involved. When those circumstances are present, as they appear to be when a new organism is used in a new program or project located at a facility located at UHM or UHH (state lands), an EA is required to determine whether the proposed project or program is likely to have a significant impact on the environment. However, certain activities may be eligible for "exemption" under provisions established through the Environmental Council, State Office of

Environmental Quality Control (OEQC), provided that the project or program is determined to have little or no impact on the environment.

Exemption from EA: In September 2008, the Department obtained the concurrence of OEQC's Environmental Council for exemption from EA for those Plant Quarantine Branch import permits and related authorizations that satisfy certain criteria, including conditions to minimize risk to agriculture, horticulture, the environment, or animal or public health. The exemption from EA for animals applies to the import of animals for various purposes according to their placement on lists maintained by the Board of Agriculture (Board) and subject to permit conditions appropriate to eliminate or minimize risks associated with the animals and their use. (See Exemption Class #10., item 3.f. of the Department's exemptions, under the links for exemptions for state agencies at: http://oeqc2.doh.Hawaii.gov/Agency_Exemption_Lists/Forms/AllItems.aspx). Permit conditions address matters such as health requirements, special precautions, and safeguarding from escape, theft or release. Under the exemption, purposes for importation of animals include, but are not limited to, direct sales as food or for aquaculture production for food, fish, feed, pet trade (tropical fish); for animal import for purposes such as scientific research by qualified entities and universities in standard research settings; municipal zoo or aquarium exhibition; captive breeding programs by qualified entities; animal feed (mealworms and crickets); pet trade or individual possession. The exemptions from EA are only applicable when a project or program will probably have minimal or no significant effect on the environment. Under OEQC's rules and the Departments' exemption list, exemptions are inapplicable when the cumulative impact of planned successive actions in the same place, over time, is

significant, or when an action that is normally insignificant in its impact on the environment may be significant in a particularly sensitive environment.

PQB Process for Exemption from EA: When seeking an exemption from EA for an import and release request that requires the full Board review process, the Department must obtain the advice of other outside agencies or individuals having jurisdiction or expertise as to the propriety of the exemption. (Section 11-200-8(a), HAR) The Board review process already includes recommendations and comments from the technical consultants (Advisory Subcommittee Members) and the Advisory Committee on Plants and Animals (Advisory Committee). The representation of outside agencies such as the Hawai‘i Department of Land and Natural Resources, University of Hawai‘i, and Hawai‘i Department of Health, OEQC, on the Advisory Committee provide opportunities for these agencies' input on the public health and environmental aspects of the import and appears to meet the consultation requirement of OEQC's rule. In addition, the input received from the Department's technical consultants on the Advisory Subcommittees, as individuals with expertise on the subject matter and the presence of individuals from the Hawai‘i Department of Land and Natural Resources, University of Hawai‘i, and the Honolulu Zoo (retired director) on the Advisory Committee appears to meet the consultation requirement.

Where the recommendations from the technical consultants and Advisory Committee support exemption from an EA, the Department may prepare a declaration of exemption, which includes a description of the import request, lists of consultants, consultants' recommendations and comments, and the basis for the Department's determination of "probably minimal or no significant effect on the environment." The declaration of exemption from EA is submitted to the Board together with the import request. Where the recommendations from the technical consultants and Advisory Committee support an EA, the Department may require an EA as a prerequisite for Board review.

Analysis of Application re EA: Under the above-cited court decision, the EA requirement is triggered under certain circumstances, including when an applicant proposes an action on state lands that requires agency approval and is not specifically exempted under Chapter 343, HRS. That is the case here. The applicant's request in this instance involves importation of the southern house mosquito, *Culex quinquefasciatus,* for laboratory, field-release, and area-wide mosquito suppression research based at UHM, i.e., on state lands; therefore, agency approval is required for the applicant's proposed action/activity on state lands. As PQB understands the court's analysis in the 'Ohana Pale decision, the activity proposed under this permit application would initiate a project that uses state lands, initially triggering the EA requirement. However, the project may be able to qualify for exemption from EA under an applicable Department exemption. This analysis will continue below the discussion on Advisory Subcommittee review.

IV. Advisory Subcommittee Review:

This request was submitted to the Advisory Subcommittee on Entomology for their review and recommendations. Their recommendations and comments are as follows:

1. I recommend Approval___/___Disapproval of a finding that the establishment of the Southern House Mosquito, *C. quinquefasciatus*, a vector of Avian Influenza, in Hawaii would constitute an ecological disaster.

Dr. Jesse Eiben: Recommends approval.

Comments: "The mosquito is already present in Hawaii, and is an ecological disaster due to being a vector of disease. Adding more *C. quinquifasciatus* to control the existing same species is a viable method to limit the current ecological disaster."

Dr. Peter Follett: Recommends approval.

Comments: "This mosquito species was introduced many years ago and is having a serious impact of native birds through the transmission of avian influenza."

Dr. Mark Wright: Recommends approval.

Comments: "*C. quinquefasciatus* is widespread in Hawaii from previous accidental introductions."

Dr. Daniel Rubinoff: Recommends approval.

Comments: "This is a very important project and it should be advanced as quickly as possible. My only question, repeated throughout this evaluation, is that the mosquitoes brought to Hawaii were originally from Hawaii, such that they do not bring in genetic diversity to Hawaiian mosquitoes that might have negative consequences. If other mosquitoes will be brought in, not originally from Hawaiian stock, perhaps the researchers could address why that wouldn't be a concern. But to be clear, this is critical research and it should be supported as efficaciously as possible."

> **Applicant's Response:** "With a replaced Wolbachia strain that results in cytoplasmic incompatibility the actual fitness is predicted to be frequency dependent. If imported mosquitoes with strain A (inoculated imported *Wolbachia*) were to escape, and were rarer than the mosquitoes carrying strain B (*Wolbachia* strains already established in Hawaii), which is virtually certain, then they are predicted to quickly be removed from the wild because their offspring would die off due to cytoplasmic incompatibility. As far as relative fitness in isolation (just A and just B) it is hard to know how to test this because it might depend on the lab conditions used; however, there is no a prioi reason to expect mosquitoes with a different strain of *Wolbachia* to be more fit than the ones in the wild."

Ms. Janis Matsunaga: Recommends approval.

Comments: "Research shows clear data that *Culex quinquefasciatus*-vectored avian diseases are primary contributors to endemic Hawaiian honeycreeper declines and extinctions. As these endemic birds' ecological ranges are shrinking, invasive mosquitoes' ranges are expanding and rising in elevation with warming temperatures. According to DLNR Chairperson Suzanne Case's letter of support, 'Five honeycreeper species [of only 21 species of extant forest birds] are likely to lose all or most of their range and become extinct by 2100 due primarily to avian malaria.'

The arrival of *C. quinquefasciatus* and the zoonotic diseases which they vector, the decimation of native forest habitat for Hawaiian honeycreepers, and the expansion of breeding habitat for *C. quinquefasciatus* into upper elevation forests are directly linked to the actions of humans.

This constitutes an ecological disaster to the remaining native Hawaiian forests and ecosystems for the reasons stated by Chair Case, 'These native birds serve critical ecological functions in our forests as pollinators and seed dispersers for the shrubs and trees that comprise our life-giving watersheds.' "

2. I recommend Approval___/___Disapproval to allow the importation of the southern house mosquito, *C. quinquefasciatus*, an unlisted insect, inoculated with foreign *Wolbachia* bacteria, for field-release, and area-wide mosquito suppression research, by the University of Hawaii at Mānoa.

Dr. Jessie Eiben: Recommends approval.

Dr. Peter Follett: Recommends approval.

Comments: "I approve of the petitioners' approach to use *Wolbachia* and cytoplasmic incompatibility to reduce the impact of this mosquito."

Dr. Mark Wright: Recommends approval.

Comments: "This action will create very few risks for any negative impacts in Hawaii, and has the potential to provide an effective and environmentally safe method for suppression of invasive *C. quiquefasciatus* in Hawaii."

Dr. Daniel Rubinoff: Recommends approval.

Comments: "My only concern/question is the source of the imported mosquitoes. Could they add genetic diversity to the existing populations in Hawaii with undesirable traits like cold-hardiness? If the source of all mosquitoes to be imported is Hawaii (and they were sent to Kentucky, infected, and returned) then this is a non-issue. But if the

mosquitoes are being imported from other regions and have developed for example, increased cold tolerance, that would not be a trait we want brought to Hawaii."

> **Applicant's Response:** "With a replaced Wolbachia strain that results in cytoplasmic incompatibility the actual fitness is predicted to be frequency dependent. If imported mosquitoes with strain A (inoculated imported *Wolbachia*) were to escape, and were rarer than the mosquitoes carrying strain B (*Wolbachia* strains already established in Hawaii), which is virtually certain, then they are predicted to quickly be removed from the wild because their offspring would die off due to cytoplasmic incompatibility. As far as relative fitness in isolation (just A and just B) it is hard to know how to test this because it might depend on the lab conditions used; however, there is no a prioi reason to expect mosquitoes with a different strain of *Wolbachia* to be more fit than the ones in the wild."

Ms. Janis Matsunaga: Recommends approval.

Comments: "Approval to allow the importation of *C. quinquefasciatus* with consideration to the following:

1. Subject (2) 'A*llow the Importation of the Southern House Mosquito, Culex quinquefasciatus, an Unlisted Insect, Inoculated with a Foreign Wolbachia Bacteria Species, by Special Permit, for Laboratory, Field-Release, and Area-Wide Mosquito Suppression Research, by the University of Hawaii at Mānoa*;' should be separated into clear subjects/parts.

   For example:

   a. Allow the Importation of the Southern House Mosquito, *Culex quinquefasciatus*, an Unlisted Insect, Inoculated with a Foreign *Wolbachia* Bacteria Species, by Special Permit, for Laboratory [and Area-Wide Mosquito Suppression?] Research by the University of Hawaii at Mānoa.

   b. Allow the Field-Release of the Southern House Mosquito, *Culex quinquefasciatus*, an Unlisted Insect, Inoculated with a Foreign *Wolbachia* Bacteria Species, by Special Permit, [for Area-Wide Mosquito Suppression Research].

      i. 'Area-wide mosquito suppression research' is not clearly defined anywhere in this document so I am unclear on what this means and how it relates to what we are recommending for approval. Is this part of the field-release research (Field-release and subsequent area-wide mosquito suppression research)? Should this be part of 'post-release monitoring and research'?

This is a combined application for both the import and release. I believe the permits should be separated between import and release. This is a multi-stage project and different steps and information are to be considered for each distinct action. As the applicants may need to obtain EAs and other applicable permits (including the possibility of release permits) from other agencies, I do not agree to lump everything together 'as is'."

2. *Safeguard Facility and Practices:*
   Safeguards: We will use DNA isolation and sequencing to confirm the identity of the Wolbachia strain present in the mosquitoes.

"When will this be done? Upon import only? Or upon import and prior to release?"

PQB Response: The two types of research mentioned (laboratory research and field release research) were not separated in order to make this submittal more succinct.

"Area-wide mosquito suppression research" is part of field release research.

Locally collected mosquitoes have been collected under USDA-Animal Plant and Health Inspection Service (APHIS)-Veterinary Services (VS) collection permit 16-3. Permit Condition No. 22 requires that the applicants be compliant with all federal, state, and county requirements regarding the research and release of mosquitoes under this project – including obtaining any applicable permits. Penalties for non-compliance are mentioned in Permit Conditions Nos. 24 & 25, including immediate cancelation of permit(s), devitalization of all mosquitoes, progeny, and imported *Wolbachia* strains, and possible citations.

Question No. 6: "Are the proposed permit conditions sufficient to assure the requested species, southern house mosquito, *C. quinquefasciatus*, an unlisted insect, inoculated with foreign *Wolbachia* bacteria, presents probably minimal or no significant effects on the environment?" has been asked to HDOA's Subcommittee on Entomology to determine whether an EA exemption for this project should be granted.

To address Ms. Matsunaga's concern about genetic testing occurring upon arrival of the mosquitoes to Hawaii and just prior to field release of the mosquitoes, PQB has created Permit Condition No. 13.

3. I recommend Approval___/___Disapproval to allow the release of the southern house mosquito, *C. quinquefasciatus*, an unlisted insect, inoculated with foreign *Wolbachia* bacteria, for laboratory, field-release,

and area-wide mosquito suppression research, by the University of Hawaii at Mānoa.

Dr. Jessie Eiben: Recommends approval.

Comments: "An ex-situ area-wide and in-situ lab colony population genetic study of the mosquito should be continuous and ongoing prior to new mosquito release to verify any new mosquitoes are of a variety not new to Hawaii. I recommend not releasing new genetics of mosquitoes to Hawaii. However, due to the control tactics employed here, and the varieties of Culex world-wide, even some new genetics will not constitute too

great a risk. Once the incompatibility of the Wolbachia strain is verified, I approve field release with subsequent monitoring."

Dr. Peter Follett: Recommends approval.

Comments: "Yes, I approve based on what is presented in this petition, as long as Carter Atkinson and Dennis Lapointe have also provided input and approve of the approach as presented in this petition."

PQB Response: This submittal has been given to Drs. Atkinson and Lapointe for their review and comments. As of this meeting, Dr. Atkinson has responded. His response is below:

"I've reviewed the documents that were attached to your email and found them to be technically accurate and well-written. I fully support the request for a permit to import *Culex quinquefasciatus* mosquitoes that have been inoculated with atypical strains of *Wolbachia*. I agree that release of these mosquitoes will be of little risk to native biota and will not pose a threat to Hawaiian ecosystems or human or animal health. In addition, I believe that the applicants have suitable containment procedures and secure facilities for handling imported Culex and are well-qualified for the proposed research."

Dr. Mark Wright: Recommends approval.

Comments: "I believe this action has essentially zero risks for negative consequences, and a high probability of providing a new option for suppression of an invasive mosquito species."

Dr. Daniel Rubinoff: Recommends approval.

Ms. Janis Matsunaga: Recommends approval.

Comments: "Approval to allow the release of *C. quinquefasciatus* with consideration to the following:

1. Subject (2) '*Allow the Importation of the Southern House Mosquito, Culex quinquefasciatus, an Unlisted Insect, Inoculated with a Foreign Wolbachia Bacteria Species, by Special Permit, for Laboratory, Field-Release, and Area-Wide Mosquito Suppression Research, by the University of Hawaii at Mānoa*;' should be separated into clear subjects/parts.

This is a combined application for both the import and release,. I believe the permits should be separated between import and release. This is a multi-stage project and different steps and information are to be considered for each distinct action. As the applicants may need to obtain EAs and other applicable permits (including the possibility of release permits) from other agencies, I do not agree to lump everything together 'as is'.

For example:

a. Allow the Importation of the Southern House Mosquito, *Culex quinquefasciatus*, an Unlisted Insect, Inoculated with a Foreign *Wolbachia* Bacteria Species, by Special Permit, for Laboratory [and Area-Wide Mosquito Suppression?] Research by the University of Hawaii at Mānoa.

b. Allow the Field-Release of the Southern House Mosquito, *Culex quinquefasciatus*, an Unlisted Insect, Inoculated with a Foreign *Wolbachia* Bacteria Species, by Special Permit, [for Area-Wide Mosquito Suppression Research].

2. Subject (4) addresses 'Determine the Probable Impact on the Environment if the Southern House Mosquito, *Culex quinquefasciatus*, an Unlisted Insect, Inoculated with a Foreign *Wolbachia* Bacteria Species, are **Accidently Released**;' but no subject clearly addresses 'purposefully field-released *C. quinquefasciatus*'.

Regarding release:
There should be clear sections on this submittal and separate permits because most information provided here addresses importation and lab research but not the field release of *C. quinquefasciatus*. There is no information explaining what the procedure or plan is for release of this insect. There is no explanation on where the applicants plan on releasing this organism, how they plan on releasing, when, etc.

There is just one line in the procedure regarding '*The imported mosquitos[sic] are intended for release (only males intended for release) to mitigate this disaster.*'

- Are release permits from USDA required?

- At what point will the first releases be made?

- What will the quality control standards be to determine that release stock is not contaminated prior to release?

If there is no clear release procedure or plan as of now, perhaps there should be a permit condition to include a pre-release report and SOP submitted to PQB (and approved) prior to initial release."

PQB Response: The two types of research mentioned (laboratory research and field release research) were not separated in order to make this submittal more succinct.

In response to Entomology Subcommittee Member Matsunaga's concerns about the lack of permit conditions for field release of mosquitoes, PQB has created Permit Conditions Nos. 13, 14, 15, and 16. These permit conditions should satisfy prerelease concerns about the mosquitoes. Permit Condition No. 17 addresses post-release monitoring.

The applicants will be responsible for obtaining any other permits as a requirement for issuance of an import and possession permit from PQB.

Field release of the mosquitoes will be determined by the results of laboratory research, submission of a PQB approved field release plan (as required by Permit Conditions Nos. 14 and 15), and how quickly the applicants will rear a population of Wolbachia inoculated mosquitoes sufficient enough to release.

To confirm mosquitoes reared for field release are infected with the inoculated Wolbachia bacteria, genetic testing will be required prior to field release (Permit Condition No. 13).

4. I recommend Approval___/___Disapproval to establish permit conditions for the importation of the southern house mosquito, *C. quinquefasciatus*, an unlisted insect, inoculated with foreign *Wolbachia* bacteria, for laboratory, field-release, and area-wide mosquito suppression research, by the University of Hawaii at Mānoa.

Dr. Jessie Eiben: Recommends approval.

Dr. Peter Follett: Recommends approval.

Comments: "Yes, I approve. But please also get approval from the experts in this field, Carter Atkinson and Dennis Lapointe, who have studied this system in the field for many years."

PQB Response: This submittal has been given to Drs. Atkinson and Lapointe for their review and comments. As of this meeting, Dr. Atkinson has responded and is supportive of this project. Although he did not comment on the permit conditions, they were included in the draft he reviewed.

Dr. Mark Wright: Recommends approval.

Dr. Daniel Rubinoff: Recommends approval.

Ms. Janis Matsunaga: Recommends approval.

5. If the requested species, southern house mosquito, *C. quinquefasciatus*, an unlisted insect, inoculated with foreign *Wolbachia* bacteria, is accidentally released, what is the probable impact on the environment?

___ minimal or no significant effects on the environment.
___ other (if "other", please explain).

Dr. Jessie Eiben: Minimal or no significant effects on the environment.

Comments: "Minimal accidental release does not create a sufficient influx of new genetics, Wolbachia strains, or quantity of mosquitoes to warrant a major impact on the environment."

Dr. Peter Follett: Minimal or no significant effects on the environment.

Comments: "The probable impact is beneficial. This is a[n] alien mosquito species wreaking havoc on native bird fauna. Genetic techniques to reduce its impact are appropriate."

Dr. Mark Wright: Minimal or no significant effects on the environment.

Comments: "It is very unlikely that negative impacts will arise from the accidental release of this mosquito, already broadly established in Hawaii."

Dr. Daniel Rubinoff: Minimal or no significant effects on the environment.

Comments: "Given the caveat that they are from mosquitoes sent from Hawaii originally, there would seem to be essentially no risk whatsoever."

Ms. Janis Matsunaga: Minimal or no significant effects on the environment.

Comments: "Why is this question asked only if *C. quinquefasciatus* is accidentally released and it is not asked for when this organism is purposefully released when submittal Subject (2) includes both the import AND release of this insect? Why ask this

question for the accidental release when the purposeful release permit has been lumped with the importation ….'for field-release research'? This is very confusing.

Question to the applicants:
Have there been any further sampling/studies of strains naturally occurring in HI following Atkinson et al. (2016)'s fieldwork? The authors state that sampling was minimal and should be expanded. Is there a more recent publication?"

PQB Response: This is a standard question the PQB asks permit applicants when they intend to import regulated articles for research. Entomology Subcommittee members are asked to estimate risk in the event the organism escapes or is accidentally released into the environment.

This question is also posed to Subcommittee members to determine whether an EA exemption can be issued by the PQB for the research and/or field release of the mosquitoes, as is the case in this instance.

6. Are the proposed permit conditions sufficient to assure the requested species, southern house mosquito, *C. quinquefasciatus*, an unlisted insect, inoculated with foreign *Wolbachia* bacteria, presents probably minimal or no significant effects on the environment?

___ Yes

___ No (If "No", please explain and suggest appropriate conditions).

Dr. Jessie Eiben: Yes.

Comments: "The facility listed and detailed is sufficient to maintain a colony of these mosquitoes in situ, until a time when regulated release may be allowed. I recommend a 2nd 2-door zipper screen-mesh vestibule to be added and affixed to the mosquito room for an additional protocol. But I think the current proposal is minimally sufficient."

Dr. Peter Follett: Yes.

Dr. Mark Wright: Yes.

Comments: "The applicants have submitted procedures and plans that should ensure that *C. quiquefasciatus* infected with Wolbachia are well-contained and do not present risks for negative environmental impacts."

Dr. Daniel Rubinoff: Yes.

Ms. Janis Matsunaga: No.

Comments: "Most are sufficient; however, it would be clearer and more transparent if the permits and permit conditions were separated into separate parts for the Import and the Release (such as what I stated above for the special permit approval):

1. Importation of *C. quinquefasciatus* for laboratory research.
2. Field-release of *C. quinquefasciatus* and subsequent area-wide mosquito suppression research.

For Permit Condition No. *5. "The permittee(s) shall submit samples of the restricted article(s) prior to importation to the PQB upon request.*
- How will they do this specifically prior to importation?
- What life stages?
- Alive? Dead?
- Prior to each shipment from various shippers?
- Why not submit samples from the actual shipment both at the time of import/shipment received and after adults emerge in the lab?

*If there is no clear release procedure or plan as of now, perhaps there should be a permit condition to include a pre-release report and SOP submitted to PQB (and approved) prior to initial release."

For Permit Condition No. *13. "The permittee(s) shall submit a report to the PQB on results of post release monitoring programs on a semi-annual basis.*- Semi-annually for how long?
- This should state specific data to include:
  - Pre-release quality control measures taken
  - Release data to include:
    - Species and strain of *Wolbachia* released
    - Locations released
    - Dates released
    - # of individuals released per strain
    - Generation released

What will post-release monitoring/research include?

Will this include capture and DNA extraction work to determine if populations of transinfected individuals and/or sexes are accidentally released and establish breeding populations?

How will results of the effectiveness of releases be measured?"

PQB Response: The two types of research mentioned (laboratory research and field release research) were not separated in order to make this submittal more succinct. The PQB also believes that permit

conditions currently are satisfactory to address import of the mosquitoes, laboratory research, and field release research.

Permit conditions must also be approved by the Board of Agriculture. Given that the applicants have not yet planned release dates, locations, the amount of individuals to release, data to collect, etc., the PQB did not wish to set experimental protocol for the researchers by asking the Board to approve conditions that may hinder research and future experimental design for field release and monitoring.

As the establishment of *Culex quinquefasciatus* and its ability to vector diseases that decimate Hawaii's native bird populations is likely to be seen as an ecological disaster, PQB felt that combining the permit conditions would ensure that the research could be conducted as expeditiously as possible and not add additional delays, such as requiring the applicant to go before the Board a second time for approval of permit conditions for field release research.

Permit Conditions Nos. 13 -17 were included to give the PQB time to consult with its subject matter experts in order to determine the efficacy and safety of field release(s) of *Cu. quinquefasciatus* for cytoplasmic incompatibility testing.

To address Ms. Matsunaga's question about Permit Condition No. 5, it is standard practice for the PQB to collaborate with HDOA's Plant Pest Control Branch (PPC) to confirm the identity of insects that are imported for research. The applicants will be required to submit specimens of imported insects in the life stage(s) requested by the PPC Entomologists for identification confirmation. The specific details of how that would need to occur can be done independently from the permit.

V. Proposed Special Permit Conditions

1. The restricted article(s), Southern House Mosquito, *Culex quinquefasciatus* (Say, 1823), inoculated with a foreign *Wolbachia* bacteria species, shall be used for laboratory, field-release, and area-wide mosquito suppression research, purposes approved by the Board of Agriculture (Board). Live sale or transfer of the restricted article(s), including progeny, is prohibited, except as approved by the Board.

2. The permittee(s), Dr. Floyd Reed, University of Hawaii at Mānoa (UH Mānoa) Edmundson Hall Room 216, 2538 McCarthy Mall, Honolulu, Hawaii, 96822 and Dr. Matthew Medeiros, University of Hawaii at Mānoa, 1993 East-West Road, Honolulu, Hawaii 96822 shall be responsible and accountable for all restricted article(s) imported, including progeny, from the time of receipt until their final disposition.

3. The restricted article(s), including progeny, shall be safeguarded at UH Mānoa Edmundson Hall Room 216, 2538 McCarthy Mall, Honolulu, Hawaii 96822, a site inspected and approved by the Plant Quarantine Branch (PQB) prior to importation. Movement of the restricted article(s), including progeny, to another site shall require a site inspection and approval by the PQB Chief prior to movement.

4. The restricted article(s), including progeny, shall be maintained by Dr. Floyd Reed, UH Mānoa, 2538 McCarthy Mall, Edmundson Hall Room 216, Honolulu, Hawaii 96822 and Dr. Matthew Medeiros, University of Hawaii at Mānoa, 1993 East-West Road, Honolulu, Hawaii 96822, or by trained or certified personnel designated by the permittee(s).

5. The permittee(s) shall submit samples of the restricted article(s) prior to importation to the PQB upon request.

6. Prior to the arrival of each shipment containing the restricted article(s), the permittee(s) shall provide to the PQB Chief the following information in writing:

    a. Expected arrival date;

    b. A copy of the shipping waybill or tracking numbers for each parcel;

    c. A copy of the invoice, packing list or other similar PQB approved document that states the quantity of the restricted article(s), the scientific and common name(s) of the restricted article(s), the shipper, and the consignee for the restricted article(s);

    d. The names and addresses of the shipper and permittee(s); and

    e. The total number of parcels.

7. The restricted article(s) shall be imported only through the port of Honolulu, as approved by the Board. Entry into Hawaii through another port is prohibited.

8. At least four sides of each parcel containing the restricted article(s) shall be clearly labeled in plain view with “Live Animals” and “This Parcel May be Opened and Delayed for Agriculture Inspection”, in 1/2” minimum-sized font.

9. The restricted article(s) shall be shipped in sturdy PQB-approved containers designed to be escape-proof and leak-proof.

10. Each shipment of the restricted article(s) shall be accompanied by a complete copy of the PQB permit with permit conditions for the restricted article(s), and an invoice, packing list or other similar PQB approved document listing the scientific and

common names of the restricted article(s), the quantity of the restricted article(s), the shipper, and the permittee(s) for the restricted article(s).

11. The permittee(s) shall immediately notify the PQB Chief in writing under the following circumstances:

    a. If any escape, theft, accidental release, disease outbreaks, pest emergence and/or mass mortalities involving the restricted article(s), including progeny, under this permit occurs. The department may confiscate or capture the restricted article(s) and any progeny that escapes or is found to be free from confinement at the expense of the owner, pursuant to the Hawaii Revised Statutes (HRS), §150A-7(c).

    b. If any changes are made to the approved sites, facilities or containers used to hold the restricted article(s), including progeny.

    c. If a shipment of the restricted article(s) is delivered to the permittee(s) without a PQB "Passed" stamp, tag or label affixed to the article, container or delivery order that indicates that the shipment has passed inspection and is allowed entry into the State. Under this circumstance, the permittee(s) shall not open or tamper with the shipment. Additionally, the permittee(s) shall secure all restricted article(s), shipping containers, shipping documents and packing materials for the PQB.

    d. If the permittee(s) are found in violation of any municipal, state or federal policies, rules and/or laws, pertaining to the restricted article(s).

    e. If the permittee(s) will no longer import and/or possess the restricted article(s) authorized under this permit. Under this circumstance, the permittee(s) shall inform the PQB Chief of the final disposition for the restricted article(s), including progeny, and the permit will be canceled.

12. In the event that the restricted article(s) become parasitized or infected by disease, all restricted article(s), including progeny, from which the parasitized or infected restricted article(s) originated shall be considered compromised and immediately subjected to a treatment(s) approved by the PQB Chief. All shipping containers, packing materials, equipment, and any other items used in conjunction with the compromised restricted article(s), shall also be subjected to a treatment(s) approved by the PQB Chief.

13. To ensure *Wolbachia* inoculation, the permittee(s) shall conduct DNA isolation and sequencing to determine the *Wolbachia* strains infecting the restricted article(s) and provide the results to the PQB:

    a. Within 7 days upon receipt of the restricted article(s); and

b. Within 30 days prior to removal of the restricted article(s) from all safeguarded locations.

14. At least 30 days prior to any field release of the restricted article(s), the permittee(s) shall submit a detailed plan for field release research, including release sites, monitoring procedures, data collection requirements and any other pertinent information regarding the field release research to the PQB. Field release may occur provided the information provided is reviewed and approved by the PQB Chief in writing.

15. Prior to interisland transportation, all restricted article(s) shall be presented to the PQB for inspection. The permittee(s) shall also follow Permit Conditions Nos. 6, 9, and 10 for each interisland shipment. The PQB inspector shall affix an interisland certificate of inspection to the shipment as verification of a completed inspection.

16. The permittee(s) shall submit a semi-annual report to the PQB on the results of all research including post-release monitoring programs. The report shall be submitted by the 31st of January and July of each year and shall cover the prior 6-month period.

17. The permittee(s) shall adhere to the use, facility, equipment, procedures, and safeguards described in the permit application, and as approved by the Board and the PQB Chief.

18. Any approved site, restricted article(s), progeny, and records pertaining to the restricted article(s) or progeny under permit may be subject to post-entry

    inspections by the PQB, upon arrival at the permittee(s) facility. The permittee(s) shall make the approved site, restricted article(s), progeny, and records pertaining to the restricted article(s) or progeny available for inspection upon request by a PQB Inspector.

19. The permittee(s) shall have a biosecurity manual available for review and approval by the PQB, at the time of the initial site inspection and any subsequent post-entry inspections, which identifies the practices and procedures to be adhered to by the permittee(s), to minimize the risk of theft, escape, or accidental release of the restricted article(s), including progeny, including minimizing the risk of introduction and spread of diseases and pests associated with the restricted article(s) to the environment. The permittee(s) shall adhere to all practices and procedures as stated in this biosecurity manual.

20. The permittee(s) shall submit to the PQB Chief a copy of all valid licenses, permits, certificates, or other similar documents required by other agencies for the restricted article(s). The permittee(s) shall immediately notify the PQB Chief in writing when any of the required documents are suspended, revoked, or terminated. This permit may be amended, suspended, or canceled by the PQB Chief in writing, upon

suspension, revocation, or termination of any required license, permit, certificate or similar document for the restricted article(s).

21. It is the responsibility of the permittee(s) to comply with any applicable requirements of municipal, state, or federal law pertaining to the restricted article(s).

22. The permittee(s) shall be responsible for all costs, charges, or expenses incident to the inspection, treatment, or destruction of the restricted article(s) or progeny under this permit, as provided in Act 173, Session Laws of Hawaii 2010, Section 13, including, if applicable, charges for overtime wages, fixed charges for personnel services, and meals.

23. Any violation of the permit conditions may result in citation, permit cancelation, and enforcement of any or all of the penalties set forth in HRS §150A-14.

24. A canceled permit is invalid and upon written notification from the PQB Chief, all restricted article(s) listed on the permit shall not be imported. In the event of permit cancelation, any restricted article(s) imported, including progeny, may be moved, seized, treated, quarantined, destroyed, or sent out of State at the discretion of the PQB Chief. Any expense or loss in connection therewith shall be borne by the permittee(s).

25. The permit conditions are subject to cancelation or amendment at any time due to changes in statute or administrative rules restricting or disallowing import of the restricted article(s) or due to Board action disallowing a previously permitted use of the restricted article(s).

26. These permit conditions are subject to amendment by the PQB Chief in the following circumstances:

    a. To require disease screening, quarantine measures, and/or to place restrictions on the intrastate movement of the restricted article(s), as appropriate, based on scientifically validated risks associated with the restricted article(s), as determined by the PQB Chief, to prevent the introduction or spread of diseases and/or pests associated with the restricted article(s).

    b. To conform to more recent Board approved permit conditions for the restricted article(s), as necessary to address scientifically validated risks associated with the restricted article(s).

27. The permittee(s) shall agree in advance to defend and indemnify the State of Hawaii, its officers, agents, and employees for any and all claims against the State of Hawaii, its officers, agents, or employees that may arise from or be attributable

to any of the restricted article(s) that are introduced under this permit. This permit condition shall not apply to a permittee that is a federal or State of Hawaii entity or employee, provided that the State or federal employee is a permittee in the employee's official capacity.

ADVISORY COMMITTEE REVIEW: May we request your recommendation and comments at the next meeting of the Advisory Committee on Plants and Animals.

Curriculum Vitae

Floyd A. Reed, Ph.D.

April 15, 2019

Contact

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Unofficial Website: `http://hawaiireedlab.com/wpress/`

Education

2004. Ph.D. Department of Molecular Biology and Genetics
Cornell University, Ithaca, New York, USA
Dr. Charles F. Aquadro, adviser (Population Genetics)
Committee Members (Ph.D. minors)
Dr. Richard T. Durrett (Applied Math)
Dr. Richard G. Harrison (Ecology and Evolution)
Dr. Kenneth A. R. Kennedy (Anthropology)

1996. B.A.(hons) Department of Biology
Department of Chemistry (second major)
Warren Wilson College, Swannanoa, North Carolina, USA
Dr. C. Lee Swendsen, adviser

Work Experience

2016–present. Associate Professor. Department of Biology, University of Hawai'i at Mānoa, Honolulu, Hawai'i, USA

2011–2016. Assistant Professor. Department of Biology, University of Hawai'i at Mānoa, Honolulu, Hawai'i, USA

2008–2011. Independent Group Leader. Department of Evolutionary Genetics, Max Planck Institute for Evolutionary Biology, Plön, Germany

2006–2008. Research Associate. Department of Biology, University of Maryland, College Park, Maryland, USA. Supervisor Dr. S. Tishkoff.

2004–2006. Faculty Research Assistant. Department of Biology, University of Maryland, College Park, Maryland, USA. Supervisor Dr. S. Tishkoff.

1996–2004. Various Teaching Assistant (TA) and Research Assistant (RA) positions. Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York, USA.

1993–1996. Dark Room and Printing Press Operator. Warren Wilson College Print Shop, Swannanoa, North Carolina, USA. Supervisor Rev. G. Tolleson.

1993. Outdoor Living Skills (OLS) cluster leader. Gwynn Valley Summer Camp, Transylvania Co., North Carolina, USA. Supervisor Ms. G. Powell.

1992–1993. Custodian janitor. Western Carolina University, Cullowhee, North Carolina, USA.

1992. Day Camp Counsellor and Assistant Farm Manager. Gwynn Valley Summer Camp, Transylvania Co., North Carolina, USA. Supervisor Mr. D. Robertson.

Teaching Experience

2018. Instructor. BIOL 172L + BIOL 499 SEA-PHAGES lab. University of Hawai‘i at Mānoa. fall. Co-taught with Dr. R. Chong.

2018. Seminar. ZOOL 490b Synthetic Biology (writing intensive). University of Hawai‘i at Mānoa. fall. Co-organized with Dr. J. Walguarnery.

2018. Seminar. ZOOL 490b Origin and Future of Life (writing intensive). University of Hawai‘i at Mānoa. spring. Co-organized with Dr. J. Walguarnery.

2014–present. Co-lecturer. ZOOL 780 Foundations of Ecology and Evolution. University of Hawai‘i at Mānoa. alternate fall semesters (with Drs. A. Wright and R. Thomson)

2013–present. Instructor. BIOL 375L Genetics Lab (biology major core class), University of Hawai‘i at Mānoa. fall semesters

2011–present. Lecturer. BIOL 375 Genetics (biology major core class). University of Hawai‘i at Mānoa. fall semesters

2014–2016. Lecture. BIOL 650 Population Genetics. University of Hawai‘i at Mānoa. alternate spring semesters

2014. Seminar. BIOL 490 GMO’s: Science and Society (ethics focus). University of Hawai‘i at Mānoa. spring. Co-organized with Dr. H. De Couet.

2011. Co-lecturer. Evolutionary Genetics. Max Planck Institute for Evolutionary Biology. (Dr. J. Bains, Dr. D. Greig)

2005. Co-lecturer. Human Genetics. University of Maryland, College Park. (Dr. S. Tishkoff)

1993. Outdoor Living Skills Team Leader and Instructor. Gwynn Valley Summer Camp Transylvania Co., North Carolina. Supervisor Ms. G. Powell.

Teaching Training

2018. Workshop for the SEA-PHAGES, HHMI, discovery-based undergraduate research course, cohort 11b. University of Maryland, Baltimore County. Sponsored by the Howard Hughes Medical Institute.

> An international summer workshop to learn about how to conduct a discovery-based undergraduate research course in undergraduates’ freshman year.
> `https://seaphages.org/`

2014. Workshop, Genome Consortium for Active Teaching, GCAT: Synthetic Biology. University of Maryland, Baltimore County. Sponsored by the Howard Hughes Medical Institute and The National Science Foundation.

A national summer workshop to learn about how to incorporate synthetic biology, along with active learning approaches, into undergraduate teaching labs. http://www.bio.davidson.edu/GCAT/GCATSynBio.html

2013. Workshop, The National Academies Summer Institutes on Undergraduate Education. University of Hawai'i at Mānoa. Sponsored by the Howard Hughes Medical Institute and The National Academies.

A summer workshop for individuals from institutes around the West Coast to learn about and practice aspects of scientific teaching. http://www.academiessummerinstitute.org/

2002. Teaching Assistant, BIOGD 282 Human Genetics, Instructor Dr. M. Hamblin, nonmajors

1998. Teaching Assistant, BIOGD 481 Population Genetics, Instructor Dr. C. Aquadro, majors

1997. Teaching Assistant, BIOGD 281 Introduction to Genetics, Instructor Dr. R. MacIntyre, majors

1996. Teaching Assistant, BIOGD 281 Introduction to Genetics, Instructor Dr. M. Goldberg, majors

Publications

As of October 14, 2018 I have an H-index of 22 and an i10-index of 24 from 36 publications with 4,544 total citations (Google Scholar Profile). A "☯" symbol denotes an equal contribution. (Articles "in preparation" are not listed here except for our book contract.)

Pending 2019-2020. Á. J. Láruson & F. A. Reed. *Population Genetics with R: A Practical Guide*. Writing contract with Oxford University Press.

2018. F. A. Reed, T. G. Aquino-Michaels, M. S. Costantini, Á. J. Láruson, & J. T. Sutton. RPM-Drive: A robust, safe, and reversible gene drive system that remains functional after 200+ generations. arXiv preprint arXiv:1806.05304. (submitted to *Proceedings of the National Academy of Sciences USA*)

2018. Á. J. Láruson, S. E. Coppard, M. H. Pespeni, F. A. Reed. Gene expression across tissues, sex, and life stages in the sea urchin *Tripneustes gratilla* [Toxopneustidae, Odontophora, Camarodonta]. *Marine Genomics* 41: 12–18. doi:10.1016/j.margen.2018.07.002

2018. S. E. Kingston, P. Martino, M. Melendy, F. A. Reed, and David B. Carlon. Linking genotype to phenotype in a changing ocean: inferring the genomic architecture of a blue mussel stress response with genome-wide association. *Journal of Evolutionary Biology* 31(3): 346–361. doi:10.1111/jeb.13224

2017. Bryk, J., Reeves, R. G., Reed, F. A., & Denton, J. A. Transcriptional effects of a positive feedback circuit in *Drosophila melanogaster*. *BMC Genomics* 18(1): 990. https://bmcgenomics.biomedcentral.com/articles/10.1186/s12864-017-4385-z

2017. F. A. Reed. Evolutionary Genetic Engineering in the Indo-Pacific: Conservation, Humanitarian, and Social Issues. https://arxiv.org/pdf/1706.01710.pdf

2017. F. A. Reed. CRISPR/Cas9 Gene Drive: Growing Pains for a New Technology. *Genetics* 205: 1037–1039. doi:10.1534/genetics.116.198887

2016. Láruson, Á. J. and F. A. Reed. Stability of Underdominant Genetic Polymorphisms in Population Networks. *Journal of Theoretical Biology* 390: 156–163. doi:10.1016/j.jtbi.2015.11.023.

2016. Shaefer, A., J. Wolf, P. C. Alves, L. Bergström, G. Colling, *et al.* Reply to Garner *et al. Trends in Ecology and Evolution* 31: 83–84. doi:10.1016/j.tree.2015.11.010

2015. Shaefer, A., J. Wolf, P. C. Alves, L. Bergström, M. W. Bruford, *et al.* Genomics and the challenging translation into conservation practice. *Trends in Ecology and Evolution* 30: 78–87. doi:10.1016/j.tree.2014.11.009

2014. Tabios, M., L. Boell, and F. A. Reed. A new mutation of PDA synthase, *sepia*, isolated from wild *Drosophila melanogaster*. *Drosophila Information Service* 97: 176–177. http://www.ou.edu/journals/dis/DIS97/Tabios%20176.pdf

2014. Reeves, R. G., J. Bryk, P. M. Altrock, J. A. Denton, and F. A. Reed. First Steps Towards Underdominant Genetic Transformation of Insect Populations. *PLoS ONE* 9: e97557. doi:10.1371/journal.pone.0097557

2014. Gokhale, C. S., R. G. Reeves, and F. A. Reed. Dynamics of a combined medea-underdominant population transformation system. *BMC Evolutionary Biology* 14: 98. doi:10.1186/1471-2148-14-98

2013. Reed, F. A., A. Traulsen, and P. M. Altrock. Underdominance. In *Encyclopedia of Genetics*, S. Brenner & J. H. Miller, Eds., Elsevier Science, Inc.

2012. Reeves, R. G., J. Denton, F. Santucci, J. Bryk, and F. A. Reed. Scientific Standards and the Regulation of Genetically Modified Insects. *PLoS Neglected Tropical Diseases* 6: e1502. doi:10.1371/journal.pntd.0001502

2012. Reed, F. A. Modern Human Migrations: The First 200,000 Years. In *Migrations: Interdisciplinary Perspectives*, M. Messer, R. Schroeder & R. Wodak, Eds., Springer. doi:10.1007/978-3-7091-0950-2

2012. Traulsen, A. and F. A. Reed. From genes to games: Cooperation and cyclic dominance of meiotic drive alleles. *Journal of Theoretical Biology* 299: 120–125. doi:10.1016/j.jtbi.2011.04.032

2011. Altrock, P. M., A. Traulsen, and F. A. Reed. Stability Properties of Underdominance in Finite Subdivided Populations. *PLoS Computational Biology* 7: e1002260. doi:10.1371/journal.pcbi.1002260

2011. Haubold, B., F. A. Reed, and P. Pfaffelhuber. Alignment-free estimation of nucleotide diversity. *Bioinformatics* 27: 449–455. doi:10.1093/bioinformatics/btq689

2011. Stemshorn, K. C., F. A. Reed, A. W. Nolte, and D. Tautz. Rapid formation of distinct hybrid lineages after secondary contact of two fish species (*Cottus* sp.). *Molecular Ecology* 20: 1475–1491. doi:10.1111/j.1365-294X.2010.04997.x

2010. Altrock, P. M., A. Traulsen, R. G. Reeves, and F. A. Reed. Using underdominance to bi-stably transform local populations. *Journal of Theoretical Biology* 267: 62–75. doi:10.1016/j.jtbi.2010.08.004

2010. Allaby, R. G., F. R. Friedlaender, F. A. Reed, K. K. Kidd, J. R. Kidd, *et al.* Prehistoric Pacific Population Movements. pp. 143–157 in *The Global Origins and Developments of Seafaring*, A. Anderson, J. H. Barrett & K. V. Boyle, Eds., ISBN: 978-1-902937-52-6, McDonald Institute for Archaeological Research, Cambridge, UK.

2009. Tishkoff, S. A., F. A. Reed, F. R. Friedlaender, C. Ehret, A. Ranciaro, *et al.* The Genetic Structure and History of Africans and African Americans. *Science*

324: 1035–1044. doi:10.1126/science.1172257

2008. Milinski, M., R. Sommerfeld, H.-J. Krambeck, F. A. Reed, and J. Marotzke. The collective risk social dilemma, and the prevention of simulated dangerous climate change. *Proceedings of the National Academy of Sciences USA* 105: 2291–2294. doi:10.1073/pnas.0709546105

2008. Reed, F. A. Are Humans Still Evolving? In *Encyclopedia of Life Sciences: Handbook of Human Molecular Evolution*, D. N. Cooper & H. Kehrer-Sawatzki, Eds., John Wiley & Sons, Ltd., Chichester, UK. `http://www.els.net` doi:10.1002/9780470015902-.a0020794

2008. Friedlaender, J. S., F. R. Friedlaender, F. A. Reed, K. K. Kidd, J. R. Kidd, *et al.* The Genetic Structure of Pacific Islanders. *PLoS Genetics* 4: e19. doi:10.1371/journal.pgen.0040019

2007. Tishkoff, S. A., M. K. Gonder, B. M. Henn, H. M. Mortensen, N. Fernandopulle, *et al.* History of click-speaking populations of Africa inferred from mtDNA and Y chromosome genetic variation. *Molecular Biology and Evolution* 24: 2180–2195. doi:10.1093/molbev/msm155

2007. Reed, F. A. Two-locus epistasis with sexually antagonistic selection: A genetic Parrondo's paradox. *Genetics* 176: 1923–1929. doi:10.1534/genetics.106.069997

2007. Gonder, M. K., H. M. Mortensen, F. A. Reed, A. de Sousa, and S. A. Tishkoff. Whole mtDNA Genome Sequence Analysis of Ancient African Lineages. *Molecular Biology and Evolution* 24: 757–768. doi:10.1093/molbev/msl209

2007. Tishkoff, S. A.[◕], F. A. Reed[◕], A. Ranciaro, B. F. Voight, C. C. Babbitt, *et al.* Convergent adaptation of human lactase persistence in Africa and Europe. *Nature Genetics* 39: 31–40. doi:10.1038/ng1946

2006. Reed, F. A. and S. A. Tishkoff. African human diversity, origins and migrations. *Current Opinion in Genetics & Development* 16: 597–605. doi:10.1016/j.gde.2006.10.008

2006. Reed, F. A. and C. F. Aquadro. Mutation, selection and the future of human evolution. *Trends in Genetics* 22: 479–484. doi:10.1016/j.tig.2006.07.005

2006. Kontanis, E. J. and F. A. Reed. Evaluation of real-time PCR amplification efficiencies to detect inhibitors. *Journal of Forensic Sciences* 51: 795–804. doi:10.1111/j.1556-4029.2006.00182.x

2006. Reed, F. A. and S. A. Tishkoff. Positive selection can create false hotspots of recombination. *Genetics* 172: 2011–2014. doi:10.1534/genetics.105.052183

2005. Reed, F. A., J. M. Akey, and C. F. Aquadro. Fitting background-selection predictions to levels of nucleotide variation and divergence along the human autosomes. *Genome Research* 15: 1211–1221. doi:10.1101/gr.3413205

2005. Reed, F. A., R. G. Reeves, and C. F. Aquadro. Evidence of susceptibility and resistance to cryptic X-linked meiotic drive in natural populations of *Drosophila melanogaster*. *Evolution* 59: 1280–1291. doi:10.1111/j.0014-3820.2005.tb01778.x

2004. Reed, F. A. *Characterizing Diversity Reducing Selection in Humans and Fruitflies*. Ph.D. Dissertation, Cornell University, Ithaca, NY. `http://wwwlib.umi.com/dissertations/fullcit/3149436`

2003. Reed, F. A., E. J. Kontanis, K. A. R. Kennedy, and C. F. Aquadro. Ancient DNA prospects from Sri Lankan highland dry caves support an emerging global pattern. *American Journal of Physical Anthropology* 121: 112–116. doi:10.1002/ajpa.10211

2001. Aquadro, C. F., V. L. Bauer DuMont, and F. A. Reed. Genome-wide variation in the human and fruitfly: a comparison. *Current Opinion in Genetics & Development* 11: 627–634. doi:10.1016/S0959-437X(00)00245-8

Field Experience

2010. Preliminary arrangements for future human DNA sample collection in Mali. December.

2007. Processing regional government permit applications for future human DNA sample and phenotype data collection in Addis Ababa and Awassa, Ethiopia. November.

2006. Human DNA sample and phenotype data collection in Marsabit, Dirib Gombo, North Horr and Lake Turkana, Kenya. May-July.

1996. Cherokee Archaeology Field School. Warren Wilson Site, Swannanoa, NC. Supervisor D. Moore, North Carolina state archaeologist. June-July.

Other Experience and Professional Memberships

2018–present. Affiliate faculty of the Department of Biology, University of Hawai'i at Hilo. `https://hilo.hawaii.edu/depts/biology/`.

2017–present. Affiliate faculty of the Anthropology Graduate Program, University of Hawai'i at Mānoa. `http://www.anthropology.hawaii.edu/`.

2016. Mosquitoes in Hawaii: Novel approaches to confront mosquito vectors and mosquito-borne pathogens in the Hawaiian Islands. Hawai'i Volcanoes National Park. Invitation-only. September.

2016–present. Associate Editor for the *Journal of Heredity*.

2016. Gene Drives: A Deliberative Workshop to Develop Frameworks for Research and Governance. NCSU Genetic Engineering and Society Center. Invitation-only. February.

2016–present. Member faculty of the Ecology, Evolution, & Conservation Biology (EECB) Graduate Specialization Program, University of Hawai'i at Mānoa. `http://www.hawaii.edu/eecb/`.

2015–present. Member of an inter-agency Hawaiian Forest Bird Conservation Genetic Pest Management Technologies working group.

2013–present. Member. Society for the Study of Evolution

2013–present. Member. American Indian Science and Engineering Society (AISES)

2011. Review Committee. *Deutshe Forschungsgemeinschaft* (German National Research Foundation) for the research unit "Natural selection in structured populations"

1999–2004. Member. American Indian Program of Cornell University

Grants

2018. U.S. National Institutes of Health COBRE: Integrative Center for the Earth's Microbiome and Human Health. One of five JI's with a total award of $2,366,501 for five years. Total Reed lab allocation of $212,250 in year one. Role JI

2017. U.S. National Science Foundation (NSF). REU Site: Environmental Biology for Pacific Islanders. (Three years, final funding details to be determined.) PI's Michael Hadfield, Matt Medeiros. Role Participating Lab.

2016. State of Hawai'i, Department of Land and Natural Resources with matching funds from the U.S. Fish and Wildlife Service. Wolbachia replacement for cytoplasmic incompatibility in *Culex quinquefasciatus*. One year. $90,585.46. Role Co-PI with Dr. J. Sutton of the University of Hawai'i Hilo.

2016. U.S. National Science Foundation (NSF). REU Site: Undergraduate Research Experiences in DNA-based discoveries in Hawai'i's biodiversity. Award 1560491. 3 years, $347,580. PI's Stuart Donachie, Stephanie Kraft-Terry. Role Participating Lab.

2012. Victoria S. and Bradley L. Geist Foundation, administered by the Hawai'i Community Foundation, Medical Research Program. Engineering Underdominance in *Culex quinquefasciatus*. 12ADVC-51343. 18 months, $50,000. Role PI

2010. *Deutshe Forschungsgemeinschaft* (DFG , German National Research Foundation). *Die Entstehung von Resistenzen gegen genetisch induzierte Sterilitt bei Insekten*. (The evolution of resistance to genetically induced sterility in insects.) RE-3062/2-1. 2 years, €59,385+BAT IIa/E13 pay-scale personnel support (approximately €120,000 total, or $170,000 equivalent). Role PI

2006. U.S. National Institutes of Health (NRSA). Characterizing a genetic history of African populations. F32HG003801/F32HG03801. 2 years, $98,224. Role Postdoc.

2002. Sigma Xi Grants-in-Aid of Research. Optimization and discrimination of background-selection and hitch-hiking predictions on the human X-chromosome. $640. Role Grad. Student

Patent Applications

Max-Planck-Gesellschaft zur Frderung der Wissenschaften E.V. (Max-Planck-Society for the Advancement of Science), applicant. Reeves, R. G. and F. A. Reed, inventors. (2012) Stable transformation of a population and a method of biocontainment using haploinsufficiency and underdominance principles. WO2014096428

University of Maryland, applicant. Tishkoff, S. A. and F. A. Reed, inventors. (2008) Single nucleotide polymorphisms and the identification of lactose intolerance. WO2008057265

Honors and Awards

2015. Nominated for an Excellence in Teaching award in the College of Natural Sciences, University of Hawai'i at Mānoa, Honolulu, HI.

2014. Nominated for an Excellence in Teaching award in the College of Natural Sciences, University of Hawai'i at Mānoa, Honolulu, HI.

2007. Selected for a competitive award from the U. S. National Institutes of Health, Loan Repayment Program for Health Disparities Research. (forced to decline because of subsequent employment outside of the U.S.)

2004. Supported by an award from The Center for Bioinformatics and Computational Biology, University of Maryland, College Park, MD.

2001. Fitch prize finalist. Reed, F. A., and C. F. Aquadro. The effects of deleterious mutations on levels of variation in the human genome. Annual meeting of the Society for Molecular Biology and Evolution. July 2001, Athens, GA.

2000. Selected for the NSF Training Grant "Evolution from DNA to the Organism: The Interface between Evolutionary Biology and the Mathematical Sciences." Administered by the Department of Biometrics, Cornell University, Ithaca, NY.

2000. Fitch prize finalist. Reed, F. A., and C. F. Aquadro. Detecting recent selection in humans using microsatellites. Joint meetings of the Society for Molecular Biology and Evolution & the American Genetics Association. June 2000, New Haven, CT.

1996. Recipient of a competitive summer semester tuition waiver for students of Cherokee heritage. Cherokee Archaeology Field School. Warren Wilson Site, Swannanoa, NC.

1996. Elected class speaker for the Warren Wilson College commencement ceremony. May 1996, Swannanoa, NC.

1996. The Edward C. Jeffrey Award in Biological Sciences. Department of Biology, Warren Wilson College, Swannanoa, NC.

1996. First Place in Undergraduate Research Presentations, Biological Sciences IV. The 93rd annual meeting of the North Carolina Academy of Science, March, Wake Forest University, Winston-Salem, NC.

1994. CRC Press Freshman Chemistry Award. Warren Wilson College, Swannanoa, NC.

Invited Talks

2018 Reed, F. A., J. T. Sutton, J. A. Denton. Robust, safe, and reversible gene drive. Entomology Society of America annual meeting. Vancouver, Canada. November. (The presentation time was missed because of a late flight.)

2018 Reed, F. A. Protecting Hawaiian birds from avian malaria. International Ornithological Congress. Vancouver, Canada. August. (Declined because of insufficient travel funds and teaching responsibilities.)

2018 Reed, F. A. Robust, safe, and reversible gene drive. American Malacological Society, Western Society of Malacologists joint meeting, Honolulu, Hawai‘i. June.

2017 Reed, F. A. Genetic pest management in Hawai‘i. Conservation Genetics Workshop. Hawaiian Institute for Marine Biology, Kāne‘ohe, Hawai‘i. February.

2017 Reed, F. A. Emerging opportunities for genetic pest management in Hawai‘i. Hawaiian Entomological Society meeting. Honolulu, Hawai‘i. February.

2015 Reed, F. A. Genetic Engineering for Species Conservation Applications in Hawai‘i. Okinawa Institute of Science and Technology, Okinawa, Japan. August.

2014. Reed, F. A. Genetic Engineering for Species Conservation Applications in Hawai‘i. ConGenOmics Workshop. Uppsala, Sweden. March.

2014. Reed, F. A. Genetic Engineering for Species Conservation Applications in Hawai‘i. Department of Entomology, North Carolina State University, Raleigh, NC. March.

2012. Reed, F. A. Genetically transforming a population using underdominance. Hawai‘i Institute of Marine Biology, Kane'ohe, Hawai‘i, September.

2011. Reed, F. A. Underdominance Predictions and Genetically Transforming a Population. Department of Zoology, University of Hawai‘i at Mānoa. February.

2011. Reed, F. A. Underdominance Predictions and Population Transformations. Department of Biology, Ludwig Maximilian University, Munich, Germany. January.

2010. Reed, F. A. On GeneCulture Coevolution: Language and Music. Technological, dialectological and theoretical linguistics meeting. Department of Lingusitics and

Scandinavian Studies. University of Oslo, Oslo, Norway. September.

2010. Reed, F. A. On GeneCulture Coevolution: Adult Lactose Tolerance in Africa. Department of Tumor Biology, Institute for Cancer Research, The Norwegian Radium Hospital, Oslo, Norway. September.

2010. Reed, F. A. Modern human migrations: the first 200,000 years. *Interdisziplıäres Dialogforum*, Migrations: Interdisciplinary Perspectives. University of Vienna, Vienna, Austria. July.

2009. Reed, F. A. Underdominance Predictions and Population Transformations. Institute for Population Genetics, University of Veterinary Medicine Vienna. Vienna, Austria. November.

2009. Reed, F. A. Underdominance Predictions and Population Transformations. Department of Evolutionary Biology, Uppsala University, Uppsala, Sweden. October.

2009. Reed, F. A. Underdominance and Population Transformations. Mind the gap: joining theoretical and empirical population genetics. VW-Stiftung Workshop, Freiburg, Germany. October.

2008. Reed, F. A. and M. Schönbrunn. Gene-Culture Coevolution: A Focus on Music in Africa. Geniale Science Festival, Department of Art and Music, Beliefeld University, Beliefeld, Germany. October.

2007. Reed, F. A. The Structure and Migrations of Human Populations in Africa. The African Society of Human Genetics, Cairo, Egypt. November.

2007. Reed, F. A. The Genetic Structure of Human Populations in Africa. Aquavit V meeting, The Max Planck Institute for Evolutionary Biology, Plön, Germany. March.

2007. Reed, F. A. A Microsatellite Based Likelihood-Approximation with Simultaneous Mutation, Demographic and Selective Inference. The Institute for Genetics, University of Cologne, Cologne, Germany. March.

2007. Reed, F. A. Using microsatellites to characterize human population structure in Africa and simultaneously infer selection and demography in Drosophila. The Department of Genetics, North Carolina State University, Raleigh, North Carolina. January.

2002. Reed, F. A. Levels of human polymorphism are consistent with weak background-selection. The Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany. December.

2000. Reed, F. A., and C. F. Aquadro. Detecting recent selection in humans using microsatellites. LSU Ecology & Evolution Department, Baton Rouge, Louisiana. November.

2000. Reed, F. A. The genetic history of the Jacob breed of sheep. National meeting of the Jacob Sheep Breeders Association, Gilbertsville, New York. June.

Broader Media Impact

I was interviewed for an article on commercial genetic testing by Tam, N. (2017) "23 Defines You." *Hoʻa Oʻahu* `https://www.hoaoahu.com/genetic-testing`

Our laboratory work on mosquito Wolbachia replacement in Hawaiʻi has been reported in various media sources including:

Anonymous (2017) 'Birth control' targets Hawaii's mosquitoes to protect native birds. *The Garden Island* `http://thegardenisland.com/news/state-and-regional/`

birth-control-targets-hawaii-s-mosquitoes-to-protect-native-birds/article_cb3e8482-f958-11e6-8f93-4be24a81ac33.html

Anonymous (2017) Hawaii implements mosquito 'birth control' to protect native birds. *KHON2* http://khon2.com/2017/02/22/hawaii-implements-mosquito-birth-control-to-protect-native-birds/

Anonymous (2017) Hawaii implements mosquito 'birth control' to protect native birds.. *WAVY.com* http://wavy.com/2017/02/23/hawaii-implements-mosquito-birth-control-to-protect-native-birds/

Anonymous (2017) Birth control for mosquitoes targeted at saving Hawaiian birds. *University of Hawai'i News* http://www.hawaii.edu/news/2017/02/22/birth-control-for-mosquitoes-targeted-at-saving-hawaiian-birds/

Anonymous (2017) DLNR NEWS RELEASE: "Birth control" for mosquitoes targeted at saving unique, imperiled Hawaiian birds. *State of Hawai'i Governor's Office* http://governor.hawaii.gov/newsroom/latest-news/dlnr-news-release-birth-control-for-mosquitoes-targeted-at-saving-unique-imperiled-hawaiian-birds/

Anonymous (2017) State pursues mosquito 'birth control' to save native birds. *Hawai'i News Now* http://www.hawaiinewsnow.com/story/34578965/state-pursues-mosquito-birth-control-to-save-native-birds

Anonymous (2017) Mosquito 'Birth Control' Targets Saving Hawai'i's Birds. *Big Island Now* http://bigislandnow.com/2017/02/22/mosquito-birth-control-targets-saving-hawaii-birds/

Anonymous (2017) VIDEO: Mosquito "Birth Control" Under Development At UH. *Big Island Now Video News* http://www.bigislandvideonews.com/2017/02/25/video-mosquito-birth-control-under-development-at-uh/

Anonymous (2017) Mosquito 'Birth Control' Aims to Protect Native Birds. *Maui Now* http://mauinow.com/2017/02/22/mosquito-birth-control-aims-to-protect-native-birds/

Anonymous (2017) Birth control for mosquitoes targeted at saving Hawaiian birds. *ScienceBlog* https://scienceblog.com/492388/birth-control-mosquitoes-targeted-saving-hawaiian-birds/

Ashe, I. (2017) Game-changer; UH research looking at unique way to stop mosquito-borne diseases in Hawaiian birds. *Hawaii Tribune-Herald* http://www.hawaiitribune-herald.com/news/local-news/game-changer-uh-research-looking-unique-way-stop-mosquito-borne-diseases-hawaiian

Else, J. (2017) New technique could save endangered birds on Kauai. *The Garden Island* http://thegardenisland.com/news/local/new-technique-could-save-endangered-birds-on-kauai/article_d8e36bf5-bd24-57a4-93f8-8db5fdc50ba5.html

Murray, M. M. (2017) Lab-altered mosquitoes may save rare birds. *Frontiers in Ecology and the Environment* 15(3): 120.

Nabarro, M. (2017) Birth control for mosquitoes, effort to save native birds. *KITV* http://www.kitv.com/story/34580499/birth-control-for-mosquitoes-effort-to-save-native-birds#

Our laboratory work on mosquito genetic engineering in Hawai'i has been reported in

various media sources including:

Harvey, C. (2016) This new gene technology could wipe out entire species – to save others. *Washington Post* https://www.washingtonpost.com/news/energy-environment/wp/2016/09/07/this-new-gene-technology-could-wipe-out-entire-species-to-save-others/

Goldman, J. G. (2016) Harnessing the Power of Gene Drives to Save Wildlife. *Scientific American* http://www.scientificamerican.com/article/harnessing-the-power-of-gene-drives-to-save-wildlife/

Schughart, A. (2016) *Beschleunigte Vererbung mit Gene Drive: Unkontrollierbar oder Lebensretter?* (German language, English translation: Accelerated Heredity with Gene Drive: Uncontrollable or Lifesaver?) *WIRED* https://www.wired.de/collection/science/beschleunigte-vererbung-mit-gene-drive-unkontrollierbar-oder-lebensretter

Áki Láruson (Reed lab) and Caitlyn Genovese (Moran lab) were interviewed about their sea urchin research in 2014 by Jay Fidell in a "Research at UH Mānoa" segment on "ThinkTech Hawai'i." https://youtu.be/R1BJ0vZZTBc

Reeves *et al.* (2012) was the subject of an editorial, two expert commentaries and initiated the PLoS Genetically Modified Insect Collection of PLoS Collections http://www.ploscollections.org/GMInsect. This work was also featured in several news sources:

von Bredow, R. (2012) *Armee der Killermücken. Der Spiegel* 5:100-102. (German print version)

von Bredow, R. (2012) The Controversial Release of Suicide Mosquitoes. Spiegel Online: http://www.spiegel.de/international/world/0,1518,812283-2,00.html (English online version)

Callaway, E. (2012) What should the public know about GM insect trials? Nature News Blog http://blogs.nature.com/news/2012/01/what-should-the-public-know-about-gm-insect-trials.html

Hoffman, E. (2012) Genetically engineered mosquito buzz continues. Friends of the Earth Blog http://www.foe.org/news/blog/2012-02-ge-mosquito-buzz-continues-concerns-around-transpare

Ledger, W. (2012) GM mosquito release not transparent, say scientists. Cayman-NewsService http://www.caymannewsservice.com/science-and-nature/2012/02/02/gm-mosquito-release-not-transparent-say-scientists

Webb, C. (2012) Time to regulate the release of GM mosquitoes and here's how. The Conversation http://theconversation.edu.au/time-to-regulate-the-release-of-gm-mosquitoes-and-heres-how-5062

Tishkoff *et al.* (2009) was featured on the cover of Science and was the subject of several newspaper, magazine and online articles including:

Achenbach, J. (2009) African's Have World's Highest Genetic Diversity, Study Finds. *The Washington Post* (published online http://www.washingtonpost.com/wp-dyn/content/article/2009/04/30/AR2009043002485.html).

Gibbons, A. (2009) Africans' Deep Genetic Roots Reveal Their Evolutionary Story. *Science* 324: 575. http://science.sciencemag.org/content/324/5927/575

Gill, V. (2009) Africa's genetic secrets unlocked. BBC News (published online http://news.bbc.co.uk/2/hi/science/nature/8027269.stm).

Kwok, R. (2009) Africa's genetic history unraveled. Nature News (published online doi:10.1038/news.2009.426)

Wade, N. (2009) Eden? Maybe. But Where's the Apple Tree? *The New York Times* Section A, Page 6, May 1 (published online http://www.nytimes.com/2009/05/01/science/01eden.html?ref=world).

Milinski *et al.* (2008) was featured in the commentary section of PNAS and picked up by news agencies.

Dreber, A. and M. A. Nowak (2008) Gambling for global goods. *PNAS* 105: 2261–2262.

Leahy, S. (2008) Climate Change: A Game With Too Many Free Riders. *Inter Press Service News Agency*, April 4, (published online http://ipsnews.net/news.asp?idnews=41859).

Friedlaender *et al.* (2008) was the subject of several newspaper and magazine articles including:

Handwerk, B. (2008) Polynesians Descended From Taiwanese, Other East Asians. *National Geographic News*, (published online) January 17. http://news.nationalgeographic.com/news/2008/01/080117-polynesian-taiwan.html

Holden, C. (2008) Polynesians Took the Express Train Through Melanesia to the Pacific. *Science* 319: 270.

Wilford, J. N. (2008) Pacific Islanders' Ancestry Emerges in Genetic Study. *The New York Times*, Section A, Page 6, Column 1, January 18.

My presentation at 2007 The American Anthropological Association meeting was featured online by Nature.

Callaway, E. (2007) Music is in our genes. Nature News (published online) December 10. http://www.nature.com/news/2007/071210/full/news.2007.359.html doi:10.1038/news.2007.359

Tishkoff *et al.* (2007) was the subject of several newspaper and magazine articles including:

Check, E. (2006) How Africa learned to love the cow. *Nature* 444: 994–996.

Gibbons, A. (2006) Human Evolution: There's More Than One Way to Have Your Milk and Drink It, Too. *Science* 314: 1672.

Wade, N. (2006) Lactose Tolerance in East Africa Points to Recent Evolution. *The New York Times* Section A, Page 15, Column 1, Dec. 11.

Weiss, R. (2006) The Key to Lactose Tolerance. *The Washington Post* Section A, Page 8, Column 1, Dec. 11.

My presentation at 2005 The American Association of Physical Anthropology meeting was featured in the news section of Science (later published as Tishkoff *et al.* (2009) above).

Culotta, E. (2005) Human Relations. *Science* 308: 491.

I consulted for and briefly appeared in a 2001 Discovery Channel documentary "The Ultimate Guide: Mastodon in Your Backyard," aired in October 2001.

Supervisory Experience

Postdoctoral

Sutton, Jolene T. 2013–2015. Engineering underdominance in Culex mosquitoes. University of Hawai'i at Mānoa.

Denton, Jai A. 2010–2013. Mutation screens for the evolution of resistance to genetic pest management techniques. Max Planck Institute for Evolutionary Biology.

Reeves, R. Guy 2008–2013. Engineering underdominance to safely and reversibly modify insect pest populations. Max Planck Institute for Evolutionary Biology.

Graduate

Sung, Helen 2019–present. M.S. Zoology Program, U.H. Mānoa

Wallstrom, Michael A. 2017–present. M.S. Zoology Program, U.H. Mānoa

Costantini, Maria 2016–present. Ph.D. Zoology Program, U.H. Mānoa

Láruson, Áki J. 2013–2018. Ph.D. Zoology Program, U.H. Mānoa

Schukies, Stella S. 2011–2012 Masters Diploma. *Christian-Albrechts-Universität zu Kiel*, Dept. of Evolutionary Ecology and Genetics. Laboratory research conducted at the Max Planck Institute for Evolutionary Biology.

Babiker, Hiba M. A. 2010–2012 Ph.D. The International Max Planck Research School for Evolutionary Biology.

Graduate Committees

Current

Sean Canfield (PhD), Elena Hughes (PhD), Tom Iwanicki (PhD), Jared Nishimoto (MS), Alina Pang (PhD), Kirill Vinnikov (PhD), Van Wishingrad (PhD)

Completed

Silvia Beurmann (PhD), Helena De Souza Brasil Barreto (MS), Carly Fitzpatrick (MS), Elizabeth Henry (MS), Jessica Maxfield (PhD), Emilie Richards (MS), Orion Rivers (PhD), Michael San Jose (PhD), Janna Zoll (MS)

Undergraduate

Galvizo, Glenn. 2018. BIOL 499 Directed Research. Approximate Bayesian Computation with human microsatellite data.

Aquino-Michaels, Todd. 2017. BIOL 499 Directed Research. Environmental RNA interference in *Culex* mosquitoes.

Ajifu, Rumer. 2017. Summer REU program. Opsin expression and popualtion genetics in *Tripneustes gratilla*.

Quiogue, Zachary. 2017. Summer REU program. Oviposition preference of *Aedes albopictus*.

Barton, Casey. 2016–present. BIOL 499 Directed Research. Analysis of Toxopneustidae sea urchin test morphology.

Holcomb, Angelina. 2016–present. BIOL 499 Directed Research. Effects of telomere complex disruption on longevity in *Drosophila melanogaster*.

Sharp, Victoria. 2016. Summer REU program. Analysis of Toxopneustidae sea urchin jaw morphology.

Lau, Alyssa. 2016. Summer REU program. Testing alternative Culex mosquito feeding methods.

Paulino, Stacey. 2015–2016. Isolating and characterizing novel *Vibrio coralliilyticus* lysing Vibriophage.

Wallstrom, Michael. 2014–2015. BIOL 499 Directed Research. Describing Hawaiian Porifera with phylogenetics.

Wagner, Chelsea. 2014. BIOL 499 Directed Research. Sea urchin larvae survival under cold stress.

Asao, Kenton. 2014. BIOL 499 Directed Research. Hawaiian damselfly karyotyping.

Roup, Fabrece. 2013–2015. Testing migration–selection equilibrium in an underdominant Drosophila system.

Tabios, Myles. 2013. BIOL 499 Directed Research. Characterizing a novel spontaneous mutant of *sepia* in *Drosophila melanogaster*.

Müller, Hagen. 2009–2010. Trans-generational Influence of Tetracycline on *Drosophila melanogaster*. Bachelor Thesis. Fachhochschule Bingen, University of Applied Sciences, Dept. of Life Sciences and Engineering. Bingen, Germany.

Professional

Möller, Anita 2008–2011. Part-time laboratory technician. Max Planck Institute for Evolutionary Biology.

Gorsler, Vanessa 2010–2011. *Auszubildende als Biologielaborantin* (certificate training for laboratory work in biology). Max Planck Institute for Evolutionary Biology.

Klocksin, Carlos 2010. *Auszubildende als Biologielaborantin* (certificate training for laboratory work in biology). Max Planck Institute for Evolutionary Biology.

Langer, Katharina 2008–2009. *Auszubildende als Biologielaborantin* (certificate training for laboratory work in biology). Max Planck Institute for Evolutionary Biology.

Weiß, Katharina 2008. *Auszubildende als Biologielaborantin* (certificate training for laboratory work in biology). Max Planck Institute for Evolutionary Biology.

References

Dr. Charles F. Aquadro, Professor, and Director, Cornell Center for Comparative and Population Genomics. Department of Molecular Biology and Genetics, Cornell University, Ithaca, NY. Email: cfa1@cornell.edu

Dr. Mohamed A. F. Noor, Professor, Department of Biology, Duke University, Durham, NC. Email: noor@duke.edu

Prof. Dr. Arne Traulsen, Director of the Department of Evolutionary Theory, Max Planck Institute for Evolutionary Biology, Plön, Germany. Email: traulsen@evolbio.mpg.de

MATTHEW CHRISTOPHER IKAIKA MEDEIROS
CURRICULUM VITAE

I. PERSONAL INFORMATION

Assistant Professor
Pacific Biosciences Research Center
University of Hawai‘i at Mānoa
Honolulu, HI

mcmedeir@hawaii.edu
Phone: (808)-956-8187

II. EDUCATION

2007-2013 Doctor of Philosophy, Biology
University of Missouri-St. Louis
Department of Biology
Program in Evolution, Ecology and Systematics
Advisor: Robert E. Ricklefs
Dissertation: *Elucidating the Factors that Modulate the Distribution of Avian Haemosporida Parasites across a Community of Hosts*

2001-2006 Bachelor of Science
University of Hawai‘i at Mānoa
Major: Zoology

III. IDIOMS

English: Native language
Portuguese: Understand well, read well, and speak fair
Hawaiian: familiar

IV. EXPERIENCE

2019- Co-Director
Center for Microbiome Analysis through Island Knowledge and Investigation (C-MĀIKI)
University of Hawai‘i at Mānoa
Honolulu, HI

2019- Chief Scientist
Insectary for Scientific Training and Advances in Research (InSTAR)
University of Hawai‘i at Mānoa
Honolulu, HI

2016- Assistant Professor
Pacific Biosciences Research Center
University of Hawai‘i at Mānoa
Honolulu, HI

2015-2016 Post-doctoral Researcher
Department of Entomology
Texas A&M University
College Station, TX
Advisor: Gabriel L. Hamer, Ph.D.

2014-2015 Post-doctoral Researcher
Laboratório de Ecologia e Conservação de Aves

Departamento de Zoologia
Universidade de Brasília
Advisor: Miguel Â. Marini, Ph.D.

2012-2013 Graduate School Dissertation Fellow
University of Missouri-St. Louis

2007-2012 Graduate Assistant
Department of Biology
University of Missouri-St. Louis
Activities: Teach and coordinate a Human Anatomy and Physiology lab course for undergraduate students.

2006-2007 Fieldwork coordinator
Department of Zoology
University of Hawai'i at Mānoa
Activities: Organized and coordinated a bird mist-netting operation on O'ahu, Hawaii

V. TEACHING

Courses

2018-2019 Instructor
Introduction to Systems Biology (OEST 103)
University of Hawai'i at Mānoa
Activities: Develop curriculum, deliver lectures, administer examinations

2014 Field Instructor
Techniques in ornithology field course (Pantanal, Brazil)
Universidade de Brasília
Activities: Demonstration and instruction on field methods associated with the study of birds.

2007-2012 Lab Instructor
Human Anatomy and Physiology Laboratory (BIOL 1131)
University of Missouri-St. Louis
Activities: Lectures on relevant topics associated with laboratory activities; instruction on practical exercises (i.e. dissection, anatomical structure identification); setting up, administering, and grading practical exams.

2006 Teaching Intern
Animal Evolution (ZOOL 480)
University of Hawai'i at Mānoa
Instructor: John Stimson

Student Mentoring and Training.

2021- Francisca Rodríguez, PhD student, University of Hawai'i at Mānoa, Botany
Committee member
Project: Aquatic microbiomes of bromeliad axial environments

2021- Spencer Alascio, Master's student, University of Hawai'i at Mānoa, Zoology
Committee member
Project: Small lizard life-history traits and its role in invasion biology.

2021- Jose Carranza, Master's student, University of Hawai'i at Mānoa, Zoology
Committee member
Project: Diet of small lizards in an introduced lizard community.

2021- Jordan M Gossett, PhD student, University of Hawai'i at Mānoa, Zoology
Committee member
Project: Evolutionary biology of cave dwelling insects.

2020- Chasen Griffin, PhD student, University of Hawai'i at Mānoa, Zoology

PhD advisor
Project: Heterogeneity in the vectorial capacity of mosquito populations

2020- Danya Weber, Masters student, University of Hawai‘i at Mānoa, Zoology
Masters Advisor
Project: Avian malaria and bird conservation in Hawai‘i

2019 Jeromalyn Santos, REU Intern, University of Guam
Mentor
Project: Wolbachia genetic diversity in *Culex quinquefasciatus* mosquitoes on O‘ahu

2019 Kahiwahiwa Davis, REU Intern, Gonzaga University
Mentor
Project: Larval mosquito development and microbiota assembly across different plant sources of detritus

2018 Kristen Feato, REU intern, Chaminade University
Mentor
Project: The avian microbiome of O‘ahu forest birds

2018 Ma. Vida Amor Echaluse, REU intern, Northern Marianas College
Mentor
Project: Angiostrongylus transmission in suburban environments across an environmental gradient

2017-2020 Alex Ching, Masters student, University of Hawai‘i at Mānoa, Entomology
Committee member
Project: Microbiome of tephritid flies.

2017- Randi Rollins, PhD student, University of Hawai‘i at Mānoa, Zoology
Committee member
Project: The ecology of *Angiostrongylus* transmission.

2017- Maria Costantini, Ph.D. candidate, University of Hawai‘i at Mānoa, Zoology
Committee member
Project: Role of the avian microbiome in conservation.

2017-2018 Stevie Kennedy-Gold, Masters student, University of Hawai‘i at Mānoa, Zoology
Committee member
Project: Behavioral changes in lizards across different communities

2016-2018 Rachel Sommer, Masters student, University of Hawai‘i at Mānoa, Zoology
Committee member
Project: Two invasive veronicellid slugs in the Hawaiian Islands: life history and microbiome

2017 Rachelle Tom, REU intern, Kapiolani Community College
Mentor
Project: The distribution of mosquitoes across an elevational gradient

2016- Priscilla Seabourn, Ph.D. candidate., University of Hawai‘i at Mānoa, Entomology
PhD Advisor
Project: Ecology of mosquitoes on Maui, Hawai‘i

2016- Robyn Screen, Ph.D. candidate, University of Hawai‘i at Mānoa, Zoology
Committee member
Project: Behavioral and stress responses of *Anolis sagrei* to urban habitats

2015-2019 Andrew Golnar, Ph.D. student, Texas A&M University, Entomology
Committee member
Project: The influence of coinfection on arbovirus transmission ecology

2014-2015 Nicole Dubois, Master’s student, Universidade de Brasília
Data analysis training
Project: Adaptive nest site choice in *Aratinga aurea*

2014-2015 Gabriela Correa, Master’s student, Universidade de Brasília
Data analysis training
Project: Changes in avian community structure after fire

2010-2011 Jon-Erik Hansen, Undergraduate student, University of Missouri-St. Louis
Laboratory training
Project: *Avian Haemosporida in mosquito vectors*.
Currently a laboratory technician at Monsanto, Inc.

2010 Genevieve Pang, Undergraduate student, Washington University
Laboratory training
Project: *Avian Haemosporida in Panamanian bird.*
Currently a graduate student at Michigan State University.

2005- Supervised more than 30 assistants, undergraduate students, and graduate students in ornithological fieldwork techniques.

graduate students for whom I serve as committee chair are bolded

VI. RESEARCH

***Overall research statement*:** I am a natural historian at heart who is fascinated with the complexity of nature. This fascination compels me to maintain diverse interests in ecology, evolution, and conservation biology. While my research retains distinct foci, I am eager to explore questions that broaden my appreciation for how organisms interact with each other and their environment. A central theme of my work is to combine field, laboratory, and mathematical and theoretical techniques to answer questions in ways that could not be achieved through one technique alone. Currently, my primary research focuses on the proximate and ultimate drivers of infectious disease transmission across various ecological scales, from dynamics within host individuals, to those that manifest within and between ecological communities across heterogeneous landscapes. By elucidating these mechanisms that modulate infectious disease dynamics, we aim to inform strategies that limit opportunities for infectious disease emergence and develop evidence-based approaches that mitigate transmission risks.

VII. PROFESSIONAL SERVICE, ACTIVITIES, AND IMPROVEMENT

Peer Reviewer

The Auk
Biological Invasions
Behavioral Ecology and Sociobiology
Ecohealth
International Journal of Parasitology
Journal of Animal Ecology
Ecology
Journal of Parasitology
Malaria Journal
Parasites and Vectors
PeerJ
PLoS NTD
The ISME Journal
Microbiome

Classes and Workshops

2019 National Science Foundation DEB REU workshop in in Roslynn, VA
2018 National Science Foundation GEO REU workshop in Boulder,CO
2009 Data Analysis and Presentation in R workshop
University of Missouri-St. Louis

Working Groups

2017- Hawai‘i Bird Conservation Forum
2016- Hawai‘i Mosquito Working Group
2016- All-Mosquito Working Group (invitation only)
A gathering of local, national, and international experts to review options for mosquito population suppression in Hawai‘i.

International Meetings and Presentations

2019 American Society of Tropical Medicine and Hygiene Annual Meeting (poster presentation)

2008	American Association for the Advancement of Science-Pacific regional meeting (oral presentation)
2005	Cooper Ornithological Society Annual Meeting 2005 (oral presentation)
2004	Cooper Ornithological Society Annual Meeting 2004 (oral presentation)

Invited Talks

2020	Ecology, Evolution, and Conservation Biology, University of Hawai‘i at Mānoa
2019	Hawaii Department of Health Vector Control Workshop Keynote
2019	Natrual Resource and Environmental Management, University of Hawai‘i at Mānoa
2018	Pacific Birds meeting
2018	University of Hawai‘i-West O‘ahu Math+Science+*X* seminar
2017	Department of Biology seminar series, University of Hawai‘i at Mānoa
2017	Pacific Biosciences Research Center, University of Hawai‘i at Mānoa
2016	Pacific Biosciences Research Center, University of Hawai‘i at Mānoa
2015	Wildlife Disease Association-Texas A&M Student Chapter
2015	Vector Seminar Series, Texas A&M University

VIII. GRANTS, FELLOWSHIPS, and SCHOLARSHIPS AWARDED

Grants-Awarded

2020-	$378,652	National Science Foundation DBI 1659889 REU SIE: Environmental Biology for Pacific Islanders (PI: Medeiros)
2018-	$1,061,250	National Institutes of Health P20GM125508-01 Integrative Center for Environmental Microbiomes and Human Health (Role: Project Leader) Project: Microbiome Diversity in Insect Vectors and its Influence on Pathogen Transmission (PI: Medeiros)
2017-2018	$700,000	Strategic Investment Initiative , Office of the Vice Chancellor for Research, University of Hawai‘i at Mānoa Microbiomes of Hawaiian ahupua‘a (ridge-to-reef) watersheds: Data acquisition and mathematical analysis to discover the basis of sustainability across vital Hawaiian landscapes Awarded to C-MĀIKI - Center for Microbiome Analysis through Island Knowledge and Investigation (Role: Investigator/ Member of C-MĀIKI Leadership Team)
2017-2019	$414,604	National Science Foundation REU Site DBI 1659889 Environmental Biology for Pacific Islanders (Role: Co-PI, PI: M. Hadfield)
2016-2018	$222,750	National Institutes of Health R21 AI128953-01 Social-ecological factors influencing receptivity to Zika virus and the efficacy of interventions in communities along the Texas-Mexico border. (Role: Investigator, PI: G.L.Hamer)
2016-2017	$250,000	Lawrence Livermore National Laboratory (05/01/16-04/30/19) Integrated vector-animal-human test bed for surveillance of high-consequence trans-boundary infectious diseases (role: Investigator, PI: G.L. Hamer)

Research Grants-Pending

2020-2024	$20,000,000	EPSCoR RII Track-1 MIDAAS- Microbiomics Integrated with Data Analytics to Advance Sustainability. (PI: Gwen Jacobs, Role: Project Leader; Mosquitoes: Biocontrol of invasive species through microbiome assisted rearing.)

Scholarships and Fellowships

2012-2013	Dissertation Fellowship

University of Missouri- St. Louis
University of Missouri-St. Louis Graduate School Fellowship in the amount of $15,000 to support a student during the final stages of a dissertation

2010 Raven Fellowship
University of Missouri- St. Louis
Department of Biology Fellowship in the amount of $7500 to support a student while conducting research for a semester.

2004-2005 Minority Access to Research Careers research student
University of Hawai'i at Mānoa
Tuition and stipend, advisor: Rebecca Cann, Leonard A. Freed; project: Avian malaria among a lowland community of forest birds on O‘ahu, Hawaii

2004 Research Experience for Undergraduates student
University of Notre Dame
Summer stipend, advisor: John Adams, project: Isolation and characterization of MAEBL (merozoite apical erythrocyte-binding ligand) in rodent malaria

2003-2004 Haumana Biomedical Program research student
University of Hawai‘i at Mānoa
Stipend, advisor: Leonard A. Freed; project: Reproductive Biology of the Hawai'i ‘Ākepa (*Loxops coccineus coccineus*)

2003-2004 Presidential Scholar
University of Hawai‘i at Mānoa
Tuition and stipend

IX. PUBLICATIONS

Golnar, A, **Medeiros, M.C.I.**, Rosenbaum, K., Bejcekm J. Hamer, S.A., & Hamer, G.L. (2021). Vector-borne blood-parasites of the great-tailed grackle (*Quiscalus mexicanus*) in east-central Texas, USA. *Microorganisms*, *9:* 504.

Juarez, J.G., Garcia-Luna, S. , **Medeiros, M.C.I.,** Dickinson, K.L.**,** Borucki, M.K., Frank, M., Badillo-Vargas, I., Chaves, L.F., & Hamer, G.L. (2021). The eco-bio-social factors that modulate *Aedes aegypti* abundance in South Texas border communities. *Insects* 12:183.

Rollins, R. L., Cowie, R. H., Echaluse, M. V., & **Medeiros, M.C.I.** (2021). Host snail species exhibit differential *Angiostrongylus cantonensis* prevalence and infection intensity across an environmental gradient. *Acta Tropica* 216: 105824. https://doi.org/10.1016/j.actatropica.2021.105824.

Seabourn, P.S., Spafford, H., Yoneishi, N.M., & **Medeiros, M.C.I.** (2020). The *Aedes albopictus* (Diptera: Culicidae) microbiome varies spatially and with Ascogregarine infection. *PLoS Neglected Tropical Diseases* 14(8): e0008615. https://doi.org/10.1371/journal.pntd.0008615

Medeiros, M. C., Rollins, R. L., Echaluse, M. V., & Cowie, R. H. (2020). Species identity and size are associated with rat lungworm infection in gastropods. *EcoHealth*, *17*(2), 183-193.

Poh, K. C., **Medeiros, M. C**.I., & Hamer, G. L. (2020). Landscape and demographic determinants of Culex infection with West Nile virus during the 2012 epidemic in Dallas County, TX. *Spatial and Spatio-temporal Epidemiology*, 33, 100336.

Cowie, R.H., Rollins, Randi L., **Medeiros, M.C.I.,** & Christensen, C.C. (2019) New records of Clausiliidae: *Tauphaedusa tau* (Boettger, 1877)(Gastropoda: Heterobranchia) on O‘ahu, Hawaiian Islands, and the first global record of infection of a clausiliid land snail with *Angiostrongylus cantonensis* (Chen, 1935), the rat lungworm. Bishop Museum Occasional Papers 126, 11–18.

Martin, E., **Medeiros, M.C.I.**, Carbajal, E., Valdez, E., Juarez, J.G., Luna, S.G., Salazar, A., Qualls, W.A., Hinojosa, S.,

Borucki, M.K. & Manley, H.A. (2019). Surveillance of *Aedes aegypti* indoors and outdoors using Autocidal Gravid Ovitraps in South Texas during local transmission of Zika virus, 2016 to 2018. Acta Tropica 192, 129-137.

Martin, E., Chu, E., Shults, P., Golnar, A., Swanson, D. A., Benn, J., Kim, D., Schneider, P., Pena, S., Culver, C., **Medeiros, M. C. I.**, Hamer, S.A., & Hamer, G.L. (2019). *Culicoides* species community composition and infection status with parasites in an urban environment of east central Texas, USA. Parasites & Vectors, 12(1), 39.

Hynson N., Frank K., Alegado R., Amend A., Arif M., Bennett G., Jani A., **Medeiros M.**, Mileyko Y., Nelson C., Nguyen N., Nigro O., Prisic S., Shin S., Takagi D., Wilson S., & Yew J. (2018) Synergy among microbiota and their hosts: leveraging the Hawaiian archipelago and local collaborative networks to address pressing questions in microbiome research. *mSystems* 3, e00159-17.

Ricklefs, R. E., Ellis, V. A., **Medeiros, M. C.I.,** & Svensson-Coelho, M. (2018) Duration of embryo development and the prevalence of haematozoan blood parasites in birds. *The Auk*, 135, 276-283.

Fecchio, A., Svensson-Coelho, M., Bell, J., Ellis, V.A., **Medeiros, M.C.I.**, Trisos, C.H., Blake, J.G., Loiselle, B.A., Tobias, J.A., Fanti, R., Coffey, E.D., de Faria, I.P., Pinho, J., Felix, G., Braga, E.M., Anciães, M., Tkach, V., Bates, J., Witt, C., Weckstein, J.D., Ricklefs, R.E., & Farias, I.P. (2017). Host associations and turnover of haemosporidian parasites in manakins (Aves: Pipridae). *Parasitology* 144, 984-993.

Bertram M.R., Hamer G.L., Hartup B.K., Snowden K.F., **Medeiros M.C.I.**, & Hamer S.A. (2017). Haemosporida prevalence and diversity are similar in endangered wild whooping cranes (*Grus americana*) and sympatric sandhill cranes (*Grus canadensis*). *Parasitology* 144, 629-640.

Ricklefs, R. E., **Medeiros, M.C.I.**, Ellis, V. A., Svensson-Coelho, M., Blake, J. G., Loiselle, B. A., Soares, L., Fecchio, A., Outlaw, D.C., Marra, P.P, Latta, S.C., Valkiūnas, G., Hellgren, O., & Bensch, S. *ahead of print.* Avian migration and the distribution of malaria parasites in New World passerine birds. *Journal of Biogeography* 44, 1113-1123.

Medeiros, M.C.I*, Boothe, E.*, Roarke, B., & Hamer, G.L. (2017) Dispersal of male and female *Culex quinquefasciatus* and *Aedes albopictus* mosquitoes using stable isotope enrichment. *PLoS Neglected Tropical Diseases* 11: e0005347. *These authors contributed equally.

Bertram, M., Hamer, S.A., Hartup, B.K., Snowden, K.F., **Medeiros, M.C.I**., Outlaw, D.C., & Hamer, G.L. (2017) A novel Haemosporida clade at the rank of genus in North American cranes (Aves: Gruiformes). *Molecular Phylogenetics and Evolution* 109, 73-79.

Ellis, V.A., **Medeiros, M.C.I.**, Collins, M.D., Sari, E.H.R., Coffey, E.D., Dickerson, R.C., Lugarini, C., Stratford, J.A., Henry, D.R., Merrill, L., Matthews, A.E., Hanson, A.A., Roberts, J.R., Joyce, M., Kunkel, M.R., Ricklefs, R.E. (2017) Prevalence of avian haemosporidian parasites is positively related to the abundance of host species at multiple sites within a region. *Parasitology Research,* 116 (1), 73-80.

Castellanos, A.A.*, **Medeiros, M.C.I**.*, Hamer, G.L., Morrow, M.E., Eubanks, M.D., Teel, P.D., Hamer, S.A., Light, J.E. (2016) Decreased small mammal and tick abundance in association with invasive red imported fire ants (*Solenopsis invicta*). Biology Letters, 12, 20160463. *These authors contributed equally.

Medeiros, M.C.I., Ricklefs, R.E., Brawn, J.D., Ruis, M.O., Goldberg, T.L., Hamer, G.L. (2016) Overlap in the seasonal infection patterns of avian malaria parasites and West Nile virus in vectors and hosts. *American Journal of Tropical Medicine and Hygiene,* 95, 1121-1129.

Lopes, L.E. Fernandes, A.M., **Medeiros,M.C.I**., Marini M.A. (2016) A classification scheme for avian diet types. *Journal of Field Ornithology, 87*(3), 309-322.

Meyers, J.I.,Pathikonda, S., Popkin-Hall, Z.R., **Medeiros, M.C.I.**, Fuseini, G., Matias, A., Garcia, G., Overgaard, H.J.,

Kulkarni, V., Reddy, V.P., Schwabe, C., Lines, J., Kleinschmidt, I., Slotman, M.A. (2016) Increasing outdoor host-seeking in *Anopheles gambiae* over 6 years of vector control on Bioko Island. *Malaria Journal*, *15*(1), 1.

Freed, L. A., **Medeiros, M. C.I**., Cann, R. L. (2016) Multiple reversals of bill length over 1.7 million years in a Hawaiian bird lineage. *The American Naturalist*, *187*(3), 363-371.

Medeiros, M. C. I., Ricklefs, R.E., Brawn, J.D., Hamer, G. L. (2015) *Plasmodium* prevalence across avian host species is positively associated with exposure to mosquito vectors. *Parasitology,* 142(13), 1612-1620.

Ellis, V.A., Collins, M.D., **Medeiros, M.C.I.**, Sari, E.H.R., Coffey, E.D., Dickerson, R.C., Lugarini, C., Stratford, J.A., Henry, D.R., Merrill, L., Matthews, A.E., Hanson, A.A., Roberts, J.R., Joyce, M., Kunkel, M.R., Ricklefs, R.E. (2015) Local host specialization, host-switching, and dispersal shape the regional distributions of avian haemosporidian parasites. *Proceedings of the National Academy of Sciences*, 112(36) 11294-11299.

Boothe, E., **Medeiros, M. C. I**., Kitron, U. D., Brawn, J. D., Ruiz, M. O., Goldberg, T. L., Walker, E.D., Hamer, G. L. (2015) Identification of avian and hemoparasite DNA in blood-engorged abdomens of *Culex pipiens* (Diptera; Culicidae) from a west Nile virus epidemic region in suburban Chicago, Illinois. *Journal of Medical Entomology*, *52*(3), 461-468.

Medeiros, M.C.I., Ellis, V.A., Ricklefs, R.E. (2014) Specialized avian Haemosporida trade reduced host breadth for increased prevalence. *Journal of Evolutionary Biology*, *27(11)*, 2520-2528.

Ricklefs, R.E., Outlaw, D.C., Svensson-Coelho, M., **Medeiros, M.C.I.**, Ellis, V.A., Latta. S. (2014) Species formation by host shifting in avian malaria parasites. *Proceedings of the National Academy of Sciences of the United States of America, 111 (41*), 14816-14821.

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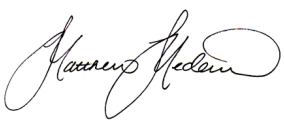
I acknowledge that this CV is the most current and correct as of the date of the signature.

Signature  Date: 4 March 2020

ATTACHMENT 3

Standard Operating Procedures: Mosquitoes Updated: March 9, 2021 Reed Research Group, School of Life Sciences, University of Hawai‘i at Mānoa
Lead PI: Dr. Floyd Reed

Standard Operating Procedures

Mosquitoes

Mosquito rearing protocol: Mosquito eggs and/or larvae are easily collected from the wild by using standard traps (containers of aged tap water and grass clippings). Egg rafts and larvae are easily identifiable to species. Larvae are reared in tap water and fed with commercially available fish food pellets. Once larvae develop to pupae, they are transferred to 1- or 2-square foot mosquito-rearing cages (*e.g.,* BioQuip; https://www.bioquip.com) to eclose (*i.e.,* former pupae emerge as adults). Adults are provided with sugar water as a food source. Adult female *Culex quinquefasciatus* and *Aedes albopictus* require blood meals for egg development. When adults are approximately one week old they are provided up to three separate blood meals. Bloodmeals are provided in the form of commercially available bovine blood (available from Lampire Biological Products; blood is obtained from healthy, adult animals; www.lampire.com). Approximately 2-7 days after bloodmeals, containers of water are added to the adult cages to provide oviposition substrate to the gravid females. Eggs are transferred to larval rearing trays. The approximate generation time is 3-4 weeks.

Safety handling practices: Latex or nitrile gloves should be worn when handling bloodmeal material. Latex or nitrile gloves should be worn when personnel put their hands into adult cages. Mosquito traps should be checked regularly (*e.g.,* at least monthly) to ensure they are functioning properly. Electric (battery operated) aspirators, rather than mouth aspirators, should be used to extract individual adults from cages as required.

Disposal: Adult cages that have produced eggs are frozen for at least 24 hours to kill the mosquitoes. Larvae can also be killed by freezing for at least 24 hours. Between uses, all materials (*e.g.,* cages) are washed with commercial dish soap. If disinfection is deemed necessary, 70% ethanol or 10% bleach solution is used as appropriate. Biological materials are autoclaved as necessary.

Authorship: This original document was primarily written by Dr. Jolene Sutton and edited by Dr. Floyd Reed as necessary to keep it up to date.

ATTACHMENT 4

DAVID Y. IGE
GOVERNOR OF HAWAII

STATE OF HAWAII
DEPARTMENT OF LAND AND NATURAL RESOURCES

POST OFFICE BOX 621
HONOLULU, HAWAII 96809

SUZANNE D. CASE
CHAIRPERSON
BOARD OF LAND AND NATURAL RESOURCES
COMMISSION ON WATER RESOURCE MANAGEMENT

ROBERT K. MASUDA
FIRST DEPUTY

M. KALEO MANUEL
DEPUTY DIRECTOR - WATER

AQUATIC RESOURCES
BOATING AND OCEAN RECREATION
BUREAU OF CONVEYANCES
COMMISSION ON WATER RESOURCE MANAGEMENT
CONSERVATION AND COASTAL LANDS
CONSERVATION AND RESOURCES ENFORCEMENT
ENGINEERING
FORESTRY AND WILDLIFE
HISTORIC PRESERVATION
KAHOOLAWE ISLAND RESERVE COMMISSION
LAND
STATE PARKS

April 30, 2020

Phyllis Shimabukuro-Geiser, Chair
Hawai‘i Department of Agriculture
1849 Auiki Street
Honolulu, HI 96819

Aloha Chair Shimabukuro-Geiser,

I am writing to support the permit applications submitted jointly by the University of Hawai‘i at Mānoa and Hilo for the importation of *Culex quinquefasciatus* (*Cx. quinquefasciatus*) mosquitoes. The Department of Land and Natural Resources understands that the University of Hawai‘i is seeking approval to import *Cx. quinquefasciatus* containing *Wolbachia* strains *wPip4*, *wAlbA,* and *wAlbB*. These permit applications are integral to a statewide initiative, of which DLNR is a part, to suppress *Cx. quinquefasciatus* using the Incompatible Insect Technique in order to prevent the imminent extinction of many native Hawaiian forest bird species from mosquito-borne avian malaria. Protection of our native forest birds is one of the highest priorities for DLNR.

The Incompatible Insect Technique acts as a form of “birth control” by releasing male mosquitoes transinfected with a strain of the bacterium *Wolbachia* that is different from the strains found in Hawai‘i. When lab-reared male *Cx. quinquefasciatus* that are infected with *Wolbachia* strains *wAlbA*, *wAlbB*, or *wPip4* mate with wild females that have the Hawai‘i *wPip5* or *wPip3* strains, little to no viable larvae are produced. This technique requires repeat releases of lab-reared males to flood the population. If releases stop, mosquito population levels will rebound. This technique is safely being used worldwide to suppress populations of human disease-carrying mosquitoes.

DLNR requests that the Department of Agriculture consider these permit applications under any and all DOA statutes and administrative rules that might help to expedite permit approval while ensuring appropriate public input and environmental safeguards. In particular, DLNR draws attention to HRS §150A-6.2, which allows the DOA to issue, on a case-by-case basis, a “special permit” for the introduction of unlisted animals, such as *Cx. quinquefasciatus*, for the purpose of remediating medical emergencies or agricultural or ecological disasters, or conducting scientific research that is not detrimental to agriculture, the environment, or humans. The importer must meet permit requirements or other guidelines as determined by the Board of Agriculture.

I. The looming extinction of numerous endemic forest birds qualifies as an "ecological disaster" sufficient to justify issuance of a special permit under HRS §150A-6.2.

The forests of Hawai‘i once held more than 100 species of native birds. Today, only 21 species of forest birds persist in the main Hawaiian Islands, with 12 of these currently endangered or threatened. Those that persist do so in greatly-diminished numbers, with severely contracted ranges mostly limited to high-elevation remnant native forests out of reach of non-native disease. These native birds serve critical ecological functions in our forests as pollinators and seed dispersers for the shrubs and trees that comprise our life-giving watersheds. Native forest birds also hold prominent places in native Hawaiian culture; many ‘ōlelo no‘eau tell of this deep cultural connection.

Avian malaria is a primary contributor to population range limitations, declines, and extinctions for Hawaiian honeycreepers. Further, this impact is predicted to be exacerbated by climate change that will allow mosquitoes to occupy currently mosquito-free high elevation forest. Five honeycreeper species are likely to lose all or most of their range and become extinct by 2100 due primarily to avian malaria: the ‘akeke‘e (945 individuals remaining), ‘akikiki (468 individuals), ‘ākohekohe (1,768 individuals), Hawai‘i ‘ākepa (13,892 individuals), and kiwikiu (157 individuals). Another two species are predicted to lose over 75% of their already greatly diminished current range by 2100: `Akiapōlā`au (1,496 individuals) and ‘Alawī (12,501 individuals).

In 2016, a multi-agency group convened in Hawai‘i to seek strategic solutions to eliminate mosquito-borne diseases affecting humans and wildlife and concluded that use of the Incompatible Insect Technique to suppress *Cx. quinquefasciatus* mosquitoes is currently the most feasible option for saving the last of Hawai‘i's forest bird species.

II. Issuance of a special permit under HRS §150A-6 is justified because the importation is for the purpose of conducting non-detrimental scientific research, specifically to explore the use of the Incompatible Insect Technique for conservation purposes.

DLNR understands that the request by the University of Hawai‘i to import transinfected *Cx. quinquefasciatus* mosquitoes will be quality tested to verify the reproductive incompatibility of the Wolbachia strain. If the ecological disaster classification is granted, the field releases of these mosquitoes will begin, while the UH labs can conduct additional research with this strain.

While the permit applications also seek approval for the eventual release of these mosquitoes, no releases will occur before first securing applicable permits from the U.S. Environmental Protection Agency. Additionally, DLNR is actively involved in the multi-agency Steering Committee for Landscape-scale Mosquito Control, including efforts to ensure meaninful community engagement about this issue. DLNR anticipates that an Environmental Assessment will be necessary before any release occurs at a landscape level to meet National Environmental Policy Act and Hawaii Environmental Policy Act requirements.

Additionally, information gained from this research is likely to yield lessons applicable to the potential use of the technique for human health purposes. During the 2019 legislative session, the Hawai‘i State Legislature demonstrated its interest in this technique by its request for a multi-

agency report on the potential use of the *Wolbachia* bacteria to control mosquitos on a landscape scale for disease control.

DLNR also notes that the permit applications seek to bring into the State a species that is already widespread in the Islands. *Culex quinqefasciatus* was the first, and one of the most serious insect pests to come to Hawai‘i, believed to have arrived on a ship in 1826. The purpose of this importation is to reduce the undeniably adverse impacts of this invasive species.

Thank you for your consideration. Please don't hesitate to ask if further clarification is needed.

Sincerely,

DGS

SUZANNE D. CASE
Chairperson

cc: Floyd Reed, Ph.D., University of Hawai‘i at Mānoa
Jolene Sutton, Ph.D., University of Hawai‘i at Hilo
Natalie Gates, DVM, Superintendent, Haleakalā National Park
Bruce Anderson, Ph.D., Hawai‘i Department of Health
Katherine Mullett, U.S. Fish and Wildlife Service
Hawai‘i Department of Agriculture, Plant Quarant

Signature: DGS

Email: david.g.smith@hawaii.gov

State of Hawaii
Department of Agriculture
PLANT QUARANTINE BRANCH
1849 Auiki Street, Honolulu, HI 96819-3100
Phone: (808) 832-0566, FAX: (808) 832-0584

For Office Use Only

Fee: $_____ Receipt No. _____

☐ Approve Permit No._____ Date: _____

☐ Disapprove ☐Other _____

Processed by:_____ Date:_____

PERMIT APPLICATION FOR RESTRICTED COMMODITIES INTO HAWAII

Date: *April 6, 2021*

In accordance with the provision of Chapter HRS 150A , Hawaii Administrative Rules of the Division of Plant Industry, Department of Agriculture, a permit is requested for the following commodities:

Please type or print clearly.

Quantity	Commodity	Scientific Name
12,500	Southern house mosquitoes containing Wolbachia	Wolbachia albopictus strain A
12,500	Southern house mosquitoes containing Wolbachia	Wolbachia albopictus strain B

RECEIVED
APR 19 2021
PLANT QUARANTINE BRANCH

NO PAYMENT
Date: 4/19/21 Initial: TSY

Name and address of shipper: _____

_____

(Mainland or Foreign address)

Approximate date of arrival: _____

Mode of Shipment: ☑ Mail ☐ Air Freight ☐ Boat

Type of Permit:
- --- Import
  - ☐ one time only ☐ multi-shipments
- --- Intrastate shipment
  - ☐ one time only ☑ multi-shipments
- ☐ Possession

Object of importation:
- ☐ Kept caged at all time
- ☐ Used for propagation
- ☐ Imported for exhibition
- ☐ Imported for liberation
- ☑ Other purposes - specify For research in laboratories at the University of Hawaii

Please type or print clearly.

Applicant's Name Floyd Reed: Matthew Medeiros

Company Name University of Hawaii
(if applicable)

Hawaii Mailing Address Please see attached

_____

Telephone number Please see attached

Facsimile number n/a

Fee Amount Enclosed (cash, check or mail order) $ Please see attac

(complete reverse side)

PLEASE COMPLETE THE FOLLOWING INFORMATION (attach extra sheet if necessary)

1. State in detail the reasons for introduction (include use or purpose).

   Please see attached.

2. Person responsible for the organism (include name, address and phone number).

   Please see attached.

3. Location(s) where the organism will be kept and used (include address, contact and phone number).

   Please see attached.

4. Method of disposition.

   Please see attached.

5. Give an abstract of the organism with particular reference to potential impact on the environment of Hawaii (include impact to plants, animals and humans).

   Please see attached.

****

I request permission to import the articles as listed on the permit application and further, request that the articles be examined by an authorized agent of the Department of Agriculture upon arrival in Hawaii.

I agree that I, as the importer, will be responsible for all costs, charges or expenses incident to the inspection or treatment of the imported articles.

I further agree that damages or losses incident to the inspection or the fumigation, disinfection, quarantine, or destruction of the articles, by an authorized agent of the Department of Agriculture, shall not be the basis of a claim against the department or the inspectors for the damage or loss incurred.

Signature [redacted] **Date** April 7, 2021

(Applicant)

State of Hawaii
Department of Agriculture
PLANT QUARANTINE BRANCH
1849 Auiki Street, Honolulu, HI 96819-3100
Phone: (808) 832-0566, FAX: (808) 832-0584

For Office Use Only

Fee: $_____ Receipt No. _____

☐ Approve Permit No._____ Date: _____

☐ Disapprove ☐Other _____

Processed by: _____ Date: _____

PERMIT APPLICATION FOR RESTRICTED COMMODITIES INTO HAWAII

Date: April 12, 2021

In accordance with the provision of Chapter HRS 150A, Hawaii Administrative Rules of the Division of Plant Industry, Department of Agriculture, a permit is requested for the following commodities:

Please type or print clearly.

Quantity	Commodity	Scientific Name
12500	Southern house mosquitoes containing Wolbachia	Culex quinquefasciatus with Wolbachia albopictus strain A
12500	Southern house mosquito containing Wolbachia	Culex quinquefasciatus with Wolbachia albopictus strain B

RECEIVED
APR 19 2021
PLANT QUARANTINE BRANCH

NO PAYMENT
Date: 4/19/21 Initial: TSY

Name and address of shipper: _____

(Mainland or Foreign address)

Approximate date of arrival: _____

Mode of Shipment: ☑ Mail ☐ Air Freight ☐ Boat

Type of Permit:
- --- Import
  - ☐ one time only ☐ multi-shipments
- --- Intrastate shipment
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- ☐ Possession

Object of importation:
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- ☐ Used for propagation
- ☐ Imported for exhibition
- ☐ Imported for liberation
- ☑ Other purposes - specify For research in laboratories at the University of Hawaii

Please type or print clearly.

Applicant's Name Matthew Medeiros

Company Name University of Hawaii
(if applicable)

Hawaii Mailing Address See attached

Telephone number see attached

Facsimile number n/a

Fee Amount Enclosed (cash, check or mail order) $ see attached

(complete reverse side)

PLEASE COMPLETE THE FOLLOWING INFORMATION (attach extra sheet if necessary)

1. State in detail the reasons for introduction (include use or purpose).
   See attached

2. Person responsible for the organism (include name, address and phone number).
   See attached

3. Location(s) where the organism will be kept and used (include address, contact and phone number).
   See attached

4. Method of disposition.
   See attached

5. Give an abstract of the organism with particular reference to potential impact on the environment of Hawaii (include impact to plants, animals and humans).
   See attached

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I agree that I, as the importer, will be responsible for all costs, charges or expenses incident to the inspection or treatment of the imported articles.

I further agree that damages or losses incident to the inspection or the fumigation, disinfection, quarantine, or destruction of the articles, by an authorized agent of the Department of Agriculture, shall not be the basis of a claim against the department or the inspectors for the damage or loss incurred.

Signature _____ **Date** April 12 2021

(Applicant)