Ultraviolet Communication in the Kangaroo Rat (*Dipodomys ordii*)

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PROJECT DESCRIPTION

Specific Aims - The objective of this project is to determine if Ord’s Kangaroo rat (*Dipodomys ordii* [family: Heteromyidae]) has the capabilities to transmit signals in the ultraviolet (UV) spectrum and perceive UV signals via their vision system. To accomplish this objective, we will conduct experiments aimed at answering two questions: (1) does *Dipodomys ordii* have the ability to perceive signals in the UV spectrum? (2) do the UV-reflective body markings of *Dipodomys ordii* correlate with any environmental elements, including morphology of a known predator (potential adaptive mimicry)? This proposal is a request for money needed to continue experiments previously funded. As such, a report of current progress is attached as a supplement to this proposal. Data collected previously supports an earlier hypothesis and compels us to continue experimentation.

METHODS

The species of interest, *Dipodomys ordii*, has no protective status under legislative statutes. Specimens will be collected with Sherman live traps (HB Sherman Co., Tallahassee, FL) under a Scientific Collector’s Permit issued to BK McDonald by the Oklahoma Department of Wildlife Conservation for 2010-2011. Animals will be euthanized by thoracic compression. All methods will follow guidelines established by the American Society of Mammalogists (Gannon et al. 2007). Field sites for the collection of *Dipodomys ordii* (experimental) and the house mouse (*Mus musculus* [positive control]) are located at the following coordinates: 35°42’04.47”N 98°28’34.74”W (Blaine County) and 35°22’38.11”N 98°06’55.14”W (Caddo County). Specimens of the Syrian hamster (*Mesocricetus auratus* [negative control]) will be obtained commercially from licensed vendors. Specimens will be obtained as needed throughout the duration of this study. The following methods are directly associated with the specific aims addressed previously.
SPECIFIC AIM #1 – “Does Dipodomys ordii have the ability to perceive signals in the UV spectrum?”

Western Blot – Western blot (Burnette 1981) analysis will be used to detect the UV-sensitive opsin in total protein isolated from retinas; the UV-sensitive opsin protein is a prerequisite for UV-vision. This analysis will also allow us to analytically compare the protein of interest to that of other retinal opsin proteins based on known molecular weights. Proteins isolated from retinas will be subjected to SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and blotted to a nitrocellulose membrane. The membrane will be treated with commercial primary antibodies synthesized to react against the proteins of interest. Results of western blot analysis will be compared to data from DNA and RNA level assays (previously funded); protein-level data will be a valuable supplement for completing Specific Aim #1. Western blot will also detect additional opsins in the retina including middle wavelength sensitive opsin (green-yellow vision), long wavelength opsin (orange-red vision), and rhodopsin (night vision). Descriptive presence/absence data for the various opsin proteins (including UV-opsin) will be a contribution to science, as the visual system of Dipodomys ordii has not been described.

Immunohistochemical Staining and Fluorescence Microscopy – In addition to determining the presence of the UV-opsin, we will use commercially available antibodies against the UV-opsin protein to map the distribution of the opsin proteins in the retina. We will follow the protocol of Williams et al (2005) and use indirect immunofluorescence microscopy to examine retinal tissue from Dipodomys ordii. Antibody staining of the retina will confirm the presence, overall distribution, and relative abundances of the UV-sensitive opsin protein in the retina as well as other relevant opsin proteins mentioned previously. This technique will allow for a more complete understanding of the kangaroo rat vision system and will help to determine the evolutionary history of the species based on comparative physiology of the eye. This is the first study to examine the molecular structure of the eye of any species in the family Heteromyidae.
Specific Aim #2 – “Do UV-reflective body markings of *Dipodomys ordii* correlate to any environmental elements?”

**Ultraviolet Photography** – Data collected thus far (see progress report) has confirmed that the body markings previously hypothesized to reflect UV-light do reflect strongly between 350-400nm; all previously discovered animal UV-communication occurs between 350-400nm. More importantly, the discrepancy in UV-reflection between the body markings of interest (experimental hairs) and the remainder of the animal’s body (control hairs) establishes the contrast necessary for potential communication in the UV-spectrum. With supporting data now available, we propose to use a UV-sensitive camera to investigate the environment (habitat and other species) of *Dipodomys ordii* for additional UV-patterns. Based on observations made previously, the UV-reflective morphological patterns of *Dipodomys ordii* are similar to markings of a known predator (rattlesnake). Previous researchers have noted unusual interactions between the kangaroo rat and rattlesnake in the form of nose-to-nose contact (Randell et al 1995). We hypothesize that the markings, which appear on both species, allow *Dipodomys ordii* to mimic a lethally venomous species (Batesian mimicry), one of many potential secondary adaptational effects of the UV-markings (i.e., exaptation). UV-photography will allow for an immediate testing of the mimicry hypothesis. The acquisition of a UV-camera outfit will also allow for the successful testing of UV-reflectance for a future field experiment where the UV-markings are manipulated and survivorship is calculated between kangaroo rats with blotted out UV-markings and those with un-manipulated body markings. This experiment will test the affects of the body markings on survival and reproduction thereby determining whether or not the body markings are under the influence of natural selection. The latter experiment will be a valuable extension allowing us to put the molecular data into a larger perspective with phenotype/environment interactions (i.e., natural selection). A UV-camera will also be used in future exploratory analyses of UV-reflective morphology of species closely related to *Dipodomys ordii*. 
BACKGROUND

Visual communication in the UV-spectrum has been observed in insects and plants, fish, amphibians, reptile, birds, and some mammals (Silberglied 1979; Kawamura S, Yokoyama 1996; Zhang 2003; Frentiu et al 2007; Hunt et al. 2007). UV-communication requires both signaling mechanisms and perception mechanisms. Signaling mechanisms include UV-reflective morphological characteristics such as hair, feathers, skin, and flowers. Such reflection of UV for communication is only known for wavelengths between 350-400nm (UV-A). For such signaling to serve as a means of communication, the vision system of the intended signal recipient must be able to perceive light wavelengths as short as 350nm. The ability of the visual system to perceive UV-light depends on many factors including transmission of UV light by the lens, cornea, and ocular media and successful absorption of UV by protein pigments (opsins) in the retina. Absorption of light energy by an opsin protein triggers a signaling cascade through the prosthetic molecule cis-retinal and optic nerve to the brain. Successful UV-vision is therefore highly dependent upon the presence of retinal opsin proteins capable of absorbing UV light.

Opsin proteins in vertebrates fall into five phylogenetic categories based on their respective wavelength sensitivities, referred to as λMAX. The five groups include: short wavelength type one (SWS1), Short wavelength type 2 (SWS2), Rhodopsin type one (RH1), Rhodopsin type two (RH2), Middle wave sensitive (MWS) and Long wave sensitive (LWS). Collectively, these opsins absorb light between 360-700nm which corresponds to UV and all visible light (colors). (Kawamura and Yokoyama 1996; Wilkie et al. 2000; Yokoyama et al. 2000; Yongsheng et al. 2001; Zhang 2003; Shi and Yokoyama 2003; Yongsheng and Yokoyama 2003; Yokoyama et al. 2007).

Ultraviolet communication in mammals remains a relatively new topic and is currently on the forefront of evolutionary biology. Evolutionary biologists often infer morphological, physiological, and behavioral character states of ancestral organisms from those of extant ones.
by principle of parsimony. However, the accuracy of such inferences is unknown due to the complexity of morphological, physiological, and behavioral traits. Recent advancements have been made at the protein and DNA sequence levels and in conjunction with computer modeling and increased power of statistical analyses, reliable inference of how such traits might evolve from ancestor to modern forms has become possible (Shi and Yokoyama 2003).

Research proposed here is aimed at examining the potential for UV-communication by *Dipodomys ordii*. This species belong to the Heteromyidae family of mammals (order: Rodentia) that has been used as an experimental model in evolutionary biology. Heteromyid rodents have been instrumental in understanding population genetics, intraspecific competition, interspecific competition, biogeography, predator-prey dynamics, chromosomal and molecular evolution, speciation, and physiological adaptations to extreme environments.

**LITERATURE CITED**


PERSONNEL INVOLVEMENT

The principle investigator in this project is BK McDonald. Dr. Dennis Frisby (CU, Dept. of Biological Sciences) will aid in molecular techniques. Undergraduate student Charles Bingham (CU) will assist in protein-level analyses. Friederike Jentoft and Mathew Wulfers (OU, Dept. of Chemical Engineering) have collected UV-reflectance data of morphological structures in the species of interest. Dr. Ramiro Moro and undergraduate student Brendon Jones (CU, Dept. of Physical Sciences) will collect data concerning transmittance of UV-light in the cornea, lens, and ocular media of Dipodomys ordii.
BENEFITS OF PROJECT

*University* - Students involved will gain first-hand experience in experimental design, data analysis, content knowledge, writing and peer-review, and presentation of results to an audience of peers. In addition, students will have the opportunity to be involved with other students and professors from collaborating institutions and academic departments thereby strengthening inter-institutional and cross-disciplinary relationships which are the cornerstone of modern science. Results of this project are anticipated to lead to publication in a national journal (e.g., Journal of Mammalogy, Evolution, and Molecular Ecology). Results will also be presented at local, state, regional, and possibly national meetings. These activities will serve to represent Cameron University in the scientific community at large. Cameron students will be given the option to use their experience in this research as their senior project for which they present their results to their respective Departments.

*Science* - Scientific advancements have often been the products of integrated approaches to scientific questions. This project is interdisciplinary, with data from Polymerase Chain Reaction (PCR), Comparative Retinal Morphology, Immunohistochemistry, UV-VIS Photospectrometry, Proteomics and field techniques. The questions addressed by this study require data across disciplinary boundaries and have thus far included results from two universities and three academic departments: (1) Cameron University Department of Biological Sciences, (2) Cameron University Department of Physical Sciences, and (3) University of Oklahoma School of Chemical Engineering. This project will contribute to the following sciences: Mammalogy, Molecular Ecology and Evolution, Animal Behavior, Physiological Ecology and Evolution, Phylogenetics, Bio-physics. This project could reveal phenomena with explanatory power in many specific fields of study in addition to those mentioned previously. Results from this project will be disseminated locally and regionally thereby confirming Cameron University’s involvement in the scientific community at large.
Professional Development - This project has encouraged and required the participants to expand beyond their respective disciplines, research experiences, and previous training. This leads to new knowledge along with application of previous knowledge in new contexts. This new-found knowledge and application finds its way into the classroom and is used to inspire students using real-world examples, therefore contributing to faculty development. The faculty participants will become engaged with other professionals from other disciplines, thereby strengthening interdisciplinary dialogue of intellectual nature.

Community - The experience gained by students associated with and involved actively in this project will serve in the interests of the local community. Students will leave Cameron University with problem-solving skills and technical knowledge which can be applied to issues affecting all arenas of life at the community level.

PROJECT DURATION

Conclusive results of this project should be available by fall, 2011.

BUDGET

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<td>Antibodies (primary &amp; secondary)</td>
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∑ = $2,600