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14. ABSTRACT
This project aimed to investigate individual variation in genes and behavior. Behavioral variation has been characterized by some in terms of Proactive and Reactive behavioral repertories, or 'personalities'. This project examined the behavior of African giant pouched rats (Cricetomys ansorgei, formerly C. gambianus) to understand their mating behavior and to determine the extent to which they demonstrate proactive and reactive personalities. The First Objective used field and lab methods to describe the basic biology of this species. The Second Objective was to assess behavior across a series of tests designed to determine the aggression, exploratory behavior, learning

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Report Title

Final Report: Predicting Behavioral Suites From Genetic Profiles: Variation in Mating, Aggression & Exploration in Pouched Rats

ABSTRACT

This project aimed to investigate individual variation in genes and behavior. Behavioral variation has been characterized by some in terms of Proactive and Reactive behavioral repertories, or 'personalities'. This project examined the behavior of African giant pouched rats (Cricetomys ansorgei, formerly C. gambianus) to understand their mating behavior and to determine the extent to which they demonstrate proactive and reactive personalities. The First Objective used field and lab methods to describe the basic biology of this species. The Second Objective was to assess behavior across a series of tests designed to determine the aggression, exploratory behavior, learning & memory, anxiety, and stress-coping responses of individuals to identify 'personality' types. The Third Objective was to identify genetic patterns associated with animals from the upper and lower quartiles tested in the second objective, in an effort to identify genetic signatures that predict behavioral predisposition for learning and exploration under different circumstances. Despite good progress, much of the project remains incomplete due to delays from importing animals and because the PI changed institutions. As a result, the state of the project is only partially complete. The project will continue under a new award (W911NF-14-1-0376) aimed at completing the aims outlined in this original project.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received   Paper

    TOTAL:

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received   Paper

    TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

(Supervised Graduate = * and Undergraduate = † students)

Cullin C, Sellers M†, Lee DN*, Ophir AG, Rogers E†, Scott K, Jackson T (2014) Occurrence of gut parasites and anthelmintic treatments in a laboratory colony of wild-caught Gambian pouched rats (Cricetomys gambianus). American Association for Laboratory Animal Science (AALAS), San Antonio, Texas, October 2014.

Cullin C, Sellers M†, Rogers E†, Scott K, Lee DN*, Ophir AG, Jackson T (2013) Depression and decreased fecal production in a Gambian pouched rat (Cricetomys gambianus). ['What's your diagnosis' session] American Association for Laboratory Animal Science (AALAS), Baltimore, Maryland, October 2013.

Scott K, Sellers M†, Rogers E†, Cullin C, Lee DN*, Ophir AG, Jackson T (2013) Blood collection techniques in Gambian pouched rats (Cricetomys gambianus). American Association for Laboratory Animal Science (AALAS), Baltimore, Maryland, October 2013.

Rogers E†, Lee DN, Ophir AG (2013) Environmental factors controlling estrous cycling in Cricetomys gambianus. NIH / Oklahoma State University. Veterinary Medicine Summer Research Training Program. Poster Symposium. July 29, 2013. Oklahoma State University. Stillwater, OK.

Rogers E†, Lee DN, Ophir AG (2013) Environmental factors controlling estrous cycling in Cricetomys gambianus. Merial-NIH National Veterinary Scholars Symposium. Michigan State University, East Lansing, Michigan. August 2012.

Number of Presentations: 5.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received   Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received   Paper

TOTAL:

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received   Paper

    TOTAL:

Number of Manuscripts:

Books

Received   Book

    TOTAL:

Received   Book Chapter

    TOTAL:

Patents Submitted

Patents Awarded

Awards

N/A

Graduate Students

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Danielle Lee	1.00
FTE Equivalent:	**1.00**
Total Number:	**1**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Alexander Ophir	0.08	
Michael Tobler	0.00	
Todd Jackson	0.00	
FTE Equivalent:	**0.08**	
Total Number:	**3**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Erin Rogers	0.00	
Matthew Sellers	0.25	
Ashley Collier	0.00	
Katherine Schwartz	0.00	
Alicia Maple	0.00	
Rhiannon Garner	0.00	
Ana Mosier	0.00	
Carlos Garcia	0.00	
Jacob Johnson	0.00	
Kathryn Collet	0.00	
Molly Fields	0.00	
Georgies Kenneth	0.00	
Cassandra Cullin	0.00	
Kathleen Scott	0.00	
FTE Equivalent:	**0.25**	
Total Number:	**14**	

Student Metrics
This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:...... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):...... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:...... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME   PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Final Report: 1 August 2011 – 31 August 2014

RE: Proposal No. 59156-LS / Agreement number W911NF1110111
PI: Ophir, AG
Title: Predicting Behavioral Suites From Genetic Profiles: Variation in Mating, Aggression & Exploration in Pouched Rats

This project was notified of award 3 February 2011 and awarded funds in 11 March 2011. Progress reports have been submitted in August 2011, 2012, and 2013. Funds for only two years of a five-year project were released and supported the work descried below. A three-year award has recently been made (W911NF-14-1-0376) to allow the completion of this project, which was prematurely terminated because the PI changed institutions.

The following achievements have been made since the last progress report.

Animal Care and Use Approval. All paperwork and permits to conduct field work in Tanzania have been acquired. We have kept our records up to date with IACUC and ACURO.

Field Work. The goal of our fieldwork experiment is to describe the social organization and nest composition of African giant pouched rats, and learn as much as we can about their natural history and basic biology. Our first field season began mid July through late September 2012. Our second field season began mid April through late June 2013. In this time, we have set up two large trapping grids and have been collecting data continuously with the help of local research assistants.

This is a large on-going project that will require at least one or two more field seasons to complete. Dr. Danielle Lee, the postdoc on this project, is planning a trip toward this end for October 2014. The research is no longer funded by the current award, but by a complementary award, designed to allow the completion of the original goals from the current project. (For practical reasons, the current project was terminated pre-maturely and the PI reapplied for funding to complete the work after changing institutions).

The state of this project is largely as it was at the time of the last progress report, but the data set has become increasingly rich. The data have qualitatively indicated that Tanzanian giant pouched rats prefer to live in forested areas near riverbeds where cover is open to moderately dense. They do not appear to inhabit or frequent the open grasslands or tall grassy shrub lands that can be found close to these niches. Females appear to nest alone or occasionally in groups with no males. Males appear to have much larger ranges that overlap multiple female home ranges and nesting sites. Young appear to stay in the natal habitat for an extended period of time and do not seem to emerge until several months old, as indicated by the weight of subjects captured so far. Dr. Lee has recently begun to compile the data for initial formal analyses. Our expectation is that data collection for this project will be complete by 2015, and analyses and publication will follow soon thereafter.

Behavioral Experiments: Social Behavior and Mating. We have two main lines of questions we plan to address with behavioral experimentation. We plan to learn more about their social behavior by performing a series of choice/preference tests and then determine if/how social influences affect breeding behavior, and to determine the reproductive behavior and reproductive biology of these animal to establish a successful breeding colony.

As we reported previously, our intention was to use APOPO's animals to run these experiments. However, Dr. Lee realized that the records for the animal colony at APOPO are in such disarray, that it is impossible to use their animals for this experiment. As many as 40% of the animals in their facility did not match the name/ID with which they are labeled. There is also a high incidence of incorrect sex identification, unexplained co-housing of non-siblings, animals identified with more than one name, and some animals that were missing based on their records. In her last visit to Tanzania, Dr. Lee organized the identification records of the breeding colony enough to identify unrelated animals that could be used in laboratory experiments. We therefore tabled this experiment and began to conduct it with our colony of animals in the US (see below).

In place of this experiment, Dr. Lee conducted a 'light-dark test' which tests stress responsiveness. This will be used in comparison to the light-dark test we conducted in the US on our sample of animals (see below).

To evaluate the reproductive behavior of this species, we initiated and have completed an exhaustive series of experiments investigating the estrus cycle of females, and what stimulates reproductive cycling. We measured the cell cytology of vaginal smears taken from cycling and non-cycling females and collected blood samples for hormonal profiling. We compared the four major cell types between these females and have identified the cell profiles and proportion of cells that characterize cycling females. Hormonal assays were delayed due to moving institution but will resume in the very near future. Sample sizes and design for this experiment may limit where it can be published, and may require further testing upon receipt of new animals under the new award, meant to allow completion of the project (see above).

We next exposed non-cycling females to a series of stimuli to determine if we could induce ovulation and have discovered that female pheromonal suppression is likely preventing other females from cycling. This suggests that breeding program should isolate females before exposing them to males (but see final paragraph of this section below).

Intentions to establish a breeding colony were thwarted due to poor institutional support from the Arts and Sciences Dean's office at Oklahoma State University after they learned the PI was leaving OSU. Plans to establish a breeding colony at the PIs new institution (Cornell University) will resume once animals have been successfully imported and behavioral assessment has been completed.

A large survey of morphological observations has led to the extremely novel discovery that young (sub-adult) females often have not developed vaginal openings. To this end, we have compared animals from the field (TZ), housed at APOPO (TZ), and lab (US) investigating the external genitalia and other morphometric measurements to assess sexual maturation in this species. We have learned from these observations that sexual maturation appears to be extremely delayed, and we believe we have observed the first example of a mammal that entirely lacks a vaginal opening postnataly. The vaginal opening appears to develop at about the time females reach 1 kg (about 1-1.5 years of age). Systematic measurements of animals across ages (within design) and between individuals are underway. We are measuring the ano-genital distances and other features of the secondary sexual characteristics of these animals. The manuscript for this project will be largely descriptive and may be married with the data on estrus cycling mentioned above. The overall picture appears to be that female suppression of cycling and possibly physical development may be maternally driven. Although no commitments have been made, an Editor at the highly prestigious journal Current Biology, has expressed interest in receiving this manuscript when it is complete. We are in the process of finalizing the data to begin writing the manuscript.

Behavioral Experiments: Individual Variation in Behavior. The second major line of behavioral experiments is to import animals from Africa, establish a breeding colony, and evaluate the individual variation in behavioral performance in a swath of tests that will determine 'personality' in these animals. Ideally this will inform how training of such animals could be carried out in a more efficient way, identifying animals that will perform better in certain tasks over others.

50 animals arrived from Tanzania in January 2013. The animals were quarantined, tested for Monkey pox (though a collaboration with the CDC) and were routinely checked for health. We initiated behavioral testing and have completed two rounds of light-dark tests (during the 'night' and 'day' for the rats), and five rounds of testing in a social reaction test, which assesses male and female reaction to unfamiliar conspecifics. Animals were successfully tested in open field test to evaluate stress responses and exploration. Attempts to test animals in T-mazes (to test learning flexibility) and in the Barnes maze (to test spatial memory) failed because the animals refused to run the mazes. We were unable to initiate the defensive burying test, forced swim, and resident-intruder tests due to timing of relocation. The intention is to add to this data set once new animals arrive at Cornell. Video analyses are nearly complete for all tests, which total over 1000 hours of observation time.

Finally, Dr. Lee co-opted a non-invasive test for assessing anxiety, known as the novelty-induced hypophagia test, in which latency to approach a novel food is used to assess anxiety. Our results were compelling. Males show significantly less anxiety compared to females. This sex difference transcended the food type, though some foods induced longer latencies than others for both males and females. This manuscript is currently in prep, and our intention is to submit it for publication in the Fall.

Genetic Mechanisms of Individual Variation. The third objective of this study is to extract DNA and RNA to identify markers of behavioral variation, and to assess the transcriptomic profiles of brain areas known to be central for anxiety, exploration, learning, social behavior, and olfaction. The project requires the completion of the behavioral assessment before it can be initiated. However the brains of all animals serving in the aforementioned behavioral experiments conducted at OSU have been collected, providing a rich resource for gene/brain profiling.

Personnel. At the outset of this project, a graduate student and postdoc were hired to work on this project. In its first year this project was delayed due to repeated complications with importing animals. The Graduate student therefore began his dissertation work on an unrelated project. The postdoc, however has been working effectively since she joined the project in January 2012. Over the course of the last two years, we hired two veterinary students (Mr. Matthew Sellers, and Ms. Erin Rogers) to work on the project. Mr. Sellers served as a research assistant through the end of the project, and has been working on most aspects of the behavioral tests described above. Ms. Rogers worked over the summer of 2012 as part of an NIH training grant run though the OSU Vet School, and her contribution to the project focused on the cell cytology experiments described above. We have also collaborated closely with the Oklahoma State University Attending Veterinarian and Director of Animal Resources (Dr. Todd Jackson). Dr. Jackson and his students have been documenting case studies as he has treated our animals and will take the lead on authoring a series of papers on the care and treatment of this species. In the final year at OSU, several excellent undergraduate students have helped with behavioral observations and collecting data from photos or video recordings of tests and experiments described above.

Summary. All three aims of this project have progressed well, despite initial setbacks at the beginning of the project limiting our ability to initiate projects, due to animal importation problems. Submitting a final report for this project is unusual because under the original agreement, we are just approaching the halfway point of this project. However because the PI changed institutions during the course of the project, completing the originally outlined project required prematurely terminating this project and

applying for a separate award through the new institution to complete the work. So although this may be a unsatisfying final report, it is worth keeping in mind that the project team has accomplished a great amount with the 2 years of funding (of 5) that has been awarded. It is also worth considering that the nature of the experiments we have been conducting are front heavy with data collection, so manuscripts and conference presentations will soon increase in frequency. To my knowledge all the allocated funds from this award have been accounted for and spent. Staff at the Oklahoma State University Contracts and Awards office should have submitted their final accounting.

Technology Transfer

We will provided animals and technical support (on animal handling and husbandry) for an ARO Funded SBIR/STTR project in 2012-2013.

Project Abstract:"Barron Associates, Inc. is developing the Rugged Automated Training (RAT) system, a cost-effective, rugged, automated environment to train and deploy small animals to detect landmines and other compounds of interest, and to evaluate their performance. The RAT system will train animals to recognize odorants in specialized, automated operant chambers controlled by custom software. A novel, wireless, non- invasive, body-worn sensor backpack will collect and relay data, in real time, from the animal to a PC base station that will employ specialized algorithms to automatically determine when the animal has discovered a substance that it has been trained to recognize. An acoustic local positioning system will be used to accurately and automatically determine the location of the animals and any detected mines within the search grid. The RAT system will also train the animals to respond to cue beacons that will guide their movements within the search field with minimal human interaction."