

CONVERSATION

In the field: an interview with Wendy Hood

Wendy Hood is Associate Professor at Auburn University, USA, where she investigates the mechanisms underlying life history variation. After her undergraduate degree in Marine Biology at the University of California, Santa Cruz, USA, she completed her PhD in Biology at Boston University with Tom Kunz, before undertaking postdoctoral research at Fordham University, USA. Hood discusses how she and her colleagues recently designed and built a mobile laboratory that allows them to investigate the mitochondrial function of species on location.

What are your scientific interests?

I am an evolutionary physiologist. Most of my work has focused on trying to understand the mechanisms that underpin life history variation within species. I only began studying mitochondria about seven years ago when I realized that they play a key role in aging. We have done a lot of work associated with the ‘trade off’ between reproduction and longevity, and we’re trying to understand how reproduction impacts mitochondrial performance in a way that could impact the aging process.

Why did you decide to take your lab to the mitochondria, rather than bring samples to the lab?

I have collaborated a lot with Andreas Kavazis, who is in the Kinesiology Department here at Auburn University. He has expertise in mitochondrial techniques, whereas I provide the ecological and evolutionary context for our studies. We also work closely with Geoff Hill, my husband, who has been investigating the role of color and, more recently, mitochondria in sexual selection. We started to think more broadly about the role of mitochondria in animal performance and adaptation. Andreas, who studies exercise physiology, suggested doing something associated with migration. We expected that there would be differences in mitochondrial capacity between migratory and non-migratory birds, so we collected preliminary data on gray catbirds and the northern mockingbird. As we live about three and a half to four hours from the US Gulf Coast, initially we drove animals back from coastal habitats to the lab. But we realized pretty quickly that stress can have negative impacts on mitochondria. When you work with mitochondria, you collect your samples and then you have a complicated process to isolate them before you measure their respiration, which has to be done within two hours of collection as samples cannot be frozen. We realized that we would have to think about ways of taking the lab to the animals, but it seemed completely pie in the sky. We didn’t think that we could include building a mobile laboratory in a standard federal grant, it was too expensive, so it was just an idea in the back of our minds.

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How were you able to find the funding to realise your dream?

When Auburn hired a new President in 2017, he wanted to push our university to Research 1 status, a designation for top research universities in the US. He came up with \$5 million and used it to support grants within the institution for collaboration across departments. When we heard about these grants, Andreas, Geoff, and I thought immediately that this might be our chance to finally get that mobile lab, so we brought in Bruce Gladden, also from Kinesiology. He invited people in the Engineering Department to participate, we applied for one of the grants and we were awarded \$600,000. That was really exciting! It allowed us not only to purchase and renovate a recreational vehicle (RV – a large camper van) but it also gave us a budget to collect our first data in the MitoMobile.

Who was involved in the design process and why did you bring those people on board?

Geoff and I thought about the types of studies that we wanted to do, what animals we wanted to study, where we’d go to study questions related to migration and speciation, and Andreas advised us what we should install in the mobile lab. Then Bruce got in touch with Mark Nelms in Electrical and Computer Engineering and asked if he would be interested in collaborating. Mark had a great guy on his team, Mike Eddy, who is really creative. Mike developed the plans for the RV renovation and did all the work along with technician John Tennant.

What equipment did you need to install in your mobile laboratory?

Isolating mitochondria and measuring their respiration requires many centrifugation steps. The refrigerated centrifuge that we need



Wendy Hood and Geoff Hill with the MitoMobile after the build was complete, two months before its maiden voyage. Photo credit: Tonia Schwartz.

is big, heavy and has to be treated well, or else you're going to damage it. Looking after the centrifuge was the biggest challenge we faced. We also use other equipment, such as Hansatech Oxytherm systems to measure mitochondrial respiration – they are small and fragile – and a plate reader to measure the reactive oxygen species that are generated by the mitochondrial electron transport chain during respiration.

How did you decide which vehicle to convert into a laboratory?

When we wrote the grant, we initially thought that we might buy the frame of an ambulance. The Oxytherm systems need a very solid source of power – you cannot have power spikes – so we thought that an ambulance would have an excellent power system. But the main challenge ended up being the centrifuge; we needed to have a very stable setup to reduce vibration, which could interfere with our respiration assays. Also, an unbalanced centrifuge can lead to metal fatigue and loosen parts of the instrument. We discovered that we would have to put self-levelling plates into an ambulance to install a centrifuge. After we got the grant, we talked about vans versus an ambulance or bus and then we realized, 'Why haven't we thought about an RV?' That turned out to be a great idea because an RV is already wired, has a generator and they have stabilising legs, which saved us from installing a pad for the centrifuge. We also wanted something that was mid-sized, that we could drive on a standard driver's license. In the end we went with a 28-foot long Jayco Redhawk, but we bought the previous year's model (2018), which saved a lot of money.

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Can you describe how you remodelled the RV?

Initially there was a queen-sized bed in the back and a large TV. They both came out and we remodelled that area with quartz laboratory countertops and space for the centrifuge. The electrical wiring was also almost completely redone to accommodate all

our equipment. There is enough room in the lab area for one person to work comfortably, two if they aren't moving around much. In addition to the centrifuge and four Oxytherm systems, we have space for an analytical balance – to weigh tissues and compounds for our assays – and the plate reader. We also got the cutest little – 80°C freezer that is two feet long and about a foot wide. In addition, we needed to carry liquid nitrogen, so that we could flash freeze samples to take back to the lab. There was a storage unit at the back of the RV that Mike extended and added a chamber that can hold two 10-gallon Dewars of liquid nitrogen. Importantly, the chamber is vented to the outside because nitrogen can displace oxygen, which is dangerous. The RV also came with a built-in refrigerator and freezer, so we kept those for storing compounds for our assays. We carry a cooler for our snacks, and we kept the dining table and chairs where we sit to process samples before isolating the mitochondria.

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When you were building MitoMobile, did you consider how much power your equipment would need?

Yes, in fact you've hit on some issues we've had. The generator that comes with the vehicle can output 4000 W of power, but the centrifuge is incredibly power hungry; it requires 1200 W of power. The engineer said right away that we would not be able to run the RV's heating, ventilation and air conditioning system at the same time as the centrifuge, so we discussed installing a second generator, but decided not to. Unfortunately, the 4000 W generator failed on our most recent trip, so we ended up replacing the built-in generator with a portable 9500 W generator. Fingers-crossed, this new generator should provide enough power to meet all our needs.

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How many people make up a MitoMobile field trip?

Four to five is a good team size. There were five people on the team when we went to a site on the edge of the University of California, Davis campus (USA) to collect data from migratory white-crowned sparrows just before they left to migrate to Alaska. One or two people collected the birds, one obtained and processed tissues and then two more worked in the lab area isolating mitochondria and measuring mitochondrial respiration. The mitochondrial respiratory measurements that we recorded are the highest that we have ever measured. When we go back to California in September 2021, we will be looking at the migratory subspecies at the end of the fall migration and the non-migrants at the end of their breeding season. Then, we plan to return to California in the following winter, when both of subspecies are in a non-migratory, non-breeding, non-moulting state, so that we can get baseline metabolic differences between them.

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How many field trips has MitoMobile been on?

We have had three trips so far. One of my former students, Amy Skibiell, has started to do mitochondrial research at the University of Idaho, USA, which is the opposite corner of the US from Alabama. She was setting up her lab, so we decided to help her out. We weren't intending to take measurements in the vehicle, but we wanted to have all of our equipment with us in case she had trouble setting things up for her experiments on dairy cows. It was an ideal first trip, because it gave us the chance to get used to driving the RV and to pack as if we were going on a real collecting trip. Five of us travelled across the country in MitoMobile and it was a pretty hard drive; it took almost four days. On our recent trip to California, there were five people, but it was in the middle of the semester, so two of the graduate students drove the RV while Geoff, my postdoc and I flew there. Once we arrive at a site, there are periods where we are working hard, all doing different things, and there are periods where there are only a couple of people in the vehicle – during the centrifugations, which last half an hour. The activity levels really fluctuate. Most of our days are about 12 hours and some were 14–15 hours long – it is intense.

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Where do you stop MitoMobile to conduct experiments?

We literally stopped on the side of the road during our trip to the University of California, Davis. We pulled off right next to a winery and people were biking and walking by; we definitely heard some 'Breaking Bad' jokes. In Davis, people knew what mitochondria are – our vehicle literally says AU MitoMobile on the side – so it gave us a chance to talk to people who were excited about and interested in our research.

How has the COVID pandemic affected your plans?

It's had a major impact. Our work on California white-crowned sparrows was initially planned for March 2020, but the departure date was just days after the university shut down, so the trip was cancelled and the MitoMobile just sat for a long time. Eventually we were able to do the trip to the coast of Alabama in Fall 2020, but we had a smaller team than we had planned originally; only one person could process, isolate and run respiration experiments and we were careful wearing masks. Fortunately, Alabama prioritized vaccines for educators, so we were all vaccinated before we went to California in April 2021, which allowed us to work as usual.

What other areas of physiology are you investigating?

Another question that has come up is speciation. Mitochondria are thought to play a role in adaptation, particularly to new environments and new metabolic challenges which can cause changes in both the mitochondrial and nuclear genomes that could ultimately prevent animals from one population from successfully mating with animals from another population. We have a trip planned to New York state in June 2021 when we are going to compare Muscovy ducks and mallards. Those two distantly related species can be mated to produce young – moulards – that are relatively healthy but sterile. We know that the hybrid offspring are prone to storing fat in their liver, which is a probable indicator of mitochondrial dysfunction. We are going to collect tissues from these animals to test our hypothesis that liver mitochondria in moulards have lower mitochondrial performance than the liver mitochondria of the muscovy and mallard parents.

At present, we are only beginning to address questions associated with mitochondrial adaptation, speciation and life history variation. The MitoMobile creates opportunities to integrate cell biology into studies of wild animals in natural environments, opening a world of new opportunities for organismal biologists. Our unique setup is a gateway to new collaborations with field biologists who have great systems for answering basic evolutionary questions, but lack the equipment and knowhow to include mitochondrial physiology. Our most fruitful collaborations will likely come from working with teams who have established a strong understanding of the basic biology of the animals they are studying. To our colleagues: don't hesitate to contact us about potential collaborations!

Wendy Hood was interviewed by Kathryn Knight. The interview has been edited and condensed with the interviewee's approval.