

The bodies of humans and animals are home to millions of microbial communities - bacteria, fungi and protozoa - known as the microbiome. The microbiome plays an essential role in multiple processes related to the host's health and well-being. Although our knowledge about the relevance of the microbiome to the functioning of the host is quickly expanding, there is a lot still to discover. One of such poorly understood aspects is how the microbiome may modulate mate choice in the host.

In many animals, mate choice is mediated by pheromones or chemical signals. Animals may synthesize pheromones through endogenous enzymatic reactions along metabolic pathways. However, in some vertebrates, such as amphibians and mammals, smell signals involved in intraspecific communication may be synthesized by symbiotic microbes inhabiting the host.

The main source of pheromones in lizards are femoral glands – epidermal organs situated on the ventral side of the hind limbs. It has been demonstrated that the volatile compounds produced by male lizards' femoral glands are used as signals for females in the selection of sexual partners. Despite the fact that the microbiome is not characterized in lizard femoral glands, microbes are ubiquitous in mammalian scent glands and suggests an analogous situation in reptiles. Bacteria are known to be able to produce a wide variety of volatiles, but it is yet unknown whether some of the compounds produced in the lizards' glands may have a microbial origin.

Previous studies have focused on describing the bacterial microbiome and pheromone compounds and some have explored the potential relation between the two. However, our knowledge in this area is still patchy and currently there is no integrative study to unambiguously demonstrate whether sexual selection is driven by host microbial microbiome. In the proposed project, we plan to use the sand lizard (*Lacerta agilis*) to understand how symbiotic bacteria may mediate mate choice through chemical signals in vertebrates by using modern genomic and microbiological techniques, along with chemical analyses and behavioral bioassays. We believe that sand lizards are a suitable model to test the hypothesis about the effect of microbes on chemical communication, due to the sand lizard's excellent olfactory abilities and well-known reproductive cycle.

Our first aim is to characterize the inherent bacterial microbiome associated with the femoral glands and assess any differences between the sexes. Secondly, microbial community dynamics will be examined during lizards' reproductive cycle to explore the possible existence of a core microbiome – one which is stable during the entire mating season, assuring adequate production of pheromones. After achieving these goals, we plan to isolate and culture the bacteria found in the femoral glands in the laboratory to identify any chemical compounds released by these taxa. Once the chemical composition has been characterized, we will be able to test whether more diverse microbiomes produce richer cocktails of compounds, considering the widespread ability of bacteria to produce chemicals.

In the final part of the project, we will carry out behavioral bioassays to empirically test whether mate choice is mediated by scents produced by bacteria. For that purpose, lizards will be exposed to scents released either by individuals who have previously been treated with antibiotics to alter their microbiome or by untreated individuals. The behavior of each animal in contact with the stimulus will be quantified in captivity. Thus, we will be able to test whether lizards can discriminate between individuals with a perturbed and impoverished microbiome from those harboring an unaltered and functional one.

In summary, our project will shed light on the functioning of the bacterial microbiome in animals and answer the key question whether pheromone-based mate choice is modulated by the microbiome of the host. We will also understand how antibiotics may impact animal mating systems that is an important issue considering the global increase of antibiotic consumption. This research will be among the first ones in the world and will be of significant research importance.