Network analyses of the dynamics and biodemography of microbial associations in ageing hosts to characterize the principles of organization of the ageing host-microbiome collective

Animal ageing is connected to the development of its microbial communities[1–6], as host-associated microbiomes contribute to the inflamm-ageing and to the ageing of their hosts[7–11]. However, evolutionary explanations of the role played by the microbiome in host ageing and of the role played by the ageing host on the evolution, and on the potential ageing of its own microbial communities are lacking. This is because the main evolutionary theories of ageing, namely the Mutation Accumulation theory by Medawar, the Antagonistic Pleiotropy theory by Williams and the Disposable Soma Theory by Kirkwood, have been developed within a classic population genetics framework, in which interspecific interactions, such as hosts and microbiomes interactions, are not explicitly modeled[12–14], and so is the ageing of the microbiomes associated to organisms. However, importantly, all these theories postulate that the strength of natural selection acting upon a host decreases with time[15], a selection shadow, which, by progressively altering the normal functioning of the ageing host, should alter the selection exerted by the ageing host on its microbiome. Yet, if the selection imposed by an ageing host[16] decreases, the host-associated microbiome could progressively emancipate from the guardianship of its host, resulting in a functional disintegration of the ageing host-microbiome collective, with possible impact on the ageing of the microbiomes. This 'host selection shadow hypothesis' and this 'ageing microbiomes hypothesis' are currently not integrated in the classic evolutionary theories of ageing. Precisely, **this project, at the interface of bioinformatics, evolutionary biology and biodemography will use new approaches of co-occurrence network comparison and recently proposed analyses of biodemography of ageing, namely the biodemographic study of systems to which organisms are connected, which goes beyond the traditional biodemographic analyses of organismal ageing, to better understand the fate of the collective composed of an ageing host and of its microbiome while considering that in addition these associated microbiomes themselves may age.**

In short, we will use publicly available metagenomics data from human and mice gut microbiome to produce time-series of co-occurrence networks, that collectively describe the structure of microbial associations over the course of these host's lives. Hosts will be pooled in age-classes and sex, e.g., 20-30 years old men. Then, microbial co-occurence networks will be constructed for each host-class using state-of-the-art approaches[17]. In these networks, depending on the granularity of the analysis, each node represents a microbial lineage present in the microbiome of hosts from a given age-class. Two nodes are directly connected by weighted edges, when they co-occur in significantly correlated proportions within individual samples from a host age-class. Such correlations may reflect a (direct or indirect) strong functional association between pairs of microbial lineages. This simple formalism is very powerful[17–20], and original network comparisons will be used to finely track the changes in the microbial associations that occur during ageing. The comparisons will identify which microbial associations are robust to host ageing, i.e. which sets of nodes and edges persist over continuous age-classes, ii) which microbial associations are resilient, i.e., which sets of nodes and edges are found in non-consecutive age-classes, e.g. are present in younger hosts then again in older hosts, and iii) which microbial associations are exclusively observed at a given host age, e.g. microbial associations corresponding to signature of extremely old hosts. Moreover, we will use the topological information of the network time-series at two levels, i.e., at the global level – the general architectural properties of the network-, and at the local level – focusing on specific edges and nodes to test the 'host selection shadow hypothesis' by searching for possible phase transition, considering that the structure of host-associated microbial communities may then dramatically changes with host ageing. For this, we will perform statistical analyses of the time-series of microbial co-occurrence network to detect phase transitions at the global and at the local levels, e.g. a phase transition in the average clustering or in the average robustness of the microbial co-occurrence networks and a phase transition in the centrality of specific nodes, when a microbial taxa becomes involved in significantly less central associations as the host ages.

Furthermore, this network modeling of microbiome dynamics in ageing hosts will provide an original material to determine the paces and shapes of ageing (I;e., the demographic ageing) of the microbiomes under investigation. This will then be the first time that biodemographic 'laws of ageing' will be unveiled and matched with the process of host ageing. As this project will develop the tools and concepts to support such an analysis, it will achieve a great progress, brigding a fundamental gap because

current theories of ageing jump across biological organisation scales. Namely, biodemographic studies of ageing are typically disconnected from microbiome studies, whereas it seems intuitive that ageing at one level of biological organisation (e.g., that of host organisms) may constrain ageing at another level of biological organisation (e.g. that of microbiomes). More precisely, this project will test whether the selection shadow affecting hosts organisms has a quantifiable effect on the 'laws of ageing' of their microbiomes, and significantly alters the paces and rates of ageing of microbiomes.

Ultimately, this interdisciplinary PhD project will generate novel explanations of the ageing host-microbiome collective, **providing a new kind of evidence to debate upon the relevance to model host-microbiome collective as a unit of selection throughout the entire lifespan of a host, hence** upon the theoretical relevance of the notion of holobiont in ageing studies, as well as expand current evolutionary theories of ageing.

Research plan

This PhD will involve a constant, tight collaboration between the Baudisch and Bapteste labs, which already have a record of collaborations (one review asa co-author, under consideration at Ageing Research Reviews, and two grants as co-PIs). The PhD candidate will spend time in both labs, a bit more so in Bapteste's lab than in Baudisch's lab, but Baudisch will regularly travel to Paris in the context of this collaboration (with her own funds) and Zoom meetings will be organized, every two weeks, between the two PIs and the PhD candidate throughout the entire project. **First**, in the Bapteste lab, the PhD candidate will **gather publicly available metagenomic datasets** from (mostly gut, and sometimes skin) **microbiomes of humans and mice**[21-24] **to generate time-series of high-quality host-associated microbiomes**. The PhD candidate will critically select such datasets based on their quality, and group selected samples by host age-classes to produce the highest number of age-classes with at least 15 samples per age class. **Second**, the PhD candidate will **construct co-occurrence networks for multiple stringency thresholds** of correlation significance and for correlation strength, as routinely done in the Bapteste lab. Dataset constitution will last approximately eight months. **Third**, in the Baudisch lab, during the next three months, the PhD candidate will delve into the literature on biodemography to **design biodemographical analyses of microbial co-occurrence networks**. In short, the PhD candidate will borrowing from a classic analytical framework from the biodemography toolkit[25–28], able to uncover different shapes and rates of ageing for cohorts of entities under study. In classic biodemography analyses, the individuals under study are conventional individual that are born once and die once. Thus, organisms x can be classified as senescent, negligibly senescent, or non-senescent. But in principles, nothing opposes to ask similar questions regarding other biological organisations than organisms. Here, each interaction that are appear one time in the system and that disappear once in the system will be considered as an individual entity of choice. By applying the tools of biodemography to cohorts of microbial interactions (rather than to cohorts of organisms), the PhD candidate will be able to determine whether microbial co-occurence networks are composed of cohorts of interactions with remarkable law-like biodemographic profiles. **Fourth**, from months 11-17, the PhD candidate will contribute to **implement these topological and biodemographical analyses** in the Bapteste lab, where all the required bioinformatic skills are present, and **get actual measures of rates and shapes of ageing** from the microbiome co-occurrence networks. In particular, the PhD candidate will compute the survival probability of each edge in the microbial co-occurrence networks, by assessing which edge born in the first host age class die during the first host age class, or die during the second host age class, or during the third one, etc. The PhD candidate will then assess whether this survival probability decays exponentially with chronological time (meaning that the probability of dying follows a Gompertz' law) and compute the associated pace and shape parameters[28]. **Fifth**, from months 18-23, in the Baudisch lab, the PhD candidate will **interpret these results to articulate how they enhance the biodemography of ageing studies**. **Sixth**, from months 24-28, in the Bapteste lab, the PhD candidate will interpret the results from a biological and evolutionary viewpoint, by **testing the 'host selection shadow' hypothesis**, according to which the structure of microbial associations significantly changes with host age, using topological evidence (e.g. network fragmentation along the least robust edges of the network and decrease in overall network robustness), compatible with a decrease of selective pressures from ageing hosts[15]. **Seventh**, during months 29-32, in the Baudisch lab, the PhD candidate will **start to expand the evolutionary theory of ageing to integrate his/her novel results on ageing**. By considering the microbiome as an additional 'genome' associated with a host, the PhD candidate will develop an expanded Mutation Accumulation theory (in which late expressed genes with deleterious effect may accumulate in the microbiome in addition to the host genome), an expanded Antagonistic Pleiotropy theory (in which in addition to host genes, microbial taxa and microbial genes can perform pleiotropic antagonistic actions on host health) and an expanded Disposable Soma theory (in which components of the microbiome may manipulate their host ageing, turning the host into an expanded disposable soma, favouring the persistence

of some microbial associations). The PhD candidate will also frame his/her novel topological results on ageing into conceptual debates on holobionts (i.e., host-microbiome collectives understood as a *bona fide* unit of selection) to **determine if the concept of Holobiont accurately captures the dynamics of the ageing host-microbiome collective, and, if not, to develop new concepts** to better comprehend this dynamics during host ageing. **Finally**, the last four months will be mainly devoted to the **writing of the PhD manuscript**.

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