

Evaluation of the Function of Wild Animal Gut Microbiomes Using Next-Generation Sequencing and Bioinformatics and its Relevance to Animal Conservation

Evolutionary Bioinformatics
Volume 15: 1–3
© The Author(s) 2019
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/1176934319848438



Ran Yao¹, Lianglaing Xu¹, Guoqing Lu² and Lifeng Zhu¹

¹College of Life Sciences, Nanjing Normal University, Nanjing, China. ²Department of Biology, University of Nebraska Omaha, Omaha, NE, USA.

ABSTRACT: The relationship between animal conservation and the animal gut microbiome is a hot topic in current microbial ecology research. Our group has recently revealed that the occurrence of diverse combinations of gut microbial compositions and functions (metagenomics) in Père David's deer (*Elaphurus davidianus*) populations is likely to lead to increased evolutionary potential and resilience in response to environmental changes. Thus, considering the effects of diet on the gut microbiome and the importance of a stable gut microbial community to host health, we suggest that a transitional buffer period (with feeding on a regular diet and a diet from the translocation habitat) is needed before animal translocation. When the gut microbiome enters into relatively stable stages and adapts to the new diet from the translocation site, the time is suitable for translocation. Long-term monitoring of the gut microbiomes of translocated animals (by collecting fresh feces and carrying out next-generation sequencing) is still necessary after their translocation.

KEYWORDS: next-generation sequencing, bioinformatics, gut microbiomes, metagenomics, animal translocation, conservation biology

RECEIVED: April 3, 2019. **ACCEPTED:** April 15, 2019.

TYPE: Metagenomics and the Analysis of Microbiomes – Commentary

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by grants from the National Natural Science Fund (31222009, 31570489) and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Lifeng Zhu, College of Life Sciences, Nanjing Normal University, Nanjing 210046, China. Email: zhulf@ioz.ac.cn

COMMENT ON: Wang L, Ding J, Yang Z, et al. Père David's deer gut microbiome changes across captive and translocated populations: Implications for conservation. *Evol Appl.* 2019;12(3):622–635. Published 2019 Jan 3. doi:10.1111/eva.12743. PubMed PMID: 30828378; PubMed Central PMCID: PMC6383733. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6383733/>

Population maintenance and survival are dependent on the occurrence of rapid evolutionary responses to changing selection conditions.^{1,2} These evolutionary changes can occur concomitantly with changes in ecological interactions, implying potential for ecoevolutionary feedback mechanisms.^{3–5} Animals predominantly obtain their nutrition and energy from food sourced from wild ecosystems. However, they can also adapt to different foods provided in captive environments by humans. The mechanism that underpins this type of ecoevolutionary feedback in vertebrate mammals requires further elucidation. One of the predominant connections between vertebrate mammals and their food occurs through symbiotic gut microorganisms, which play an important role in host nutrition and development.^{6,7} In recent years, we have seen a dramatic increase in the number of coevolutionary ecology studies investigating the interrelationships between animal diets and symbiotic gut microbial communities. One such study introduced a model community of 10 sequenced human gut bacteria to gnotobiotic mice, and alterations in species abundance and microbial gene expression in response to randomized perturbations of 4 defined ingredients in the host diet were successfully predicted.⁷

Next-generation sequencing, also known as high-throughput sequencing, includes Illumina (Solexa) sequencing, Roche 454 sequencing, Ion torrent: Proton / PGM sequencing, SOLiD sequencing, and PacBio sequencing. In 2010, one of the key-stone works on symbiotic gut microbial function performed

using next-generation sequencing (Illumina sequencing) and an associated bioinformatics pipeline was published in *Nature*: “A human gut microbial gene catalogue established by metagenomic sequencing.”⁸ Since then, an increasing number of studies on the animal gut microbiome have used next-generation sequencing (eg, 16S ribosomal RNA gene MiSeq and metagenome sequencing). Some of the pilot studies focused on the gut microbial function of wild mammals using next-generation sequencing. For example, in 2011, a metagenomics analysis of wild giant panda feces (based on Illumina sequencing) revealed that some gut microbes may contribute to bamboo cellulose (main diet) digestion.⁹ However, studies on the function of wild animal gut microbiomes (especially for these endangered animals) are ongoing.¹⁰

Père David's deer (*Elaphurus davidianus*) became extinct in its native China in the early 20th century. The current population of Père David's deer stems from 18 individuals brought to Woburn Abbey in England in 1894 and 1901. The Dafeng Nature Reserve (China) has the largest population of this species in the world (approximately 2800 individuals), living in 3 core areas.¹¹ Core areas I (DFI) and II (DFII) are captive environments (Figure 1). The main dietary plants in DFI include *Pennisetum alopecuroides*, *Imperata cylindrica* var. *major*, and *Phragmites australis*. The main dietary plants in DFII are *I. cylindrica* var. *major*, *P. alopecuroides*, and *P. australis*. From winter to the next early spring, these animals depend on foraged



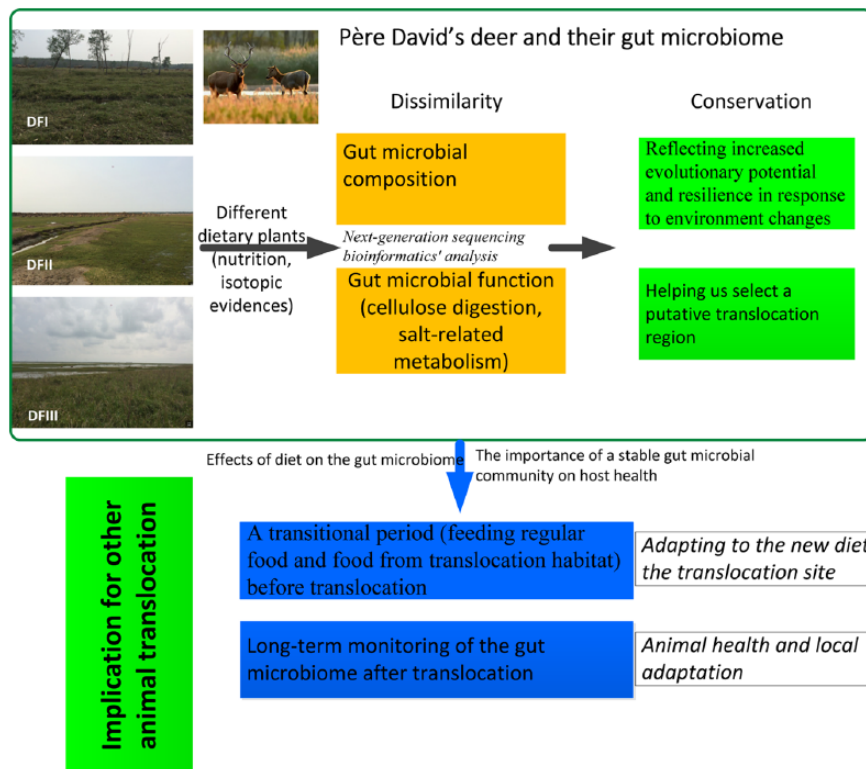


Figure 1. Père David's deer gut microbiomes and their relevance to animal conservation (eg, translocation). DFI, core area I (captive) in the Dafeng Natural Reserve; DFII (captive), core area II in the Dafeng Natural Reserve; DFIII, core area III (translocation region) in the Dafeng Natural Reserve. The copyright of the Père David's deer photograph belongs to Yuhua Ding.

materials provided by humans. DFIII harbors the translocated population living in wild habitat, and the current translocated population size has increased to 215.¹¹ The main dietary plants in this area are *Spartina alterniflora*, *P australis*, *Suaeda glauca*, *P alopecuroides*, and *I cylindrica var. major*. Considering the function of animal gut microbiomes and their potential contribution to host adaptation and conservation,^{12,13} the changes in gut microbial composition and function that have occurred in this translocated population could be one of the consequences of translocation. However, it is unknown whether this is the case under the current strategy for Père David's deer conservation.

In our study, we used several different macro- and micro-ecological approaches (nutritional methods, microscopy, isotopic analysis, MiSeq, and metagenomics approaches) to address the ecoevolutionary relationship between Père David's deer and their gut microbiomes.¹⁴ We found some differences in gut microbiome composition (16S MiSeq) and function (metagenomics) between the captive and translocated populations within the same season. For example, ruminal cellulolytic bacteria (eg, *Ruminococcus*) were enriched among the captive population gut microbiomes compared with those of the translocated population. We then investigated the genes involved in cellulose digestion and found that the proportion of genes encoding putative endoglucanases, beta-glucosidase, and cellulose 1,4-beta-cellobiosidase was higher in the gut

microbiome of the captive populations than in that of the translocated population, which might be caused by the high proportions of cellulose and hemicellulose in the dietary plants of the captive populations. Moreover, we found a potential correlation between some functional pathways of the gut microbiome with a special diet: the gut microbiomes of the deer in the translocated population consuming a high-salt diet (eg, *S alterniflora*) were enriched in 2 enzymes (natA and natB) involved in the sodium transport system. Genome-wide analysis of the Père David's deer genome showed that several genes under positive selection were associated with controlling the reabsorption of sodium in the body, blood pressure regulation, cardiovascular development, cholesterol regulation, glycemic control, and thyroid hormone synthesis.¹⁵ Thus, the selective pressure on these genes in Père David's deer and the enrichment of particular pathways of the symbiotic gut microbiome might buffer the adverse effects commonly associated with a high-salt diet. The large area of wetlands along the Yellow Sea might be a possible region for future translocations.

Here, we speculate that the occurrence of diverse combinations of gut microbial compositions and functions is likely to lead to increased evolutionary potential and resilience in response to environmental changes. We further emphasize one of the hot topics regarding the relationship between animals and their gut microbiomes: the contribution of gut microbiomes to animal

conservation measures, such as translocation. Our study reveals the potential contribution of the gut microbiomes to the local adaptation of deer (eg, related to a new diet) at a fine-scale level: all of the investigated core regions are located close together and share some dietary plants. However, some translocation strategies move the animals to different habitats with different nutritional components. Considering the effects of diet on the gut microbiome and the importance of a stable gut microbial community to host health, we suggest that a transitional buffer period is needed before animal translocation (Figure 1). In this period, the candidate animals will feed on a regular diet along with added food from the translocation habitat, and they will finally feed on a diet from the translocation habitat. During this period, we can monitor the dynamics (composition and function) of the gut microbiomes of these candidate animals using next-generation sequencing and bioinformatics. When the gut microbiome enters into a relatively stable stage, the timing will be suitable for moving to the translocation habitat. After translocation, we still need to monitor the gut microbiome by collecting fresh feces from the animals and carrying out next-generation sequencing. The gut microbial composition and function reflect an animal's health and local adaptation to some extent.

Author Contributions

All authors contributed to the discussion and manuscript writing. RY and LX contributed equally to this work.

REFERENCES

1. Cousyn C, De Meester L, Colbourne JK, Brendonck L, Verschuren D, Volckaert F. Rapid, local adaptation of zooplankton behavior to changes in predation pressure in the absence of neutral genetic changes. *Proc Natl Acad Sci U S A*. 2001;98:6256–6260.
2. Declerck SA, Malo AR, Diehl S, et al. Rapid adaptation of herbivore consumers to nutrient limitation: eco-evolutionary feedbacks to population demography and resource control. *Ecol Lett*. 2015;18:553–562.
3. Hairston NG, Ellner SP, Geber MA, Yoshida T, Fox JA. Rapid evolution and the convergence of ecological and evolutionary time. *Ecol Lett*. 2005;8:1114–1127.
4. Schoener TW. The newest synthesis: understanding the interplay of evolutionary and ecological dynamics. *Science*. 2011;331:426–429.
5. Hiltunen T, Becks L. Consumer co-evolution as an important component of the eco-evolutionary feedback. *Nat Commun*. 2014;5:5226.
6. Ley RE, Hamady M, Lozupone C, et al. Evolution of mammals and their gut microbes. *Science*. 2008;320:1647–1651.
7. Faith JJ, McNulty NP, Rey FE, Gordon JI. Predicting a human gut microbiota's response to diet in gnotobiotic mice. *Science*. 2011;333:101–104.
8. Qin J, Li R, Raes J, et al. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature*. 2010;464:59–65.
9. Zhu L, Wu Q, Dai J, Zhang S, Wei F. Evidence of cellulose metabolism by the giant panda gut microbiome. *Proc Natl Acad Sci*. 2011;108:17714–17719.
10. Wei F, Wu Q, Hu Y, Huang G, Nie Y, Yan L. Conservation metagenomics: a new branch of conservation biology. *Sci Chin Life Sci*. 2018;1:11.
11. Ding YH, Ren YJ, Wen HJ, Li PF, Chang Q. Research on recovery and conservation of wild Pere David's deer population in China. *Chin J Wildlife*. 2014;35:228–233.
12. Redford KH, Segre JA, Salafsky N, Del Rio CM, Mcaloose D. Conservation and the microbiome. *Conserv Biol*. 2012;26:195–197.
13. Trevelline BK, Fontaine SS, Hartup BK, Kohl KD. Conservation biology needs a microbial renaissance: a call for the consideration of host-associated microbiota in wildlife management practices. *Proc Roy Soc B*. 2019;286:20182448.
14. Wang L, Ding J, Yang Z, et al. Père David's deer gut microbiome changes across captive and translocated populations: implications for conservation. *Evol Appl*. 2019;12:622–635.
15. Zhu L, Deng C, Zhao X, et al. Endangered Père David's deer genome provides insights into population recovering. *Evol Appl*. 2018;11:2040–2053.