

4-1604

Studies on Gut Bacteria of Japanese Rock Ptarmigans for Its Potential Use in In-situ and Ex-situ Conservation Protocol

Kazunari USHIDA

Chubu University, Academy of Emerging Sciences (CUAES)

1200 Matsumoto-cho, Kasugai-City, Aichi 487-8501, JAPAN

E-mail: k_ushida@isc.chubu.ac.jp

Key Words: Japanese rock ptarmigans, Ex-situ protection, Re-introduction, Intestinal microbes, Beneficial microbes of wild rock ptarmigans

Japanese rock ptarmigans (*Lagopus muta japonica*) are birds that only inhabit the alpine areas of Japan's main island. The birds are recognized as endangered, prompting the endorsement of their national conservation program. We conducted a series of studies to contribute to the conservation program in particular their ex-situ protection.

The birds have a pair of big ceca, in which dense bacterial populations efficiently ferment the food consumed by the host. We established that the anti-herbivory chemicals, synthesized by the alpine plants, were efficiently degraded by these intestinal bacteria thus promoting the birds' survival in the alpine environment.

Therefore, we analyzed the cecal microbial composition of wild living Japanese rock ptarmigans, and also their dominant cecal microbial structure was defined. The mechanisms to maintain these important gut microbes for generations are evolutionarily advantageous. Coprophagy is one effective way to transmit microbes across generations. We observed the coprophagous habits exhibited by the Japanese rock ptarmigan chicks feeding on their mothers' cecal feces. In 2016 and 2017, this study succeeded in defining the coprophagy period manifested by chicks and the developmental process of the cecal microbiome in chicks under an in-situ protection.

Amongst all chicks, coprophagy was only done between 3 and 18 days of age. The number of bacterial species at 1-week-old chicks was the same as that of the adults. Most of the predominant species in the adult individuals were already existent in the 1-week-old chicks. These results indicate that coprophagy in this precocial bird contributes to early colonization of cecal bacteria that are essential for the digestion of food and, hence, survival of the chicks. We have successfully isolated 1,024 strains from the wild Japanese rock ptarmigans and selected one *Lactobacillus apodemi*, one *Streptococcus gallolyticus* and one *Escherichia fergusonii* as our probiotic candidates. Feeding of these probiotics to the chicks from 1 to 14 days old was a successful substitute for antibiotic use in terms of disease prevention. In addition, dried leaves of persimmon of high tannin content were supplemented to their diet stimulate the growth of the probiotic strains, because *L. apodemi* and *S. gallolyticus* were characterized as potent tannin degraders, and *E. fergusonii* as xylanolytic. The supplementation also reduced excessive body weight gain, which often caused leg problems under captivity.

Publications

- 1) S. TSUCHIDA, K. MURATA, M. OHKUMA and K. USHIDA: J. Gen. Appl. Microbiol, 63, 3, 195-198 (2017), Isolation of *Streptococcus gallolyticus* with very high degradability of condensed tannins from feces of the wild Japanese rock ptarmigans on Mt. Tateyama.
- 2) S. TSUCHIDA, Y. OHARA, K. KURAMOCHI, K. MURATA and K. USHIDA: Jpn. J. Zoo Wildlife Med, 22, 3, 41-45 (2017), Effective degradation of phenolic glycoside rhododendrin and its aglycone

rhododendrol by cecal feces of wild Japanese rock ptarmigans.

- 3) M. MATSUBAYASHI, S. TSUCHIDA, K. USHIDA and K. MURATA: Int. J. Parasitol. Parasites Wildl, 7, 2, 134-140 (2018), Surveillance of *Eimeria* species in wild Japanese rock ptarmigans, *Lagopus muta japonica*, and insight into parasitic seasonal life cycle at timberline regions of the Japanese Alps or at the Japanese alpine regions.
- 4) M. MATSUBAYASHI, S. TSUCHIDA, A. KOBAYASHI, T. SHIBAHARA, H. NAKAMURA, K. MURATA and K. USHIDA: Int. J. Parasitol. Parasites Wildl, 7, 2, 243-250 (2018), Molecular identification of two *Eimeria* species, *E. uekii* and *E. raichoi* as type B, in wild Japanese rock ptarmigans, *Lagopus muta japonica*.
- 5) A. UEDA, A. KOBAYASHI, S. TSUCHIDA, T. YAMADA, K. MURATA, H. NAKAMURA, K. USHIDA: Microorganisms 6, 3, 77. <https://doi.org/10.3390/microorganisms6030077>. (2018), Cecal Microbiome analyses on wild Japanese rock ptarmigans (*Lagopus muta japonica*) reveals high level of coexistence of lactic acid bacteria and lactate-utilizing bacteria.
- 6) A. KOBAYASHI, S. TSUCHIDA, A. UEDA, T. YAMADA, K. USHIDA and H. NAKAMURA: 14th International Grouse Symposium Utah State University (2018), The Significance of Coprophagy in Chicks of Japanese Rock Ptarmigan.
- 7) S. TSUCHIDA, A. KOBAYASHI, K. MURATA and K. USHIDA: 14th International Grouse Symposium Utah State University (2018), Isolation of Cecal Bacteria from Wild Japanese Rock Ptarmigans and their Functionalities.
- 8) A. KOBAYASHI, S. TSUCHIDA, A. UEDA, T. YAMADA, K. MURATA, H. NAKAMURA and K. USHIDA: J. Vet. Med. Sci., 81, 9. <https://doi.org/10.1292/jvms.19-0014>. (2019), Role of coprophagy in the cecal microbiome development of an herbivorous bird Japanese rock ptarmigan.

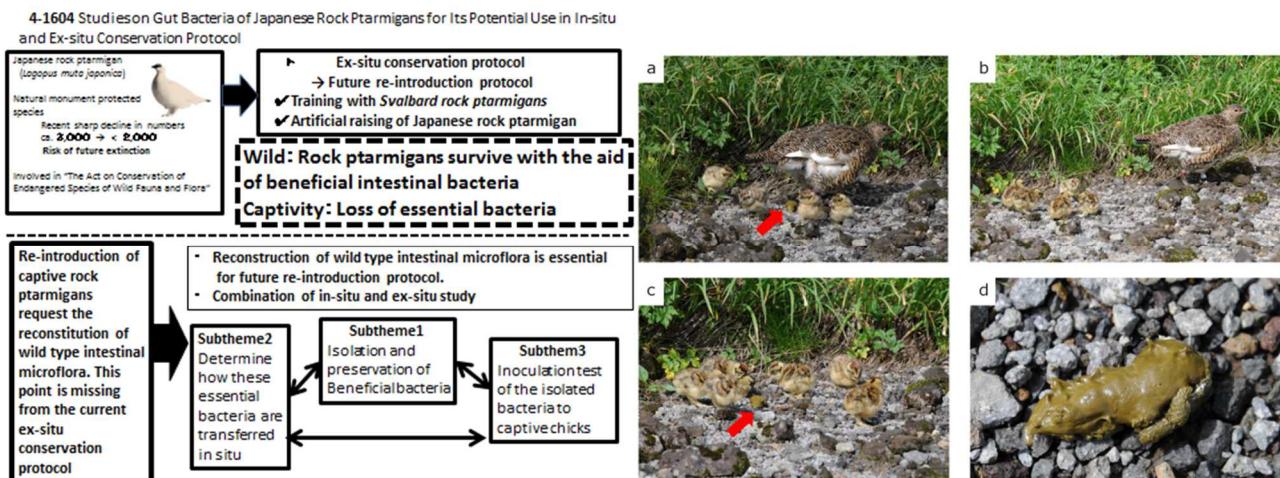


Fig. Coprophagy behavior of Japanese rock ptarmigan chicks. a) Excretion of the cecal feces, just after brooding to warm the body temperature of the chicks. b) congregation of chicks around cecal feces, rather than around the hen. c) Pecking of the hen's cecal feces by chicks. Pecking behavior was observed for all chicks. d) Cecal feces left behind after pecking by the chicks. Traces of pecking are seen on the surface of the feces. Red arrows indicate the cecal feces of hens. (Kobayashi et al. 2019, J. Vet. Med. Sci. doi.org/10.1292/jvms.19-0014.)