

## 博士論文要約 (Summary)

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タイトル	Animal biology from feces: ecological and endocrinological research in Siberian flying squirrels <i>Pteromys volans</i>
<p>The animal's sign is often focused on when difficult-to-observe animals are researched. Feces are among the most common for animal research, and are used to reveal the basic ecology in various studies. Furthermore, recently fecal steroid hormone analysis that is non-invasive has been developed over the past two decades to profile the endocrine patterns. Feces can therefore be used as a tool for studying difficult-to-observe animals, and the results obtained can help to elucidate various aspects of animal biology, such as life history, genetic structure, and endocrine patterns.</p> <p>The Siberian flying squirrel, <i>Pteromys volans</i>, a member of the Family Sciuridae, is difficult to observe. However, its feces are distinctive and can be easily identified. Despite the diversity of information offered by fecal analysis, there has been little research on feces in Siberian flying squirrels. Research focusing on fecal characteristics could help to elucidate the ecology and endocrinology of this species.</p> <p>Here, with a focus on the benefits of using feces, author studied the ecology and endocrinology of Siberian flying squirrels. First, author established the confirmation method for presence of Siberian flying squirrels by using feces (Section 1). Then, author validated the use of fecal progesterone analysis for predicting pregnancy (Section 2). Finally, author investigated progesterone concentrations during lactation and the progesterone dynamics of lactating females to estimate the presence of postpartum estrus in Siberian flying squirrels (Section 3).</p> <p>1) A confirmation method for the presence of the Siberian flying squirrel via feces</p>	

There is concern about population decline and local population extinction of the Siberian flying squirrel *Pteromys volans* because of forest fragmentation. The goal of this study was to confirm a simple and efficient method of determining the presence of the squirrels to monitor the effects of forest fragmentation. Author searched for their feces in 11 fragmented forests. Author set 12 transects, each 10 m long and 4 m wide, randomly in each forest and searched for feces within each transects. First, to characterize the places where feces were found, author measured the distance between the feces and the closest tree, along with the diameter at breast height (DBH) of that tree. The feces that author found were close to large trees; author therefore found that it was efficient to mainly search for feces within 20 cm of such trees. Second, to reveal the efforts to search feces in each forest, author evaluated the relationship between the number of transects on which author found feces and forest size. The number was unrelated to forest size. Therefore, author did not need to change the research effort according to forest size. Furthermore, author found that five transects per forest gave valid results for squirrel presence.

## 2) Validation of fecal progesterone analysis for predicting pregnancy in Siberian flying squirrels *Pteromys volans*

Recently steroid hormone analysis using feces have been developed during past twenty years. Development of fecal steroid hormone analysis has facilitated to elucidate a variety of wild and zoo animals. However, there is a paucity of information on the Siberian flying squirrel's basic reproductive physiology, and there is no established method for studying it. The purpose of this study was to validate fecal progesterone analysis in this animal using a commercial enzyme immunoassay (EIA) kit for endocrine profiles in Siberian flying squirrels. First, author tested parallelism between serially diluted fecal progesterone and a standard curve to validate the EIA. Comparison of the slopes of the two regression lines to test for parallelism revealed no significant difference. Therefore, progesterone concentrations in the fecal samples of the Siberian flying squirrels were exactly measured. Second, author

compared progesterone concentrations among four groups— pregnant females, adult females in the non-breeding season, juvenile females, and adult males— to determine whether fecal progesterone analysis was useful for evaluating reproductive status using GLMM. Fecal progesterone concentrations were significantly higher in pregnant females than in other groups. These results indicated that fecal progesterone analysis in Siberian flying squirrels was valid for predicting pregnancy.

### 3) Fecal progesterone concentrations and dynamics during lactation in Siberian flying squirrels *Pteromys volans*

The reproductive strategy is a key concept of species survival. *Pteromys volans*, which is a seasonal breeder, produces up to two litters per year. But, *P. volans* is imposed to reproduce in a breeding season because of severe winter. To circumvent time constraints, *P. volans* may have a postpartum estrus similarly to a variety of small mammals. If *P. volans* has a postpartum estrus, progesterone would be secreted from formed corpus luteum (CL) after postpartum ovulation. Therefore, we investigated progesterone concentrations and dynamics during lactation in this species by using an enzyme immunoassay to test this hypothesis. To compare fecal progesterone in lactating females with pregnant females and non-reproductive females, fecal samples were collected from each individuals with different reproductive status. As a result, the 95% confidence interval of fecal progesterone concentrations in lactating females overlapped with that in pregnant and non-reproductive females. This result indicated that a part of used fecal samples included luteal phase. Furthermore, author captured six pregnant females in spring and kept them temporarily to monitor progesterone dynamics during lactation. The durations with high progesterone concentration were detected in 4 of 6 lactating females, which indicated that follicular development was reinitiated after parturition, ovulation occurred, and the formed CLs began secreting progesterone. Thus, author showed that *P. volans* has the physiological potential to mate during lactation.

These studies showed fecal analysis can help to mainly elucidate the basic biology of Siberian flying squirrels. However, the research using feces could also help to conserve this

squirrels of which the population have decreased due to forest fragmentation in the worldwide. For instance, the research in section 1 showed that feces can be used as the indicator of presence, and might be useful to monitor the lag time effect of forest fragmentation on the population of the squirrel. Furthermore, forest fragmentation has deleterious effect on the reproductive physiology of living animals in fragmented forests. Therefore, reproductive status needs to be assessed if we are to evaluate the effects of forest fragmentation on Siberian flying squirrels. Fecal progesterone analysis is used to assess reproductive status and detect pregnancy in wild populations of other animals. Also, in Siberian flying squirrels, fecal progesterone analysis could be used to predict pregnancy rates in populations in fragmented forests, and this could help to assess population dynamics in these fragmented areas.

In addition to in situ conservation, ex situ conservation is often applied to animals subjected to population decreases, i.e. endangered species. Ex situ conservation focuses mainly on captive breeding in zoological parks and aquariums to increase populations. Application of endocrine techniques can suggest problems in captive-breeding programs and can guide hormone therapy in endangered species. In section 2, author validated the use of fecal progesterone analysis for predicting pregnancy; not only pregnancy but also other reproductive status could be predicted. Furthermore, in section 3 author showed that high progesterone concentrations were detected despite lactation, and this seems to be the characteristic endocrine pattern of Siberian flying squirrels. If Siberian flying squirrels express postpartum estrus, facilitation of breeding during lactation may permit efficient captive breeding. Nevertheless, for efficient captive breeding author needs to establish a more precise diagnosis of pregnancy and will need to reveal in more detail the dynamics of progesterone during pregnancy.