氏　　名	加藤 貴大
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論 文 審 査 委 員	主　査　教授 佐々木 顕 講師 沓掛 展之 助教 寺井 洋平 准教授 相馬 雅代 北海道大学 理学研究院

(別紙様式 2)
(Separate Form 2)

論文の要旨

Summary (Abstract) of doctoral thesis contents

For sexually reproducing species, parents should allocate their investment to offspring strategically according to its sex so as to maximize their fitness. Environmental heterogeneity may favor biased offspring sex ratio (local mate competition, local resource competition, local resource enhancement, etc.). Also, social and environmental conditions, and mate attractiveness can bias offspring sex ratio adaptively. In invertebrate species, empirical studies showed an evidence of adjustment of primary sex ratio (at fertilization stage). In dioecious vertebrates like birds and mammals, many studies reported that the sex ratio at the timing of birth or hatch of offspring, which were defined of primary sex ratio, changed by breeding environment. However, those sex ratios should be labeled as the secondary sex ratio. The confusion on the stage of offspring sex ratio was caused by ambiguous definition for offspring sex ratio, which masked the timing and mechanism of biasing sex ratio. Furthermore, recent meta-analytical studies in birds reported that the biases in primary sex ratio are questionable. As such, the existence of biased primary sex ratio in vertebrate is a controversial topic.

Sex-specific mortality (SSM) of offspring is a pervasive phenomenon across animal taxa. Although SSM is one of factors biasing offspring sex ratio after fertilization stage, the growth stage at which SSM occurs, breeding contexts in which SSM occurs, and consequences of SSM remain little known. Here, I investigated (i) a growth stage at which SSM occurred, (ii) a relationship among breeding context, individual's behaviour and SSM, (iii) a hormonal cause of SSM, and (iv) a consequence and a possible function of SSM in wild population of Eurasian tree sparrows *Passer montanus* by a long-term fieldwork in Akita prefecture, Japan.

In the first study (Chapter 2), I investigated the growth stage in which SSM occurred. I found that 37.6% of eggs in a clutch failed to hatch. Observation of the inner tissue of unhatched eggs suggested that 54.9% of eggs did not show a sign of embryo development. Furthermore, 82.2% of undeveloped eggs contained many nuclei stained by fluorescent dye, indicated that most of undeveloped eggs were fertilized but died at early development stage. The sex of undeveloped eggs was biased to male (36 males, 1 females). In addition, I found that sex ratio at the stage of laying was not biased to either sex (109 males, 101 females). These results showed that offspring sex ratio at laying was unbiased, but was biased to female due to high male embryo mortality.

In the second study (Chapter 3), I investigated breeding context in which SSM occurred. I manipulated breeding density of Eurasian tree sparrows, and observed individual's behaviour for nest-site competition. I found that male embryo mortality was high under high breeding density and competitive environment. I also showed that

parental defence behaviour increased under dense and competitive environment. Furthermore, the frequency of the maternal defence was negatively associated with the embryo development success and biased secondary sex ratio toward female. These results showed that ecological conditions, such as breeding density and nest-site competition, affected the occurrence of SSM. Furthermore, parental behaviour, whose frequency changed by ecological conditions, was associated with male embryo mortality.

In the third study (Chapter 4), I investigated the hormonal cause of SSM. I predicted that observed relationship between breeding environment and embryo mortality was mediated by maternal corticosterone (stress hormone) level because dense and competitive breeding environment should induce maternal high stress level. To test this idea, I measured corticosterone level in feces of nestlings instead of maternal corticosterone level is positively reflected into that of nestling. I found that the fecal corticosterone level of nestlings was negatively associated with embryo development success of the same clutch. Furthermore, I conducted an experiment implanting a silicone tube sealed corticosterone, or metyrapone (corticosterone synthesis inhibitor) to female parents. The experiment supported the hypothesis that corticosterone was a cause of embryo development failure.

In the fourth study (Chapter 5), I investigated the consequence of SSM. I tested a hypothesis that the presence of unhatched eggs boosts offspring condition. I compared condition of nestlings among three experimental settings: the broods in which unhatched eggs were added, removed or unremoved as control experimentally. As a result of this experiment, condition of nestlings increased in the brood with unhatched eggs. Moreover, hatchlings from broods with SSM occurred grew to be “good” condition relative to one in brood where SSM did not occur when there were unhatched eggs in nest, possibly because parental food provision concentrated on survived nestlings. On the other hand, condition of nestlings was constant despite that SSM occurred or not when there were not unhatched eggs.

This study found that the secondary sex ratio was biased to female in dense and competitive situations. This pattern agrees with sex allocation theory because it may be adaptive for female parents to invest on female offspring, which is dispersive sex, under dense and competitive situation. Moreover, the presence of unhatched eggs improved condition of nestlings. My studies raised three issues composing payoff of SSM; the cost of producing unhatched eggs, trade-off between quality and quantity of offspring, the sex difference of natal dispersion. These inter-related issues should be investigated in the future study.

In conclusion, my studies provided the first data on the causes and consequences of SSM. So far, no studies investigated the relationship between SSM and breeding context in the light of the sex allocation theory, though SSM is one of the strong factors biased offspring sex ratio. At first glance, SSM seems not to be

adaptive because of a decrease in offspring number. However, I found positive effects of SSM in offspring conditions. The results in this study raised a possibility that offspring sex ratio is adjusted by SSM.

(別紙様式 3)
(Separate Form 3)

博士論文審査結果の要旨

Summary of the results of the doctoral thesis screening

学位申請者（加藤貴大）は、秋田県大潟村においてスズメ*Passer montanus*を対象とした野外調査を行い、性特異的死亡(sex-specific mortality)を多角的に研究した。

博士論文は六章からなる。第一章では、本研究の背景、対象種の基礎生態が簡潔に述べられている。その中では、スズメのオス胚が発生中に高確率で死亡すること、この性特異的死亡が起きる発生段階、原因、帰結が明らかにされていないこと、性配分(sex allocation)理論との関連も不明であることが指摘されている。二章では、スズメの発生・発達各段階の死亡率とその性差が報告されている。胚発生中の細胞学的観察と、分子実験による性判定の結果、孵化しない卵は受精卵であるが胚発生の初期に発生が停止すること、オス胚の発生停止が多いこと、その結果、二次性比がメスに偏ることが報告されている。この研究は、*Ecology and Evolution* 誌に掲載されている（博士論文に含むことは共著者による承諾済み）。

第三章では、巣箱を高密度に設置した条件と低密度に設置した条件の比較、および、営巣場所での行動観察によって、性特異的死亡の行動学的・生態学的決定要因が分析されている。その結果、胚の正常発生確率は、高巣箱密度条件下において低巣箱密度条件下よりも低く、また、同種・他種個体が巣に接近する頻度、および親個体が巣に滞在する頻度と負の関係にあった。つまり、高巣箱密度・高競争環境において、性特異的死亡が高頻度で起き、二次性比はメスに偏っていた。これらの結果は、高巣箱密度・高競争環境において、母親個体はなんらかの負の影響を受けている可能性を示している。この可能性を受けて、第四章では、母親個体の高いストレスレベルが、胚の発生不全の至近要因であるという仮説を立て、雛の糞中コルチコステロン値の測定、および母親個体へのホルモン埋め込み実験によってこの仮説を検証した。その結果、いずれの実験でも、高いコルチコステロン値が性特異的死亡と関連していることが判明し、胚の発生を決定する内分泌学的要因が母親のコルチコステロン値であることが示唆された。第五章では、性特異的死亡がもたらす帰結として、孵化した雛のコンディションが調べられている。巣に未孵化卵を加える実験操作を行った結果、未孵化卵を除去した群と比較して、未孵化卵が存在する群の雛の身体的・生理的指標（体重・免疫状態）が高いという効果が見つかった。この結果は、性特異的死亡が親の適応度に負の影響のみを与えているわけではないことを示している。

第六章では、第二章から第五章の研究を概説し、スズメにおける性特異的死亡の適応的意義が議論されている。性特異的死亡によって、出生地から分散するメスに性比が偏る点、未孵化卵の存在によって雛のコンディションが上昇する点を性配分理論に照らし合わせ、性特異的死亡が母親個体による性比調節である可能性を議論している。

本博士研究は鳥類の行動生態、繁殖生理に関する理解を広げるものであり高く評価できる。博士論文は英語で書かれており、申請者は十分な英語能力を有すると判断される。これらの点を合わせて、審査委員会は全員一致で、本論文が博士（理学）の授与に十分値するものと判定した。