High male embryo mortality biases secondary sex ratio: stage, causes, context and consequence of sex-specific mortality in Eurasian tree sparrows *Passer montanus*

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Abstract

For sexually reproducing species, parents should allocate their investment according to the sex of offspring so as to maximize their fitness strategically. Environmental heterogeneity may favor biased offspring sex ratio at an individual level (local mate competition, local resource competition, local resource enhancement, social and environmental conditions, mate attractiveness, etc.). In invertebrate species, empirical studies showed the evidence of adjustment of primary sex ratio (at fertilization stage). On the other hand, many studies of offspring sex ratio investigated secondary stage in dioecious vertebrate like birds and mammals, while those reported that the primary sex ratio was affected by the breeding environment. One of the causes of this difference was ambiguous definition for offspring sex ratio, which masked the timing and mechanism of biasing sex ratio. Furthermore, recent meta-analytical studies in birds found no or slight biases in primary sex ratio. So far, 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 occurred, breeding context 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 function of SSM in wild population of Eurasian tree sparrows *Passer montanus* by 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 showed that 54.9% of unhatched eggs did not show a sign of embryo development. Furthermore, 82.2% of undeveloped eggs contained many nuclei stained by fluorescent dye, indicating 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.

In the third study (Chapter 4), I investigated the hormonal cause of SSM. I predicted that the observed relationship between breeding density and embryo mortality (Chapter 3) was mediated by maternal corticosterone (stress hormone) because the dense and competitive breeding environment could induce a high level of maternal stress. To test this idea, I measured corticosterone level in feces of nestlings instead of maternal corticosterone level because maternal corticosterone level 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 grew to be "good" condition relative to ones 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.

Overall, 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 a dispersive sex, under dense and competitive situation. Moreover, the presence of unhatched eggs improved condition of nestlings. My studies raised three issues as to 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.