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Every Rose Has its Own Thorns? A Study on Trivers-Willard Hypothesis in Plateau Pikas

B.F. Yin, I.M. Hegab, A.Q. Wang and W.H. Wei
College of Bioscience and Biotechnology, Yangzhou University,
Yangzhou, 225009 Jiangsu, China

Abstract: This study is considered the first dealt with Trivers-Willard hypothesis in China using a wildlife population of plateau pikas (*Ochotona curzoniae*) by which we have investigated the relationship between maternal quality, litter sex ratio and litter size in a polytocous mammal. The results indicated that the reproductive time significantly affected the litter sex ratio. Although, the concentration of female adult plasma Immunoglobulin G (IgG) and Glucagon (Glu) at gestation, body weight, ear length, rear foot length and body length of adult female after gestation did not affect the litter sex ratio however, litter size has a significant negative impact on the litter sex ratio and body weight of adult females have a significant effect on the litter size. These results might give some evidence for the Trivers-Willard hypothesis in polytocous mammals.

Key words: Sex ratio variation, litter size, Trivers-Willard hypothesis, *Ochotona curzonia*, mammals, China

INTRODUCTION

Mammals usually produce approximately equal numbers of males and females offspring. However, as the proverb said, every rose has its own thorns. This proverb had been proven in a wide variety of mammalian species where the sex allocation hypothesis of Trivers and Willard has provided a rational evolutionary underpinning to adaptive changes in sex ratio. In mammals, sex allocation theory predicts maternal allocation of resources should be greater in the sex that most increases the mother's fitness by having a higher future reproductive potential, implying that maternal expenditure affects the fitness of offspring of each sex differently (Trivers and Willard, 1973; Frank, 1990; Clutton-Brock *et al*., 1981; Hardy, 2002). Especially for polygamous species, males may have more variable reproductive success than females (Clutton-Brock and Iason, 1986) because high quality males gain disproportionately higher numbers of mating trials relative to low quality males whereas female reproductive output is constrained by the number of offspring that can be produced. This expectation is an extension of the Trivers-Willard Hypothesis (TWH; Trivers and Willard, 1973) which proposes that if the cost of producing each sex differs, the offspring sex ratio will vary according to the mother's ability to allocate resources. Trivers and Willard (1973) suggested that high-quality mothers should provide a greater investment in sons than in daughters when males have a greater variance in individual fitness than females. Expressed in another way, females in good condition to produce male offspring and conversely, females in poor condition, female offspring.

Although, the literature reported abundant sex allocation patterns in mammals (Frank, 1990), controversies still surround these predictions and no general conclusions have emerged (Cameron and Linklater, 2002; Sheldon and West, 2004). Until now, Trivers-Willard hypothesis has been conformed on the species in which litter or clutch size is one but has little supported in mammals with larger ones (Cameron, 2004; Sheldon and West, 2004). If mothers produce a variable number of offspring, adaptive adjustments according to maternal condition may affect both the sex and the number of offspring within the litter or clutch and the predictions may be changed (Williams, 1979; McGinley, 1984). However, if there is a Trivers-Willard effect and good quality mothers able to allocate more resources then it will be an advantage to get more males.

In this study, the researchers examined sex allocation strategy in the plateau pikas (*Ochotona curzoniae*). The plateau pika is a small, native diurnal lagomorph that only inhabits alpine meadows 3000 m above sea level on the Qinghai-Xizang (Tibetan) plateau, People's Republic of China (Smith *et al*., 1986; Dobson *et al*., 2000). The breeding period starts April and lasts to August; the average number of litters is 3-5 sequential litters of

Corresponding Author: W.H. Wei, College of Bioscience and Biotechnology, Yangzhou University, Yangzhou, 225009 Jiangsu, China

average litter size 3-6 young with 3 weeks intervals (Smith and Wang, 1991; Yin *et al*., 2004). This species allows us to study maternal allocation to sex and number of offspring in a polygamous mammal.

The researchers firstly tested the TWM by investigating the relationship among maternal body condition, plasma hormones and the litter sex ratio. According to the TWM, the researchers hypothesized that females of good body conditions will produce more males to females. Secondly, the researchers tested whether changes in litter size will influence sex ratio variation in small polytocous mammals.

MATERIALS AND METHODS

Study area: This study was conducted at the Haibei Alpine Meadow Ecosystem Research Station of the Chinese Academy of Science (HAMERS) in Qinghai province, China from May to June in 2009 and 2010. HAMERS is located at the Northeast Qinghai-Tibet plateau, a large valley in the Qilian mountains (37°29-37°45'N, 101°12-101°33'E). The climate is continental monsoon type dominated by the southeast monsoon and high pressure from Siberia with average temperature about -1.7°C ranging between -37.1-27.6°C. Winter is long and severe and summer is short and cool. Most rainfalls occur from May to September and heavy snow packs in winter (Sun *et al*., 2005). The major vegetations include alpine meadow, alpine shrub and swamp meadow. Almost all plateau pika have lived in alpine meadow. Vegetation was *Kobresia humilis* meadow community with a variety of sedges, grasses and forbs that served as pika forage.

Experimental design: In early May and June, at the peak of reproduction, pikas were caught with string nooses, anchored to soil near the hole with chopsticks (Dobson *et al*., 2000). Blood samples (300 μL) of captured pregnant pikas (weight >190 g) were immediately collected from suborbital veins. The total handling time measured from initial caughting of an animal to the completion of the blood collection did not exceed 2 min. Blood samples were consistently taken by the same observer (The first researchers) and centrifuged at 7000 rpm for 10 min, plasma separated from blood cells and stored at -20°C until subsequent hormone assays. After the blood sampling, all pregnant pikas were taken in HAMERS and were housed individually in plastic-bottomed wire cages (20×30×20 cm high) and were provided with water and feed *ad libitum*. Pregnant animals gave birth after 1-4 days. About 1 day after pup delivery, adult female pikas were introduced into a 2.5 L jar and anesthetized through adding a piece of tissue paper impregnated with ether. After 1 min, the animal was taken out, weighted and measured for the length (mm) of body, rear foot and ear. All animals revived from anesthesia within 10 min and were transferred back to cages. About 15 days after pup delivery, the newly born pikas were checked to determine sex and figured out the sex ratio (Males/Males+Females) in the litter of each mother.

Measurement of the plasma glucagon and immunoglobulin G levels: Glucagon (Glu) and Immunoglobulin G (IgG) concentrations in the plasma were assayed by Enzyme-Linked Immuno Sorbent Assay (ELISA) with reagents supplied by Shanghai Yope Biotech Inc and according to the manufacturer's instructions. The intensity of the colored product is directly proportional to the concentration of Glu (or IgG) present in the samples. The sensitivity in the analyses was 1.0 pg mL^{-1} for Glu and 0.1 g L^{-1} for IgG. The researchers ran samples in duplicate and compared hormone concentrations to a standard curve.

Data analysis: The body weight, ear length, rear foot length, body length, Glu and IgG concentrations in the plasma were used to evaluate the maternal body condition of adult pikas. The assumption of normality was tested with Komolgorov-Smirnov test and the assumption of homogeneity of variances was tested with Levene test. If these assumptions were not met, Logarithmic (LOG) or Square-Root (SQRT) transformation was made. The variables of maternal body condition, litter sex ratios and size were compared using a two-way (Year x Month) ANOVA. Meanwhile, the effect of variables of maternal body condition on litter sex ratio and litter size were analyzed by stepwise regression. An alpha level ($\alpha<0.05$) was used for all tests. All statistical analyses were performed with the statistical software SPSS 16.0.

RESULTS

Morphological characteristic of adult female pikas: The body weight, ear, rear foot and body length of adult female pikas form May to June among 2009-2010 were shown in Table 1. Two-way ANOVA on the weight of adult female pikas as a dependent variable revealed a significant month effect ($F = 5.231$, $p = 0.014$) but non-significant year effect ($F = 1.314$, $p = 0.251$) and non-significant (Year x Month) interaction ($F = 1.262$, $p = 0.231$) were observed. On the other hand, the body, rear foot and ear lengths of adult female pikas showed neither month nor year effects nor (Year x Month) interaction ($p>0.05$).

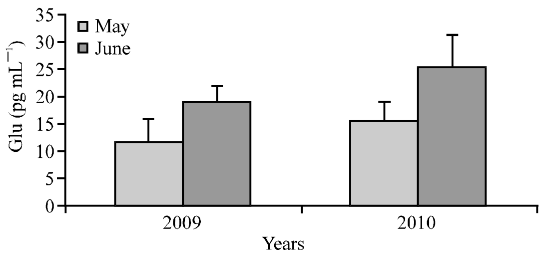

Fig. 1: The concentrations of glucagon (pg mL^{-1}) in plasma of adult female plateau pikas from May to June in 2009 and 2010

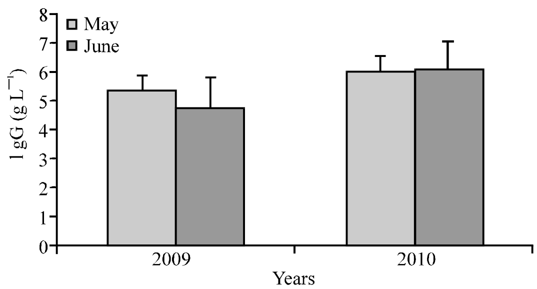

Fig. 2: The concentrations of immunoglobulin G (g L^{-1}) in plasma of adult female plateau pikas from May to June in 2009 and 2010

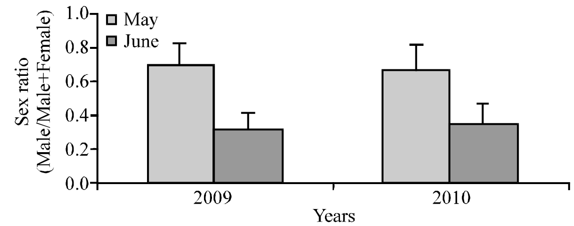

Fig. 3: The litter sex ratio (Males/Males+Females) of female plateau pikas from May to June in 2009 and 2010

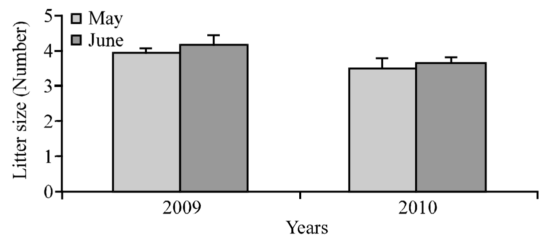

Fig. 4: The litter size (Number) of female plateau pikas from May to June in 2009 and 2010

Plasma glucagon and immunoglobulin G levels: Glu and IgG concentrations in the plasma of adult female pikas form May to June in 2009 and 2010 were shown in Fig. 1 and 2. Two-way ANOVA on the Glu and IgG concentrations of adult female pikas as a dependent variables revealed non-significant month effect ($p>0.05$), year effect ($p>0.05$) nor (Year x Month) interaction ($p>0.05$).

Litter sex ratio: Litter sex ratios of adult female pikas form May to June in 2009 and 2010 were shown in Fig. 3. Two-way ANOVA on the litter sex ratios revealed a significant month effect ($F = 21.297$, $p<0.001$) but a non-significant year effect ($F = 1.199$, $p = 0.278$) or (Year x Month) interaction ($F = 2.322$, $p = 0.131$) were observed. Stepwise regression indicated that body weight, ear length, rear foot length, body length, Glu and IgG concentrations in the plasma of adult female had non-significant effects on the litter sex ratio ($p>0.05$). However, litter size has a significant negative effect on the litter sex ratio ($t = -2.355$, $p = 0.022$).

Litter size: Litter sizes of adult female pikas form May to June in 2009 and 2010 were shown in Fig. 4. Two-way ANOVA on the litter sex ratios revealed non-significant month ($F = 5.091$, $p = 0.107$) nor year effects ($F = 0.872$, $p = 0.355$) nor (Year x Month) interaction ($F = 1.327$, $p = 0.257$). Stepwise regression indicated that body weight of adult female had a significant effect on the litter size ($t = 2.164$, $p = 0.034$) however, ear length, rear foot length, body length, Glu and IgG concentrations in the plasma of adult female had no effect on the litter size ($p>0.05$).

DISCUSSION

Up to the knowledge, this study is the first study to validate the Trivers-Willard hypothesis on a polytocous mammal in China. The original Trivers-Willard hypothesis was proven valid to species in which litter or clutch size is one (Frank, 1990; Hardy, 2002). If mothers produce a variable number of offspring, adaptive adjustments according to maternal conditions may affect both the sex and the number of offspring within the litter or clutch and the predictions may be changed (Williams, 1979; McGinley, 1984). As the number of offspring per reproductive attempt (or the number of offspring that shared limited parental resources) increases across species, fitness returns from investing in male and female offspring will become equivalent because fitness returns scaling with numbers of offspring will outweigh differences between the fitness returns of having male or female offspring (Frank, 1990). As Williams (1979) pointed out, considering both numbers and sex of

Table 1: The body weight (g), ear, rear foot and body length (cm) of the plateau pikas female adult from May to June in 2009 and 2010

Years	Months	Body weight (g)	Ear length (cm)	Rear foot length (cm)	Body length (cm)
2009	May	123.12±3.78	2.18±0.03	3.51±0.04	18.32±0.21
	June	119.55±2.78	2.12±0.04	3.35±0.03	18.43±0.31
2010	May	126.14±6.37	2.24±0.03	3.47±0.04	18.36±0.26
	June	119.12±1.49	2.23±0.03	3.48±0.03	18.75±0.16

offspring complicates predictions about the relationship between parental resources and the sex ratio under an adaptive model. Consequently, the Trivers-Willard hypothesis needs to be modified for species with multiple births. Servanty *et al.* (2007) had research the changes in sex ratio in relation to litter size in a highly polytocous species, wild boar (*Sus scrofa scrofa*). The fetal sex ratio was negatively related to increasing litter size, providing some support for TWH. The results agree with that of Servanty *et al.* (2007) to a certain extent. Although, neither body weight, ear length, rear foot length, body length nor Glu and IgG concentration influenced the proportion of male in litters however, litter size has a significant negative effect on the litter sex ratio and body weight of adult female have a significant effect on the litter size. This adjustment of offspring sex ratio in relation to litter size, involving higher sex ratio when more per capita resources are available, might support the TWH.

In the reproductive period of plateau pikas, adult females have been producing 3-5 sequential litters in 3 weeks intervals (Smith and Wang, 1991; Yin *et al.*, 2004). In the middle of May, most of adult females gave birth to the first litter. About 3 weeks later (June), the second litter has been born. So, in this research, the young pikas born in May were the first litter and the young pikas born in June were the second litter. Although, the Glu and IgG concentration of adult females were not significantly differ between the May and June, the body weight of adult females in May was significantly higher than that of June (Table 1). It is shown that the maternal body condition in May exceeded that of June. On the other hand, the proportion of male young in the first litter was significantly higher than that of second litter (Fig. 3), it suggests that the TWH could be supported.

CONCLUSION

In this study, the results for the plateau pikas are in concordance with the central idea of a higher investment in the sex with the higher reproductive potential (Trivers and Willard, 1973; Clutton-Brock *et al.*, 1981) but the predictions of TWH should be tested with caution in polytocous species where litter size adjustment may be much more important than sex ratio variation (Frank, 1990) in any case these should be examined in combination. Further studies of postnatal survival of young pikas in relation to litter composition and reproductive time are needed.

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