Operational Sex Ratio and Alternative Reproductive Behaviours in Chinese Bushcricket, *Gampsocleis gratiosa*

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Abstract

The effects of operational sex ratio (OSR) on male mating tactics in the Chinese bushcricket *Gampsocleis gratiosa* were investigated in male- and female-biased environments. We measured fresh and dry spermatophore contents and copulation duration, and counted sperm numbers of each copulation. The fresh weight of spermatophore and spermatophylax was positively correlated with male body weight. The males in a strongly male-biased environment produced significantly heavier fresh ampulla and more sperm per ejaculation, which were likely tactics for successful matings under the competition of rivals. The spermatophore might function as a structure to protect the fertilization potential of the ejaculate from rival males.

Introduction

Several recent studies demonstrated that differences between males and females in potential reproductive rate, copulation refractory time, mortality, and nutritional status, can increase or decrease the ratio of sexually active males to fertilizable females in populations of polygamous species (Kvarnemo & Simmons 1998; Okuda 1999; Kvarnemo & Forsgren 2000). Emlen & Oring (1977) defined the ratio of sexually active males to fertilizable females as operational sex ratio (OSR), and postulated that variations in sexual behaviour under different OSRs would play an important role in increasing the reproductive success and fitness. In most insect species, longer copulations predominate in male-biased environments as opposed to shorter copulations in female-biased environments (McLain 1981; Sillén-Tullberg 1981; Clark 1988). Male insects can respond to the threat of sperm competition by increasing the amount of sperm (Gage & Barnard 1996) or decreasing the mass of spermatophore (Gage 1995) transferred during copulation in a male-biased population, while constraining the ejaculate size in a female-biased population (Reinhardt & Arlt 2003).

In a male-biased environment, the likelihood that a mated male encounters another female is relatively low. Therefore, males should invest relatively more sperm per mating by ways of longer copulation. In addition to sperm transfer, longer copulations may reflect mate guarding as a form of competition for mating partners among males in a male-biased environment (Thornhill & Alcock 1983; Schöfl & Taborsky 2002). In contrast, females are likely to mate with more than one male, hence the competitive costs for males could be compensated by copulating longer and transferring relatively more sperm (Thornhill 1980; Dickinson 1986; Wolf et al. 1989). Studies using fruit flies showed that longer copulation resulted in greater number of offspring (Sisodia & Singh 1996; Singh & Singh 1999).

In bushcrickets, males donate a spermatophore to a female after copulation, which is then consumed by the female as courtship food (Gwynne 1997). The spermatophore consists of spermatophylax, ampulla and semen. Gwynne (1984) hypothesized that the spermatophore functions as paternal investment, while Simmons et al. (1993) and Wedell (1994) argued that the spermatophore might function to protect the ejaculate.

The Chinese bushcricket (*Gampsocleis gratiosa* Brunner von Wattenwyl) is widely distributed in hilly regions, and on grasslands of Northern China. Several lines of evidence indicate that great variation exists in investment of spermatophore components and sperm number during copulation in the Chinese bushcricket (Jia & Jiang 1999a,b; Jia et al. 2000) as well as in other bushcrickets (Simmons et al. 1993). In the Chinese bushcricket, a male needs 1–3 d, depending on the nutritional supply (Jia & Jiang 1999a), to produce a new spermatophore. Therefore, males require much longer mating intervals than females, which can re-mate very soon after the consumption of the spermatophore. Such difference in mating intervals between males and females influences the potential reproductive rates (Gwynne 1990), resulting in decreased OSR.

The Chinese bushcricket offers an ideal model insect for investigating variation of copulation investment by males under different OSRs. We studied the effects of OSR on the variation of copulation duration, spermatophore components, and sperm number per ejaculation, in order to determine whether males could adjust these traits to differently male- and female-biased environments.

Materials and Methods

Origin and Collections of Insects

More than 700 Chinese bushcrickets, as penultimate instar nymphs, were collected from Yi County, Hebei province, China (39°20′N, 115°30′E). They were housed individually in a transparent plastic container (640 ml bottle with two openings on two sides covered with plastic mesh screen), and fed with yellow mealworms *Tenebrio molitor*, green vegetables and water *ad libitum* with daily checking. All containers were kept in a room with a photoperiod of L:D 14:10 and a temperature of 30 ± 2°C. Seven days after the imaginal moult, most of the bushcrickets became sexually mature. Eighty-seven males and 87 females were selected for the experiment based on two criteria. (i) They had to be sexually mature and (ii) their body sizes had to be within the 10% of the mean size of the entire sample. Those insects were allowed to mate once over a period of 3 d prior to the experiment to standardize the mating status.

Experimental Design and Measurements

Male reproductive parameters were tested for five different OSRs (male:female): two treatments with male-biased sex ratio (4:1 and 2:1), two treatments with female-biased sex ratio (1:2 and 1:4), and one with equal sex ratio (1:1) as control. The mating trials were grouped into five replicates in accordance with the days the tests were conducted. Each replicate consisted of 25 tests (five tests in each of the five sex ratio treatments) conducted overnight starting at 9:00 PM, as the Chinese bushcrickets mate mostly late at night or early in the morning (pers. obs.). Forty-five males and 45 females were used in the tests within each replicate. During the trial, once the males and females were mated, they were measured and replaced with new pairs; the unmated males and females were moved into the tests of the following replicated trial.

The weight and width of the pronotum were measured for each individual before the experiment started, and all the crickets used for the trials were marked with Arabic number on the pronotum for labelling. The marked individuals were placed into a mating cage (30 × 30 × 30 cm in dimension), separated by sex with a wire mesh, and kept for 24 h with food and water supplied. The mesh was removed at the beginning of the trial, and each trial was terminated when a spermatophore had been transferred, or after a maximum of 3 h.

The copulation duration was monitored and recorded by a computer program developed by Gao & Kang (2003). Following mating, the spermatophore was removed from the female with tweezers and weighed to the nearest 0.0001 g (Sartorius 210s Electric Balance). The spermatophylax was carefully separated from the ampulla and weighed. The semen was extruded from the ampulla and dissolved into 5 ml of PBS buffer, which was then stirred vigorously to a vortex for 1 min to prevent agglutination of the sperm. The sperm number was counted using the method described by Simmons et al. (1993) and Vahed & Gilbert (1996). The spermatophylax and ampulla were dehydrated in an oven at 40°C for 4 h and weighed.

Statistical Analysis

Prior to the statistical analysis, we examined the normality of the data distribution using Kolmogorov–Smirnov tests. Univariate analysis (ANOVA) was performed to compare the numbers of mating events per replicate for each of the five OSR treatments. Relationships among spermatophore, spermatophylax, and ampulla mass, copulation duration, sperm number and male or female body weight were analysed by Pearson's correlation and least-squared

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regression. The correlation table was corrected by the Sequential Bonferroni method (Rice 1989). We used a General Linear Model approach with OSR as fixed factor, and male body weight as covariates to test the effect of OSR on copulation duration, fresh weight of spermatophore, fresh and dry weight of spermatophylax, and ampulla, as well as sperm number. This approach has the advantage of concurrently considering manipulated factors (OSR) and non-manipulated but potentially influencing mating parameters (male body weight), rather than using residuals in a separate analysis (Freckleton 2002). Then Tukey's tests were used to compare the differences among the five treatments. All values are presented as means ± SE. All statistics were done with SPSS 11.0, SPSS Inc.

Results

Ratio of Successful Mating

We recorded 52 successful mating events among a total of 87 pairs of the crickets: seven for the 4:1 OSR, 11 for the 2:1 OSR, 10 for the 1:1 OSR, 11 for the 1:2 OSR, and 13 for the 1:4 OSR. We analysed the number of mating events per replicate of each OSR treatment and found that the OSR had no significant effect on the rate of successful mating (ANOVA, $F_{1,23} = 0.248$, $p = 0.312$).

Male Reproductive Investment

The copulation duration varied from 584 to 2280 s, with an average of 1282 ± 321 s across all the trials (Table 1). The average weight of fresh spermatophores measured 466 ± 77 mg, which was 11.32% of the average weight of the male body. After dehydration, the mean weight of spermatophylax and ampulla decreased from 339 to 58 mg and from 77 to 14 mg, respectively, losing up to 80% of the fresh weight (Table 1).

The fresh weight of spermatophore was significantly positive, correlated with the fresh and dry weights of spermatophylax, the fresh weight of ampulla and the male body weight (Table 2). The fresh weights of spermatophylax and ampulla were significantly (Table 2) correlated with the dry weights of the two variables.

Table 1: Reproductive investment by male Chinese bushcricket *Gampsocleis gratiosa* under different OSRs. Values presented are means ± SE of five replicates

	OSR (male:female)				
	4:1	2:1	1:1	1:2	1:4
Fresh weight (mg)					
Spermatophore	452 ± 77	459 ± 37	465 ± 84	457 ± 45	499 ± 41
Spermatophylax	313 ± 48	349 ± 45	336 ± 57	336 ± 32	358 ± 27
Ampulla	92 ± 9	69 ± 8	71 ± 22	73 ± 3	80 ± 13
Dry weight (mg)					
Spermatophylax	52 ± 10	63 ± 15	57 ± 14	52 ± 6	65 ± 17
Ampulla	17 ± 4	14 ± 3	14 ± 4	13 ± 1	14 ± 4
Copulation duration (s)	1045 ± 321	1193 ± 130	1554 ± 431	1359 ± 252	1225 ± 173
Sperm number (4×10^4/μl)	6.16 ± 0.78	3.56 ± 0.72	2.64 ± 0.91	3.02 ± 1.62	3.42 ± 0.83

Table 2: Correlation coefficients between various reproductive variables in the Chinese bushcricket *Gampsocleis gratiosa* (n = 52)

	Fresh weight of spermatophylax	Fresh weight of ampulla	Dry weight of spermatophylax	Dry weight of ampulla	Copulation duration	Sperm number	Male weight	Female weight
Fresh weight of spermatophore	0.923*	0.562*	0.763*	0.343	0.296	0.125	0.48*	0.212
Fresh weight of spermatophylax		0.392	0.821*	0.299	0.394	0.112	0.339	0.183
Fresh weight of ampulla			0.340	0.815*	−0.074	0.091	0.227	0.012
Dry weight of spermatophylax				0.343	0.337	0.207	0.261	0.106
Dry weight of ampulla					−0.131	0.156	0.102	−0.025
Copulation duration						0.270	−0.113	0.145
Sperm number							−0.121	−0.001
Male weight								0.119

*$p < 0.05$ after sequential Bonferroni correction.

Table 3: General linear model on the mating parameters in the bushcricket *Gampsocleis gratiosa*, OSR as fixed factors and male body weight as covariables

Source	Dependent variable	df	F	Sig.	R^2
Model	Copulation duration	5	1.216	0.323	0.317
	Fresh weight of spermatophore	5	2.839	0.030	0.226
	Fresh weight of spermatophylax	5	1.662	0.171	0.157
	Fresh weight of ampulla	5	2.946	0.026	0.304
	Dry weight of spermatophylax	5	1.493	0.218	0.105
	Dry weight of ampulla	5	2.425	0.055	0.183
	Sperm number	5	2.306	0.066	0.240
Intercept	Copulation duration	1	14.402	0.001	
	Fresh weight of spermatophore	1	2.975	0.094	
	Fresh weight of spermatophylax	1	3.766	0.061	
	Fresh weight of ampulla	1	1.857	0.182	
	Dry weight of spermatophylax	1	0.652	0.425	
	Dry weight of ampulla	1	1.049	0.313	
	Sperm number	1	4.183	0.049	
Male body weight	Copulation duration	1	0.622	0.436	
	Fresh weight of spermatophore	1	11.486	0.002	
	Fresh weight of spermatophylax	1	7.210	0.011	
	Fresh weight of ampulla	1	4.212	0.048	
	Dry weight of spermatophylax	1	4.486	0.042	
	Dry weight of ampulla	1	3.746	0.061	
	Sperm number	1	0.328	0.570	
OSR	Copulation duration	4	1.395	0.256	
	Fresh weight of spermatophore	4	0.374	0.826	
	Fresh weight of spermatophylax	4	0.203	0.935	
	Fresh weight of ampulla	4	2.638	0.041	
	Fry weight of spermatophylax	4	0.410	0.800	
	Dry weight of ampulla	4	2.111	0.101	
	Sperm number	4	2.864	0.038	

The copulations did not differ significantly among the five OSR levels treatments (GLM, $F = 1.395$, $p = 0.256$, Table 3, Fig. 1). Only the fresh weight of ampulla and sperm number were affected significantly by the OSR (GLM, ampulla: $F = 2.638$, $p = 0.041$; sperm number: $F = 2.864$, $p = 0.038$). The fresh weight of ampulla was significantly greater in the strongly male-biased treatment (4:1 OSR) than other treatments (Tukey's test, $p < 0.05$, Fig. 2). The sperm numbers were greatest in the

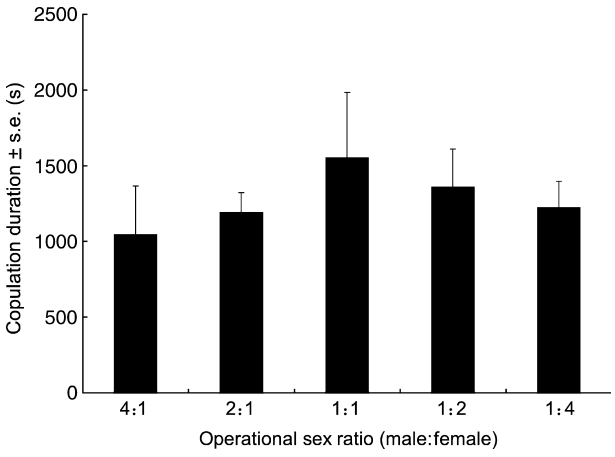

Fig. 1: Copulation duration of male Chinese bushcricket *G. gratiosa* under different OSRs (mean ± SE of five replicates)

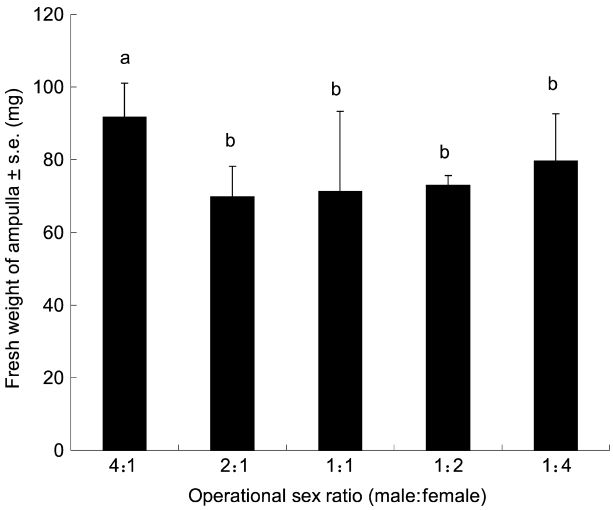

Fig. 2: Fresh weight of ampulla transferred by male Chinese bushcricket *G. gratiosa* under different OSR environments (mean ± SE of five replicates). Groups designated with the same letters are not significantly different

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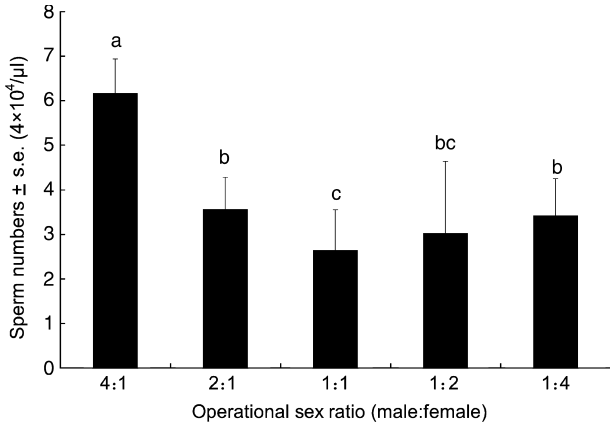

Fig. 3: Sperm numbers transferred by male Chinese bushcrickets *G. gratiosa* under different OSR environments (mean ± SE of five replicates). Groups designated with the same letters are not significantly different at $p \leq 0.05$

strongly male-biased treatment (4:1 OSR), and smallest in the equal sex ratio of 1:1 (Tukey's test, $p < 0.05$, Fig. 3).

Discussion

Operational sex ratio may affect the reproductive strategies of male insects regarding nutritional investment in mating partners, copulation duration and ejaculate size (Clark 1988; Reinhardt & Arlt 2003). Using the Chinese bushcricket as a model, we examined three aspects of the reproductive investment, copulation duration, spermatophore contents and ejaculate size and demonstrated that males follow different investment strategies with ampulla weight and sperm numbers depending on OSR, but not spermatophore weight, spermatophylax weight, and copulation duration. We found that in a strongly male-biased environment, male bushcrickets increased their investment in fresh ampulla weight and sperm number, which is likely to improve the fertilization potential of their ejaculate when competition is strong. The quantitative change of reproductive parameters under different OSR suggests that male bushcrickets adapt flexibly to their social environment. Studies in a number of other animal taxa (reviewed in Vahed 1998) such as guppies *Poecilia reticulata* (Farr 1976), water striders (Clark 1988), Mediterranean fruit fly (Gage 1991) and other bushcrickets (Simmons et al. 1993) also found the changes in ejaculate size, spermatophore size or copulation duration in response to social factors, but the effects of sex ratio has rarely be varied as systematically before. In contrast to most other studies, our results suggest that in a strongly female-biased environment males did not change their copulation duration and spermatophore contents.

In strongly male-biased environments, competition among the males for a limited number of females is expected to be intense. We observed that under such conditions, instead of fighting to keep rivals off the females, males often touched each other with antennae, body flank or even with their genitalia for extended periods of time. Sometimes males ejaculated after being touched at their genitalia. This may potentially be a tactic of males to prevent rivals from courting females. However, in contrast to the findings of other studies (McLain 1981; Sillén-Tullberg 1981; Clark 1988), the longest copulation durations were observed in the equal sex ratio treatment rather than under male-biased as it were expected, though the effect of the sex ratio on the copulation duration was not statistically significant.

The spermatophore of bushcrickets may function to retain the sperm in the female vagina, similar to copulatory plugs in certain grasshoppers (Gregory 1965) and rodents (Hartung & Dewsbury 1978). The ampulla is the hardest part of the spermatophore. With a heavier ampulla, female bushcrickets would need a longer time to consume the whole spermatophore, thus decreasing their chance for further matings. Therefore, we assume that the heavier spermatophores and ampullae served to protect the sperm in the highly male-biased environment. Increasing sperm number and ampulla mass may ensure that more sperm is transferred to the female, and hence a higher reproductive success is achieved (Simmons et al. 1993; Reinhardt & Arlt 2003).

In several insects, like the migratory locust (Reinhardt & Arlt 2003) and in fruit flies (*Drosophila* spp., Spiess 1970), the copulation duration is closely related to the rate of sperm transfer. In *Drosophila bipectinata* and *D. ananassae*, for example, the copulation duration correlated with the number of progeny produced, indicating that copulation duration and transferred sperm number are closely coupled (Sisodia & Singh 1996; Singh & Singh 1999). In contrast, in Chinese bushcrickets, there was no apparent relationship between copulation duration and sperm numbers in the spermatophore. This may be due to the fact that in bushcrickets, copulation ends shortly after spermatophore transfer, and sperm transfer occurs only at the end of copulation (Gwynne 1990).

In conclusion, our study demonstrated that, in Chinese bushcrickets, the males in a strongly

male-biased environment produced significantly heavier ampullae (fresh weight) and more sperm per ejaculation, which is likely to be a tactic to increase the mating success when competition is intense. The spermatophore might function as a structure to protect the fertilization potential of the ejaculate from rival males. Future research should reveal whether this tactic is commonly found among insects.

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