Mating tactics and related behaviors and morphology of males in the Japanese scorpionfly *Panorpa japonica.*

ヤマトシリアゲ *Panorpa japonica* における雄の交尾戦術とそれに関する行動と形態について

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CHAPTER 1

General introduction

In sexual selection, males with more developed sexual traits, such as body size and weapon traits, have been thought to be more likely to be preferred by females (Andersson 1994). However, large individual variation in the expression of male sexual traits have been observed in a wide range of animal taxa (e.g., Iwata et al. 2005; Ota et al. 2014; Kelly 2006).

Over the past two decades, it has been shown experimentally in a wide range of animal taxa that the intensity of environmental stress (e.g., population density; temperature; nutritional conditions) influences the maintenance of diversity in male sexual traits (Qvarnström 2001; Balaban-Feld and Valone 2017; Kelly 2018). For example, in a species of stalk-eyed flies, male sexual trait, an eye-span, is affected by worsening nutritional conditions during growth, leading to greater genetic variance in eye-span (David et al. 2000). It has also been shown that male sexual traits increased by environmental stress affect the strength of female preference for male sexual traits (Qvarnström 2001; Balaban-Feld and Valone 2017; Kelly 2018).

Although there are many examples of studies on identifying genetic and environmental factors involved in the diversity of male sexual traits (e.g., Engqvist and Sauer 2008; Morrow et al. 2008), there is less knowledge on the impact of differences in environmental factors among different populations for male sexual traits (Kelly 2018).

Mating tactics have been reported to be influenced by other sexual traits such as body size and weapon traits (e.g., Okada and Hasegawa 2005; Karino and Niiyama 2006). For example, males who are less competitive and have less chance of mating employ mating tactics such as satellite tactics and sneaking tactics (e.g., Moczek and Nijhout 2003; Iwata et al. 2005; Ingleby et al. 2010; Ota et al. 2014; Kelly 2006).

The rate of adoption of mating tactics has been shown to affect interspecific competition, predation pressure, population density and viability ratios, among population (e.g., Thornhill 1987 Rowell and Cade 1993; Godin 1995). In the dung beetle *Onthophagus taurus*, which uses sneaking behavior as an alternative tactic, the frequency of male mating tactics has been shown in two artificially introduced populations in North America and Australia (Moczek and Nijhout 2003; Simmons et al. 2007).

These results suggest that it is possible to predict the external environmental stressors in their respective habitats and the strength of sexual selection in each local population by comparing the proportion of mating tactics employed among populations. Therefore, it is expected to be able to predict the strength of sexual selection by comparing the rate of adoption of mating tactics among populations.

In recent years, there have been numerous examples of studies showing that the mating tactics vary with environmental factors in field populations (e.g., Puniamoorthy et al. 2012; Okuzaki 2021), as well as studies comparing the rate of adoption of mating tactics among closely related species (e.g., Missoweit and Sauer 2007; Ota et al. 2014). However, to the best of my knowledge, there are few examples of regional comparisons of the rate of adoption of mating tactics in the same species, except when artificially introduced populations are used.

One of the mating tactics is nuptial gift. Nuptial gift is one of the mating styles in which the male presents resources such as food to female, and male mates while the female consumes the gift (Thornhill and Alcock 1983). There have been numerous studies on nuptial presentation behavior in nuptial presentation species (e.g., Sakaluk 1984; Thornhill 1979; 1981; 1992). However, there is a paucity of knowledge on the selection of mating tactics and intraspecific variation of mating tactics in nuptial presenting species.

In the scorpionfly (Panorpidae), which is often used to study sexual selection and mating tactics, three types of mating tactics: (1) nuptial gifts using nutritious saliva, (2) nuptial gifts using food in natural foods such as dead insects and fruits, and (3) forced mating without saliva or food (Thornhill 1983). Males of scorpionfly have been shown to potentially use multiple tactics as alternative tactics (e.g., Thornhill 1979; 1981; 1992; Byers and Thornhill 1983; Sato and Fujiyama 2018; Missoweit and Sauer 2007).

In North American species, only males that win the male-male competition over food can perform nuptial gifts using food, loser males choose nuptial gifts using salivary masses, and males that cannot produce salivary masses due to malnutrition choose forced mating (Thornhill 1980). In addition, north American females preferred males that selected nuptial gifts using food, especially males that selected large-sized food as nuptial gifts, to males that selected nuptial gifts using salivary masses (Thornhill 1981). Thornhill (1981) concluded that the insect mating system of the North American scorpionfly is resource-defense polygyny (RDP). However, a number of studies have been reported suggesting that European species do not have RDP mating systems. For example, in *P. vulgaris* and *P. alpina*, males do not have a monopoly on food resources, and the mating period of males is longer for nuptial gifts using salivary masses than for nuptial gifts using food (Engqvist and Sauer 2003; Missoweit and Sauer 2007).

These results suggest that the adoption rate of mating tactics and the mating system are different between North American and European species. However, there are few studies on the details of mating tactics and mating systems in Japanese species.

The Japanese scorpionfly, *Panorpa japonica*, used in this study is ubiquitous throughout Honshu, Shikoku, and Kyushu islands in Japan (Nakamura 2000; Tsutsumi 2010). *P. japonica* is easy to collect and mainly uses insect carcasses as nuptial gifts (Thornhill 1992a), which makes it very suitable as a research material because it is easy to observe mating behavior in the field and Laboratory experiments.

In this study, I used the Japanese scorpionfly, *P. japonica*, first to investigate the types of mating tactics used by this species and then to compare the rates of adoption of mating tactics among different populations.

In Chapter 2, I first conducted a laboratory experiment of mating observation to investigate the types of mating tactics used by this species. I also compared the relationship between the observed adoption rates of mating tactics and male traits.

Since the laboratory observations were made in a small container, field experiments were conducted in Chapter 3 to test whether males would behave in a field environment in a similar manner to the results of the laboratory experiments.

It has also been reported that males that lose the male-male competition (loser males) wait around food occupied by winner males and try to force mating with females (Thornhill 1992a). For this reason, I also observed the behavior of loser males. In addition, previous studies using North American scorpionflies have reported the use of wing swinging behavior, in which they move their wings up and down while rotating them, as a display of food defense against the same or other species (Magner and Montgomery 2017). Therefore, I tested whether wing-waving behavior is used in inter-male fighting and courtship in this species.

The results of the above field experiment behavioral observations suggested that the behavior of loser males in the field may be different from that of loser males in previous studies (Thornhill 1992a).

Therefore, in Chapter 4, I compared the adoption rate of mating tactics by loser males by field experiments in Aichi and Okayama where the previous study was conducted.

Chapter 2

Relationships between mating tactics and male traits such as, body size and fluctuating asymmetry in the Japanese scorpionfly.

2.1. Introduction

Sexual selection leads to the evolution of male traits that are advantageous for male-male competition and female mate choice (e.g., Andersson 1994; Miller and Svensson 2014). Male body size and weapon size are related to sexual selection in many species (e.g., Calder 1984; Peters 1986; Blanckenhorn 2000). Larger males have usually an advantage in male-male competition and thus tend to have more mating opportunities with females compared with those of smaller males in insects (e.g., Thornhill and Alcock 1983; Emlen 2008). On the other hand, smaller males avoid fighting, and they use satellite or sneaking tactics instead (e.g., Arak 1988; Gross 1996). For example, during copulation in the Japanese stag beetle, *Prosopocoilus inclinatus*, the duration of mounting by smaller males is longer than that by larger males, and smaller males force mating more frequently than larger males (Okada and Hasegawa 2005). Additionally, in some insect species, such as the Japanese rhinoceros beetle, *Trypoxylus dichotomus*, and the yellow dung fly, *Scathophaga stercoraria*, a negative correlation between body and weapon size and mating duration has been reported (Ward and Simmons 1991; Karino and Niiyama 2006).

Fluctuating asymmetry (FA) influences sexual selection (Møller 1990). FA is defined as small, random deviations from perfect bilateral symmetry in a morphological trait (Møller and Pomiankowski 1993). FA is caused by low genetic quality, nutritional status, and environmental stress during growth stages (Palmer and Strobeck 1986; Leary and Allendorf 1989; Parsons 1990; Thornhill and Sauer 1992). In the Australian sheep blowfly, *Lucilia cuprina*, FA is a very sensitive indicator of environmental stresses in the larval stage because FA is strongly affected by population density and temperature stress during the larval stage (Clarke and McKenzie 1992). Additionally, in various species, reproductive success is higher in males with smaller FA than in males with larger FA (Møller 1988; 1990; Harvey and Walsh 1993).

For example, in males of the scorpionfly *Panorpa vulgaris*, FA may be an indicator of sexual selection because bilateral forewing symmetry has a genetic basis and because males with symmetrical forewings are more likely to win male-male competitions (Thornhill and Sauer 1992). However, some studies have reported no significant correlation between genetic quality or the presence or absence of environmental stress and bilaterally symmetric fluctuations (e.g., Bjorksten et al. 2000; Woods et al. 1999). Early studies of FA and mating success also failed to evaluate measurement errors and the reproducibility of FA measurements (Simmons et al. 1999). Furthermore, a meta-analysis showed that publication bias occurred in earlier studies of FA and mating success (Palmer 2000). Thus, the relationship between FA and mating success in the context of sexual selection is now questioned. If FA is related to sexual selection, the adoption rate of mating tactics might also be affected by FA. However, to my knowledge, there are no studies on the effects of FA on the adoption rate of mating tactics and mating duration.

Males of many scorpionfly (Panorpidae) species provide nuptial gifts to females during mating. A nuptial gift is food, or a nutritious secretion provided before or during copulation (Thornhill and Alcock 1983). The nuptial tactics of male scorpionflies are to attract females by sex pheromones, and then the male provide food, such as arthropod carcasses or nutritious saliva secretions, to the females (Sauer et al. 1997).

In the Japanese scorpionfly, *Panorpa japonica*, males exhibit a characteristic posture presumably for releasing sex pheromone near bait and give it to an approaching female as food for a nuptial gift before mating (Thornhill 1992a). Thornhill (1992b) observed the behavior of male Japanese scorpionflies and reported that males frequently perform male-male combat for nuptial gifts and try to catch females using the graspers at the end of their abdomen. When males approach females without a nuptial gift, this behavior is called “forced mating”. Thornhill (1992b) also observed male-male competition and then reported that FA of forewing length is significantly less in winners than in losers, while females prefer the pheromone of males of relatively low FA in forewing length (Thornhill 1992a). In addition, high FA males have fewer lifetime mating frequencies with different females than low FA males (Thornhill 1992a; 1992b).

Therefore, high FA males may usually choose other tactics (such as force mating) over nuptial gifts. A high FA male might be investing a lot in the mating duration of one copulation event. However, there are no experimental examples of the relationships between FA, the adoption rate of mating tactics, and mating duration.

Thus, in the present study, I examined the relationships between FA and male body size, grasper (weapon used for direct male-male competition) size, mating tactics, and mating duration in *P. japonica*.

2.2. Materials and Methods

2.2.1. Insects

Forty males and 40 females of *P. japonica* were collected from Handa-yama Mountain, Okayama city, Japan (latitude: 34 ° 6 ‘ N, longitude: 133 ° 9 ’ E), from 1 to 15 May 2017 to minimize the effects of sampling time on male mating tactics. The experiment was conducted for one week after collection. In addition, I investigated the effects of the date and time of collection on the mating duration and mating tactics of the individuals used in the experiment, but no correlation was found (Wilcoxon signed-rank test; $Z = 0.4437$ $p > 0.05$). Therefore, I consider no periodical effect of insect collection on the results of this study.

Each adult was reared in a plastic container (10 cm diameter × 8 cm height) in a chamber maintained at 25 °C and 16 L : 8 D until the experiment was conducted. A piece of mealworm (*Tenebrio molitor*; weight: 0.2 g; length: 5 mm) was given once every two days.

2.2.2. Observation

A randomly chosen male and female were placed into a cylindrical plastic container (10 cm diameter × 10 cm height) in which a piece of mealworm (0.2 g) had been placed on the bottom with appropriately moistened absorbent cotton. After the pair was settled, mating behaviors were recorded with a video camera (HDR-PJ590V, Sony, Tokyo Japan). The mating duration was defined as the period from the point at which the male grabbed the female's abdomen to the point when the male released the female. Each male and female pair was used for only a single observation.

2.2.3. Measurement

After the recording was finished, each individual was preserved in 70 % ethanol, and the lengths of the left and right forewings were measured using a stereoscopic microscope (× 7) (Olympus SZX12, Tokyo) and microscope camera (INOCAM-HD2, Inohara Shokai, Hiroshima) according to the method described by Thornhill (1992a) (Fig. 2.1). Before I measured the wing length, I dried the wings to remove any moisture. In the species used in this study, the forewing anal vein was unclear (see Thornhill 1992a). Therefore, herein, the forewing length was measured from the starting point of the anal vein to the third radial vein (see Fig.2.1). Body size in *Panorpa* is accurately estimated by forewing length (Thornhill 1981). Thornhill (1981) showed that forewing length can be an accurate index of body size in *Panorpa* species. For *P. japonica*, Thornhill (1992b) defined forewing length as the entire length of anal vein. However, since its distal end was unclear in my samples, I measured the length from the proximal end of anal vein to the distal end of the third radial vein in this study (Fig. 2.1). In this study, forewing size (≒ body size) refers to the sum of the left and right forewing lengths divided by 2 according to the method described by Thornhill (1992a).

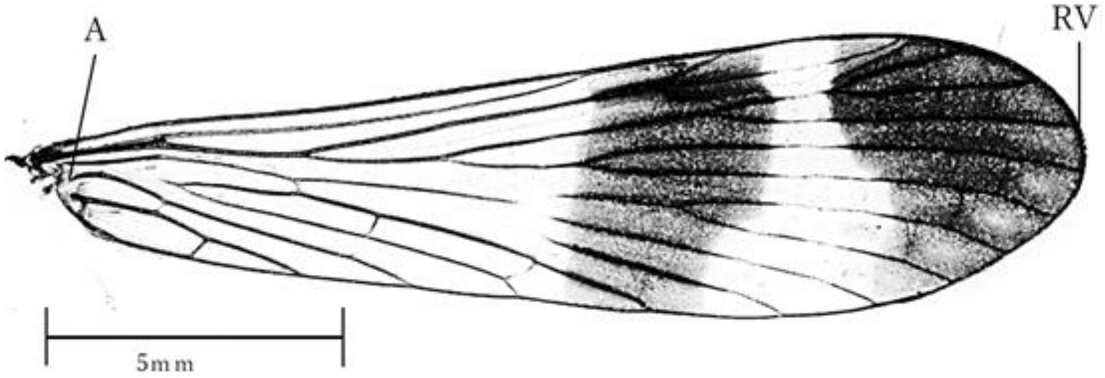


Fig. 2.1. Diagram of the right forewing of the species used in this experiment (*Panorpa japonica*). I defined the distance from the starting point of the anal vein (A) to the third radial vein (RV), as shown in the figure, as the length of the forewing.

FA was defined as the absolute values of the difference between the right and left wings. Each measurement of the forewings was taken three times. The three measurements for each forewing were used in all the analyses. Grasper size was defined as the length from the first protrusion counted from the inside (Fig. 2.2A) to the base to the tip of the grasper (Fig. 2.2B). The grasper base size was defined as the length from point A of Fig. 2.2 to the outside tip of the grasper base (Fig. 2.2C). Because the difference in body size between males and females may affect mating tactics and mating duration, I used the absolute values of the difference in body size between males and females. The ImageJ software program (Ver. 1.50i) was used for all the measurements. I adopted the absolute FA values, i. e., FA divided by body size, as additional indicators of FA. Note that all measurements of FA in the present study are absolute not relative values.

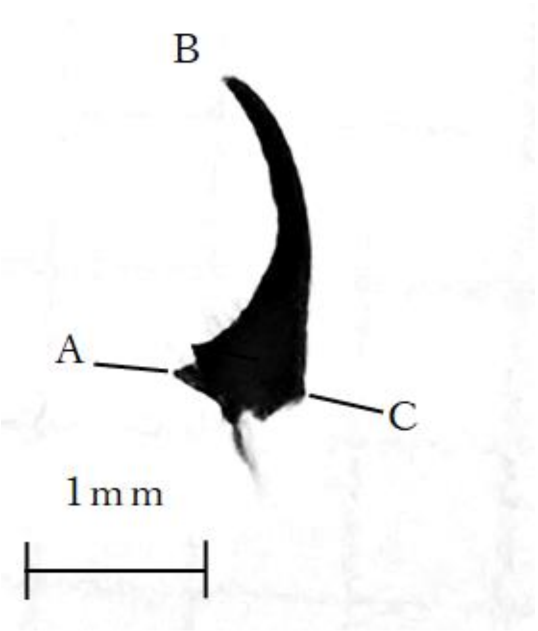


Fig. 2.2. Diagram of the right grasper of *Panorpa japonica.*

2.3. Statistical analysis

The one-way analysis of variance (ANOVA) was used to compare the mating durations of the three tactics. A mixed model ANOVA with three replicate measurements of body size and FA as a random factor was used to compare body size and FA among the tactics. Spearman's rank correlation coefficients were used to analyze the relationships between the mating duration and size parameters including FA. ANCOVA was used to assess the measurement error and reproducibility of FA in the relationships between mating duration and size parameters, including FA.

The statistical package JMP version 12.2 (SAS Institute Inc. 2015) was used for all the analyses.

2.4. Results

2.4.1. Behavioral sequences in mating

Fig. 2.3 shows a flowchart of male mating behavior. Three mating tactics were categorized based on the following criteria: (A) nuptial gifting; males found a bait, remained near the bait, released a pheromone, and succeeded in mating, (B) feeding mating; males did not release pheromones and did not wait near bait but approached a female who was eating bait and then succeeded in mating, and (C) forced mating; males approached females without releasing pheromones while the females were not eating a bait.

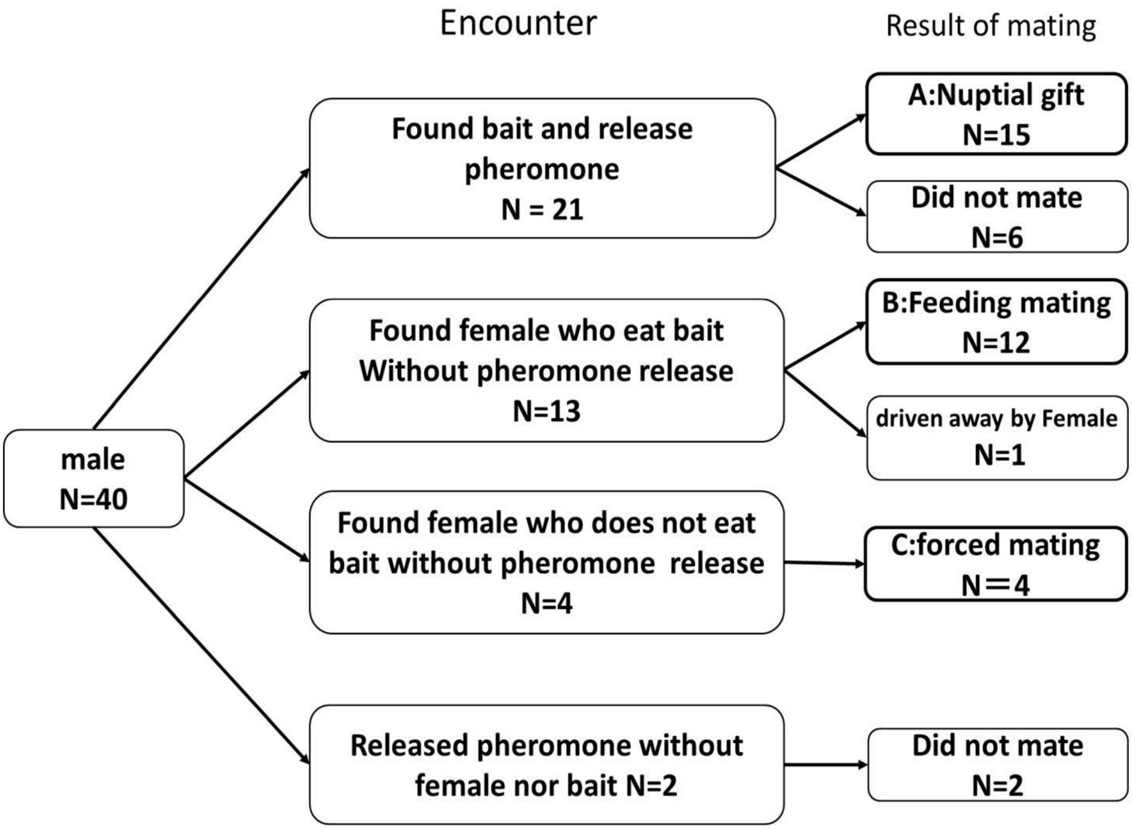


Fig. 2.3. Flowchart of the male mating sequence in *Panorpa japonica.*

The mating patterns of forty pairs were observed. At the encounter stage, 21 males found bait and released pheromones, 13 males found females that were eating the bait without releasing pheromones, 4 males found females that were not eating bait without releasing pheromones, and 2 males released pheromones without finding either a female or the bait. Of the 21 males that found bait and released pheromones, 15 presented a nuptial gift to the female and then mated with the female (A: nuptial gift), but the remaining 6 males did not mate. Of the 13 males that found a female that was eating the bait, 12 males mated with the female (B: feeding mating), but one male did not mate because he was driven away by the female. The four males that found a female that was not eating the bait proceeded to force mating (C: forced mating). The two males that released pheromones without finding a female or the bait did not mate.

In the following comparisons, I used three types of mating tactics, i. e., (A) nuptial gift, (B) feeding mating, and (C) forced mating, to compare mating duration, FA, male body size, and grasper size.

2.4.2. Relationships between mating tactics and mating duration

The mean mating durations for the three mating tactics were compared (Fig. 2.4), 3386.14 ± 524.43 (average ± standard error) (s) (N = 15) for (A) nuptial gifting, 3754.33 ± 586.33 (s) (N = 12) for (B) feeding mating, and 3307.33 ± 1312.55 (s) for (C) forced mating. No significant differences were found among the three tactics (One-way ANOVA; $F_{2,30} = 0.4303$; $p = 0.6546$). In the following analyses, (C) forced mating was removed because of the small sample size.

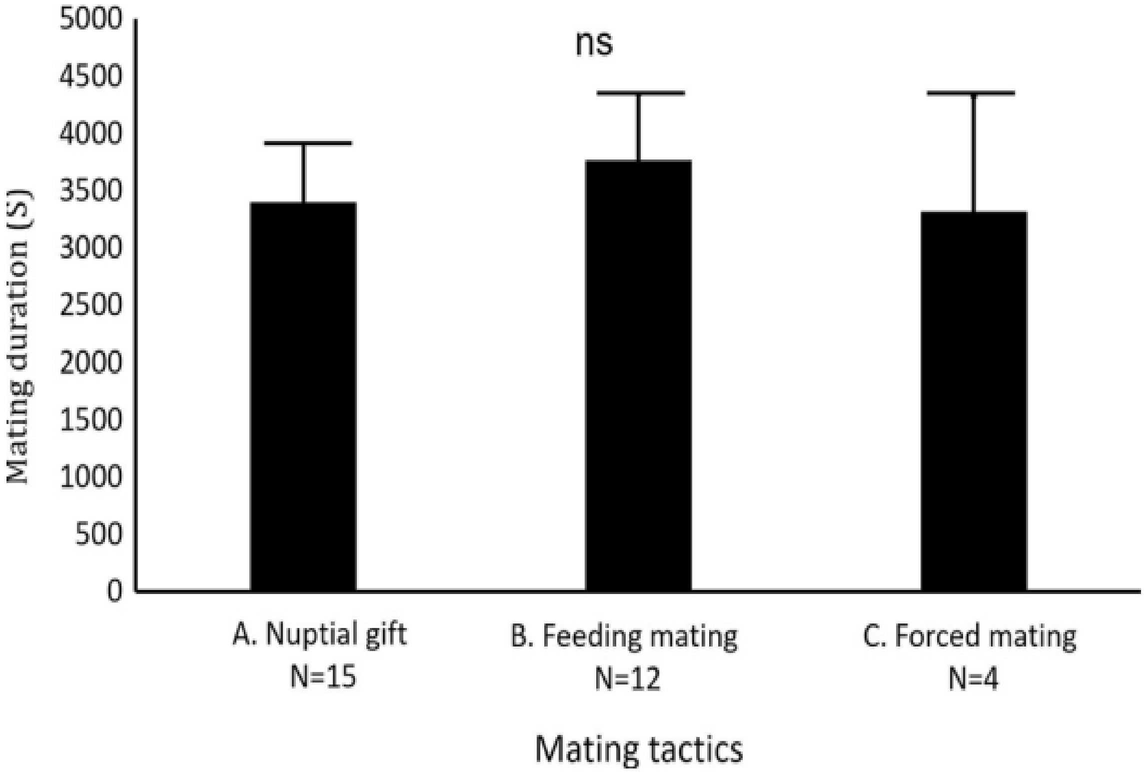


Fig. 2.4. Mating duration of the three mating tactics (Tukey's HSD test; $\alpha = 0.05$). The error bars on the graphs represents the standard error.

2.4.3. Body size and FA of males, mating duration, and grasper size in two mating tactics

I compared the FA and body size of the males that exhibited (A) nuptial gifting and (B) feeding mating (Fig. 2.5). Based on the mixed model ANOVA, which included three replicates as a random factor, significant differences were found between the two mating tactics for the absolute values of FA (Mixed ANOVA; $F_{1,25} = 8.3462$; $p = 0.0079$) (top graph of Fig. 2.5), although no significant differences were found between the two mating tactics for body size (Mixed ANOVA; $F_{1,25} = 0.1016$; $p =$

0.7525) (bottom graph of Fig. 2.5). The reproducibility of the forewing FA measurements was tested among the three replications, and the reproducibility was confirmed (ANCOVA; Table 2.1).

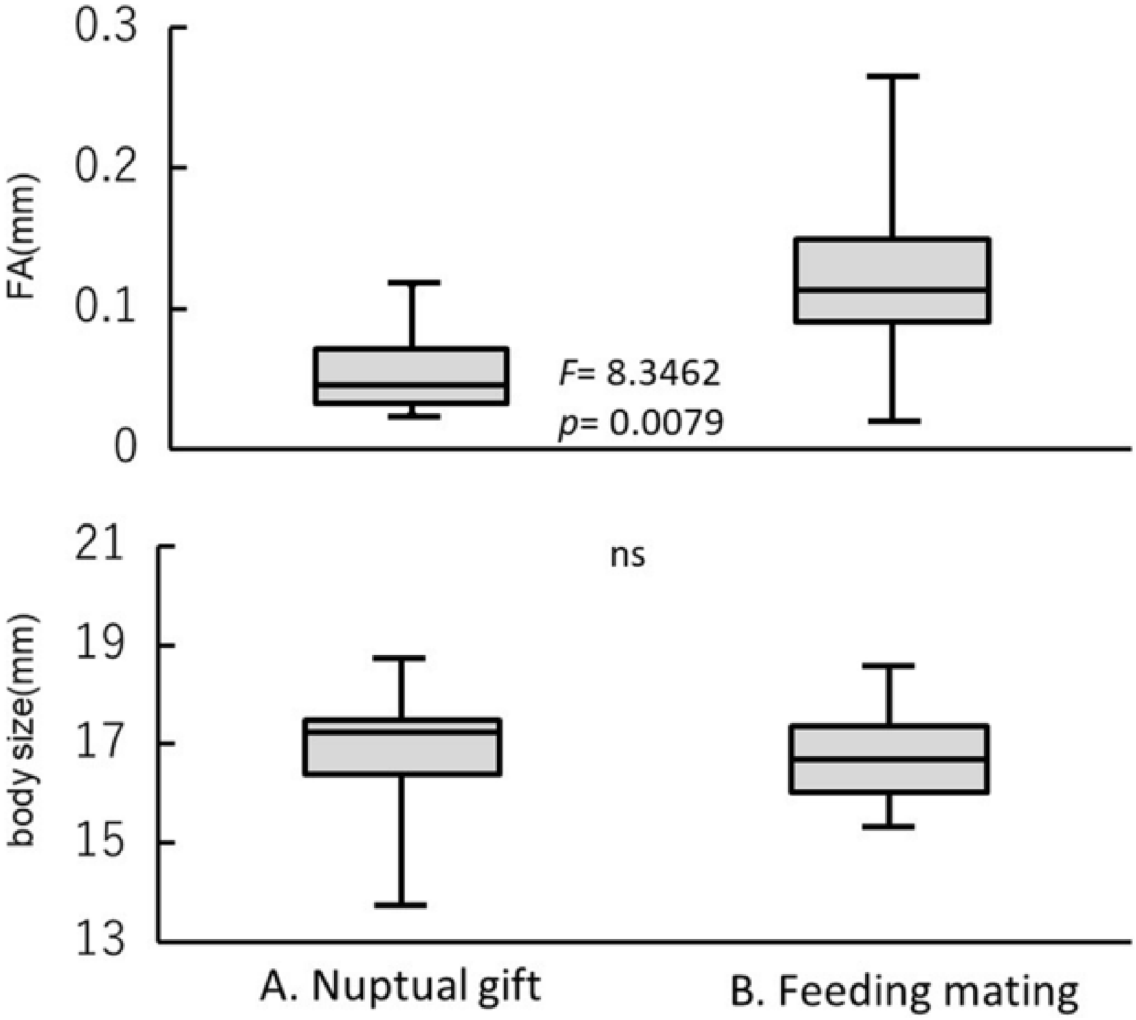
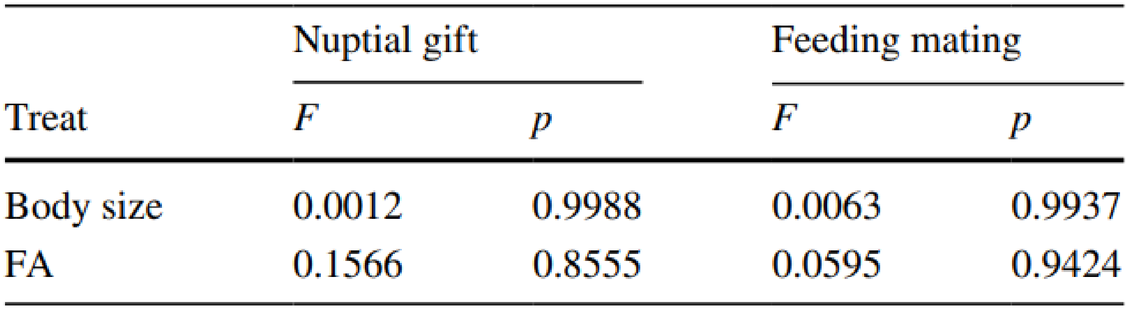


Fig. 2.5. Comparisons of FA and body size between the two mating tactics (by mixed ANOVA). The top graph shows the comparisons of the absolute value of FA between the two mating tactics, and the bottom graph shows the comparisons of body size between the two mating tactics.

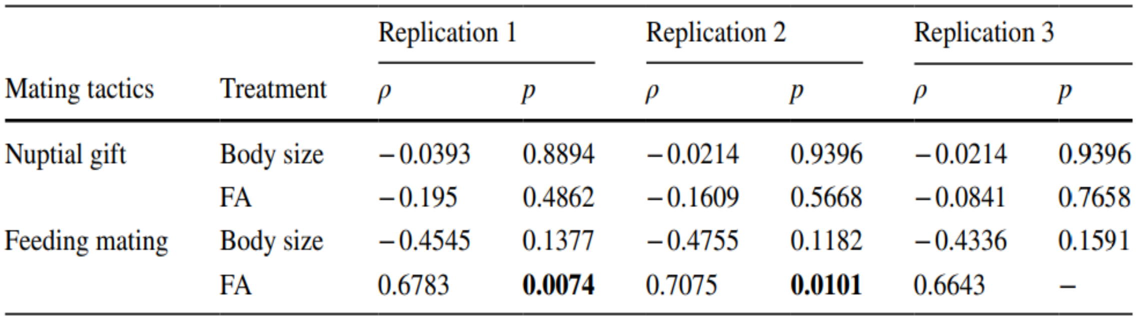
Table 2.1. Results of the reproducibility analysis of forewing FA measurement (ANCOVA).



	Nuptial gift		Feeding mating	
Treat	*F*	*p*	*F*	*p*
Body size	0.0012	0.9988	0.0063	0.9937
FA	0.1566	0.8555	0.0595	0.9424

The relationships between male body size / male FA in the three replications and mating duration are shown in Table 2.2. In nuptial gift mating, the relationships between male body size and mating duration and between FA and mating duration were not significant in any of the replications. In feeding mating, a significant negative correlation was found between the FA and mating duration in each replication. However, no significant relationships were found between male body size and mating duration in any of the replications.

Table 2.2. Relationships between male body size / male FA in the three replications and mating duration (Spearman's rank correlation coefficient)



		Replication 1		Replication 2		Replication 3	
Mating tactics	Treatment	ρ	*p*	ρ	*p*	ρ	*p*
Nuptial gift	Body size	−0.0393	0.8894	−0.0214	0.9396	−0.0214	0.9396
	FA	−0.195	0.4862	−0.1609	0.5668	−0.0841	0.7658
Feeding mating	Body size	−0.4545	0.1377	−0.4755	0.1182	−0.4336	0.1591
	FA	0.6783	**0.0074**	0.7075	**0.0101**	0.6643	–

Bold values indicate statistically significant

Fig. 2.6 shows the male grasper size (top graph) and grasper base size (bottom graph) for the (A) nuptial gifting and (B) feeding mating tactics. No significant differences were found in the male grasper size (Wilcoxon signed-rank test; *p* = 0.4155) or grasper base size (Wilcoxon signed-rank test; *p* = 0.3000) between these the two tactics.

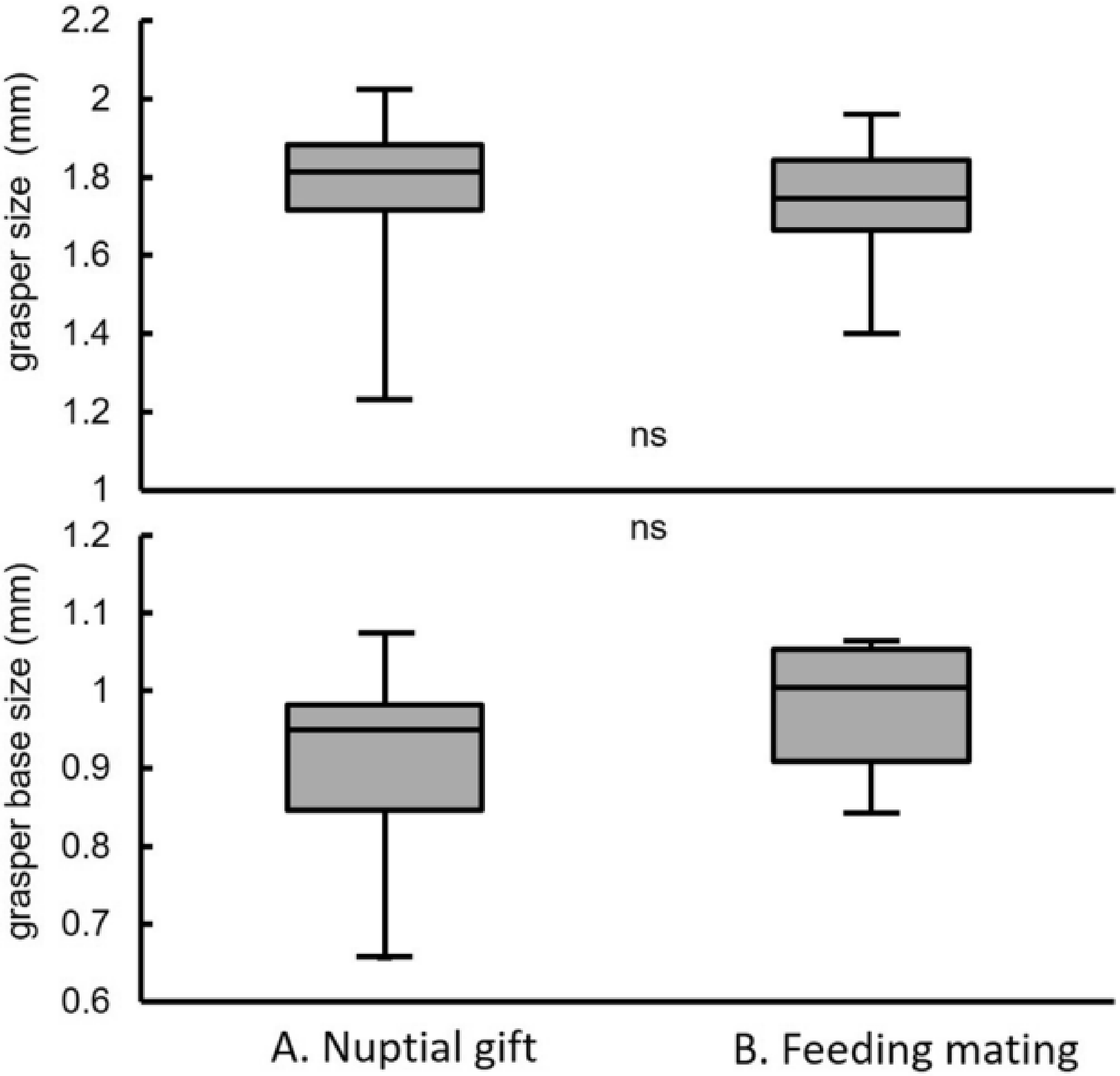
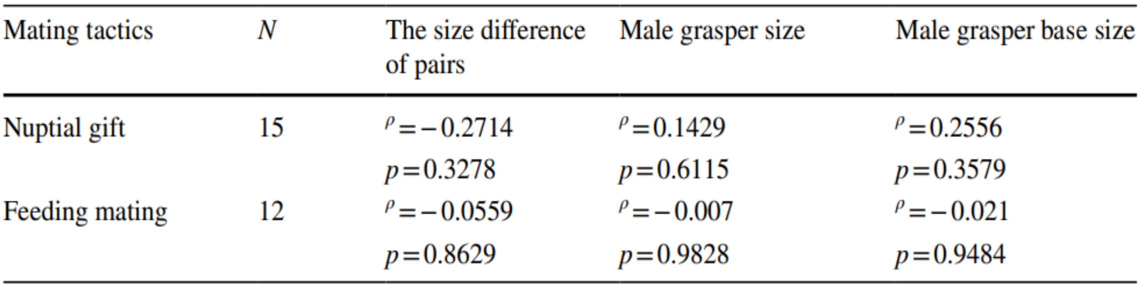


Fig. 2.6. Comparisons of male grasper size (top graph) and grasper base size (bottom graph) for the nuptial gift (A) and feeding mating (B) tactics.

2.4.4. Effect of size differences in pairs on mating duration and mating tactics

For each mating tactic, there was no significant correlation between the mating duration and the size difference of the pairs, male grasper size, or male grasper base size (Table 2.3).

Table 2.3. Relationships between mating duration and the size differences of pairs, male grasper size, and male grasper base size in each mating tactics.



Mating tactics	N	The size difference of pairs	Male grasper size	Male grasper base size
Nuptial gift	15	$\rho = -0.2714$ $p = 0.3278$	$\rho = 0.1429$ $p = 0.6115$	$\rho = 0.2556$ $p = 0.3579$
Feeding mating	12	$\rho = -0.0559$ $p = 0.8629$	$\rho = -0.007$ $p = 0.9828$	$\rho = -0.021$ $p = 0.9484$

2.5. Discussion

2.5.1. Male mating tactics

In many European scorpionflies including *P. vulgaris* which uses nutritious saliva as its nuptial gift, a positive relationship between the amount of bait used in a nuptial gift and mating duration was confirmed in *P. vulgaris* (Sindern 1996; Sauer et al. 1997; 1998; Sauer 2002).

In contrast, males of *P. japonica* use dead arthropods instead of nutritious saliva as nuptial gifts (Thornhill 1992b). The mating behavior observed in this experiment is similar to that of *P. liui*. In *P. liui*, both the male and female *P. liui* bear simple salivary glands, therefore, it is thought that this species does not use salivary masses for copulation, unlike *P. vulgaris* (Ma and Hua 2011). In *P. japonica*, as in *P. liui*, only small arthropods may be used for nuptial gifts because of the immaturity of the salivary glands of the males.

In the present study, similar to *P. vulgaris*, *P. japonica* females consumed bait during mating (Fig. 2.4). In *P. japonica*, a female may arrive and eat bait earlier than the male (I defined this as feeding mating). In this case, the males may adopt feeding mating tactics (B) rather than nuptial gift tactics (A). Moreover, because *P. japonica* males use dead arthropods as nuptial gifts. This difference in mating patterns may cause a different correlation between species of the same genus. It is necessary for future studies to further evaluate the mating durations of many species that use nutritious saliva and dead arthropods as nuptial gifts.

2.5.2. Relationship between male mating tactics and male traits

Thornhill (1992a; 1992b) found no relationships between male body size and the results of male-male competition or a female's preference for pheromones in *P. japonica*. He also reported that males that have symmetrical forewings easily win male-male competitions, and those females are attracted more to the pheromones released by the males who have symmetrical rather than asymmetrical forewings.

In this study, the males with more asymmetrical forewings were significantly more likely to adopt the feeding mating tactic than the nuptial gift tactic (top graph of Fig. 2.5). On the other hand, no difference was found in the sizes of the fly bodies or graspers, which are the male weapons (bottom graph of Fig. 2.5) between the two mating tactics. This finding suggests that male mating tactics are affected by the FA of the forewing, but that male body size and weapon size do not affect male mating tactics (Fig. 2.6). In the present study, I did not focus on nutritional conditions. In the future, it will be necessary to conduct experiments to manipulate nutritional status. Also, it is necessary to examine the effects of mating experience, male-male competition and nutritional status on mating tactics in the future.

2.5.3. Relationship between mating duration and male traits

In the present study, no significant relationship was found between mating duration and male body size or FA when nuptial gift tactics were used (Table 2.2). When feeding mating tactics were used, a significant positive relationship was found between FA and mating duration (Table 2.2), but no relationship was found between male body size and mating duration (Table 2.2). These results suggest that the FA of male forewings may affect mating duration when feeding mating tactics are used by *P. japonica*. In males with asymmetrical forewings, the mating durations were longer than those in males with symmetrical forewings.

High FA males have a lower lifetime mating frequency with different females than low FA males (Thornhill 1992b). Therefore, high FA males may increase their own mating success by increasing the mating duration. In this study, in high FA males, feeding mating had a longer mating duration than that of nuptial gift mating. High FA males may not be able to attract females with sex pheromones and can easily lose in male-male competitions (Thornhill 1992a; 1992b), making it difficult to mate with nuptial gift tactics. Therefore, high FA males may have longer mating durations when using feeding mating tactics than nuptial gift tactics. However, the relationship between the mating duration and the amount of sperm transferred by *P. japonica* was not investigated in the present study. To verify whether an increase in mating time leads to successful mating in this species, the sperm amount in spermatheca should be measured using virgin females in further experiments.

Alternatively, it may also be possible that the asymmetrical male's reproductive organs had “abnormal” growth, and thus, their mating durations were prolonged compared with those of the symmetrical males because of their malformed reproductive organs. It is necessary to increase the sample size in future studies because the present study used a small sample. In addition, it is important that the mating tactics of *P. japonica* be examined in the field in the near future.

Chapter 3

Wing-waving behaviors are used for conspecific display in the Japanese scorpionfly, *Panorpa japonica*.

3.1. Introduction

Many species use parts of the body, such as wings or legs, for visual display during courtship dances (e.g., Andersson 1994; Miller and Svensson 2014). For example, the bird of paradise (Paradisaeidae) is famous for colorful feather displays during courtship dances (Scholes 2008). In the peacock spider (*Maratus* sp.), courtship dances using the legs and abdominal flap (and they wave their midlegs during courtship) have significant impact on the mating success of males (Madeline et al. 2015).

Visual displays such as vibrating wings during courtship have been reported in many other insect species; *Drosophila melanogaster* (Cowling and Burnet 1981), *Rhagoletis pomonella* (Prokopy and Bush 1973), *Megaloprepus caerulatus* (Schultz and Fincke 2009), and *Campoplex capitator* (Benelli et al. 2019).

Nuptial gift giving is a mating behavior in which males provide gifts, such as food and nutritious secretions, to females before or during mating (Thornhill and Alcock 1983). Males of scorpionfly (Panorpidae) species attract females by a sex pheromone and provide nuptial gifts, such as a dead insect or nutritious saliva secretions, to females during courtship and mating (e.g., Sauer et al. 1997; Sato and Fujiyama 2018; Thornhill and Sauer 1992). A female allows the male to mate only when she is eating the gift (Sauer et al. 1997; Thornhill and Alcock 1983). Also, scorpionfly males fight each other for nuptial gifts.

Scorpionflies are known to perform a wing-waving (= flashing) behavior in which they move their wings up and down (Byers and Thornhill 1983). Magnier and Montgomery (2017) conducted a field experiment with the North American scorpionfly *Panorpa debilis* and discovered that the males and females use wing-waving behavior to protect a food resource against other insect species who are competing for it. Therefore, they suggested that the wing-waving behavior of *P. debilis* works as a visual display to other species. However, they did not observe whether the wing-waving behavior was used in courtship or at what time, probably because *P. debilis* only mates at night (Thornhill 1981).

Although another previous study using North American scorpionflies confirmed that males showed wing-waving behavior towards other males and females during the nuptial gift process (Thornhill 1981), there are no detailed studies on the relationships between wing-waving behavior and nuptial gifts in courtship, and between wing-waving behavior and male-male competition.

The Japanese scorpionfly, *Panorpa japonica*, has two black bands in the forewings and hind wings (Tsutsumi 2010), and this species also shows wing-waving behavior (Fig. 3.1, RI personal observation). In the *P. japonica*, males assume a pheromone-releasing posture near bait and give it to an approaching female as food for a nuptial gift before mating (Thornhill 1992a). Males of *P. japonica* often perform male-male competitions, in which males use the ends of their abdomen graspers, over food for nuptial gifts and for females (Thornhill 1992b). In *P. japonica*'s male-male competition involves butting a contestant with the head, grappling with the large genital claspers, and pinching a body part of a competitor with the sharp points of the genital claspers (Thornhill 1992a). The winner of the male-male competition remains in the food, and the loser waits near the winner after withdrawing from the contested food (Thornhill 1992a). *P. japonica* males use three mating strategies; nuptial gifts, forced mating, and feeding mating (Thornhill 1992a, see also Chapter 2). Males mainly use a dead insect as a nuptial gift (Thornhill 1992a). Males of *P. japonica* secures food for nuptial gifts for several hours (Thornhill 1992b, RI personal observation). At the end of mating with a nuptial gift, males check their food and release pheromone again to wait for females to visit (RI personal observation). Mating of *P. japonica* can be observed in the daytime (Kurokawa et al. 2012) unlike *P. debilis* (Magnier and Montgomery 2017).

Therefore, *P. japonica* is an ideal material to research the relationship between wing-waving behavior and courtship behaviors or male-male competitions.

A previous study reported that satellite males of *P. japonica* who had lost a male-male competition waited around the winners of the competitions and attempted to force mating with females attracted by the winner males (Thornhill 1992a). In laboratory experiments with *P. japonica*, it was reported that males with larger fluctuating asymmetry (FA) in the forewings adopted a feeding mating approach rather than a nuptial gift (see Chapter 2).

Thus, I hypothesized that the wing-waving behavior of *P. japonica* is used for inter- and intra-sexual displays during nuptial gift giving. In the present study, I thus examined the relationships between wing-waving behavior and courtship behaviors when giving nuptial gifts, and between wing-waving behavior and male-male competition, in the laboratory and field.

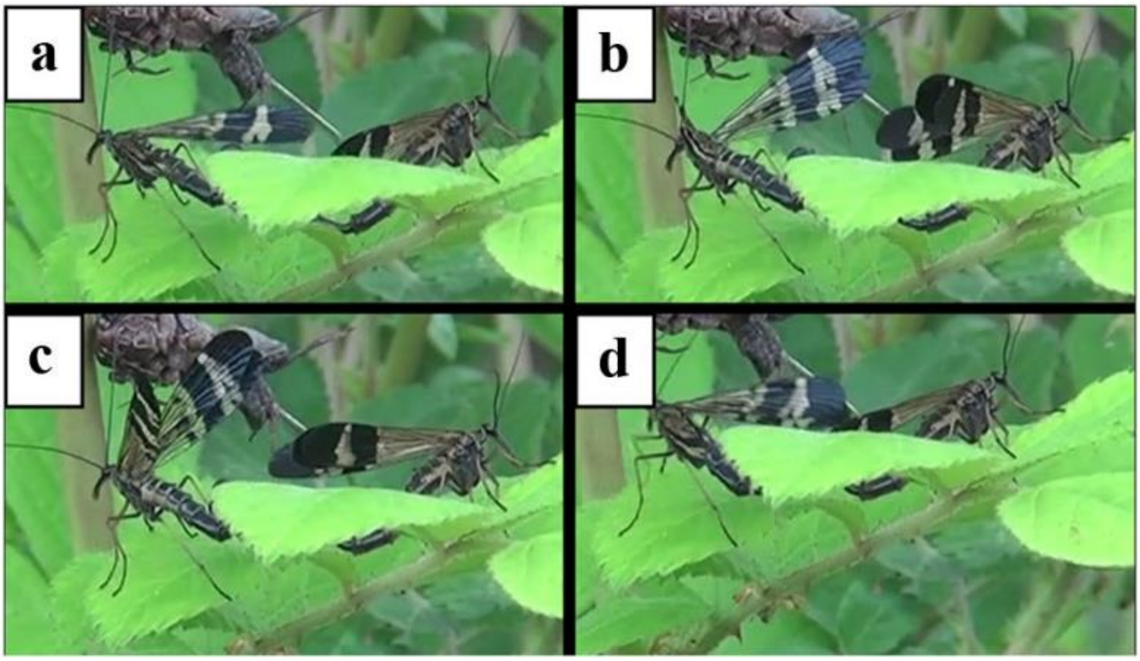


Fig. 3.1. Males of *P. japonica* in the field. (A) a male at rest on a leaf; (B), (C), (D) wing-waving behavior of a male.

3.2. Materials and methods

P. japonica, like other Panorpidae, feeds mainly on dead insects, and males that come to feed release pheromones after they eat a little (Thornhill 1992b). Females attracted to the male pheromone copulate when they begin to feed on the male's food (Thornhill 1992b).

Behavioral patterns observed in the present study were categorized into three categories: (1) "nuptial gift"; a female approaches a male keeping food for nuptial gift and develops into nuptial gift (Fig. 3.2), (2) "male-male competition"; a male approach a male carrying food for nuptial gift and develops into male-male competition (Fig. 3.3), (3) "sneaking"; a loser male attempts to re-enter the feeding area where the male-male competition took place, despite the presence of the winning male (Fig. 3.4).

Fig. 3.2. A male attempting nuptial gifting on a female visiting for food.

Fig. 3.3. Males that keep food for nuptial gift intercepting males that come for food (male–male competition).

Fig. 3.4. A defeated male in a male–male competition, trying to re-enter the bait, and a male intercepting it (sneaking). The loser male is running away from the winner male's attack.

3.2.1 Laboratory experiments

Adults (40 males and 40 females) of *P. japonica* were collected from Handayama Mountain, Okayama City, Japan (latitude: 34° 6‘ N, longitude: 133° 9’ E) from May 1 to 15, 2017. The experiment was conducted for one week after the collection. Each adult was reared in a plastic container (10 cm diameter × 8 cm height) in a chamber maintained at 25 ± 2 °C and 16 L : 8 D until the experiment. A piece of mealworm (*Tenebrio molitor*; weight: 0.2 g; length: 5 mm) was given once every two days.

Male and female (a pair) were placed in a plastic cylinder (10 cm diameter × 10 cm height) in which a piece of mealworm (0.2 g) had been placed on the bottom cotton whose surface is lightly moistened. After the pair was settled, mating behaviors were recorded with a video camera (HDR-PJ590V, Sony, Tokyo). The mating duration was defined as the period from the moment the male grabbed the female’s abdomen to the moment the male released the female. Each male and female pair was used for only a single observation. I observed 40 pairs in this experiment. In the present study, when mating was confirmed, I recorded the presence or absence of wing-waving behavior, and number and duration of wing-waving behavior acts.

3.2.2. Field experiments

The wing-waving behavior experiments were conducted from 9 am to 5 pm at the following two points: (1) from April 20 to May 19, 2019, and from April 18 to May 6, 2020, at a bank of the Asahikawa River, Okayama City (latitude: 34 ° 6 ‘ 8 "‘ N, longitude: 133 ° 9 ‘ 3 "‘ E), and (2) from May 8 to 26, 2020 at a road on Tsuneyama Mountain, Okayama City (latitude: 34 ° 5 ‘ 2 "‘ N, longitude: 133 ° 8 ‘ 8 "‘ E).

In both field experiments, the food (thawed commercially available cricket *Gryllus bimaculatus*; 2 g) was pinned to the leaves or stems of the shrubs inhabited by *P. japonica* with a metal paper clip according to the experimental method of Thornhill (1992). The food was placed from 9 am to 10 am, and a video recording was started by a video camera (HDR-PJ590V, Sony, Tokyo), when an adult was found in or near the food for 5 hours after the food was placed. In this study, 81 behaviors were observed using a pinned cricket.

The linear distances between the individuals who performed the wing-waving behavior and the other individual (who observed the wing-waving behavior) were measured. Each distance was measured by taping the ground every centimeter from the dead insects, and I recorded and determined the distances later in the video recording. The linear distance between individuals was measured by recording a video and then saving the video as an image using Image J (Ver.1.50i). In addition, when adults were found near dead insects in the field, the same recording and measuring procedures were used. A total of 18 behaviors were recorded in cases using naturally dead insects as a food resource.

When wing-waving behavior was observed, the following three parameters were recorded: (1) the number and duration of wing flashes, (2) the linear distance between the sender and receiver of wing flashes (measured from the sender's head to the receiver's head), and (3) individual behaviors during wing-waving behavior, including courtship strategies, the winners and losers of male-male competitions, and sneaking behaviors of loser males. Based on the recoded data, I calculated the frequency of wing-waving behaviors per minute, which was the number of wing flashes divided by the duration of the wing-waving behavior and then multiplied by 60. I compared the frequencies of wing-waving behaviors between two males at each sneaking behavior by defeated males after courtship, before male-male competition, and after male-male competition.

3.3. Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's HSD test were calculated using JMP version 12.2 (SAS Institute Inc. 2015). In addition, a generalized linear model (GLM) using logistic regression was used for statistical analysis of the winner or loser of male-male competition and the frequency of wing flashes. The significance level was set at $p = 0.05$ in each test.

3.4. Results

3.4.1. Description of wing-waving behavior

P. japonica mainly use wing-waving behavior for conspecific interactions. The wing-waving behaviors of *P. japonica* are similar to those of *P. debilis* (Magnier and Montgomery 2017). In detail, *P. japonica* lifts its forewings and hindwings from a stationary position so that they rotate the right wings would be going clockwise and the left counterclockwise when viewed *P. japonica* from the front. After raising the wings to an approximately 45 ° angle with the thorax as the axis, the wings are lowered vertically at about the same speed as they were raised, and the wings are returned to their resting positions. During this sequence of actions, the forewings and the hind wings are slightly separated, and the hind wings follow the forewings (Fig. 3.1). In this study, I defined this sequence of actions as a single wing-waving behavior act.

3.4.2. Laboratory experiments

Fig. 3.5 shows the frequencies of wing-waving behaviors during courtship. Of 40 pairs observed, 31 courtships by males were observed, of which, 27 cases were confirmed to mate. Wing-waving behavior was confirmed in all 31 males who were observed courting, while only four females performed wing-waving behavior (Tukey's HSD test; $p < 0.001$).

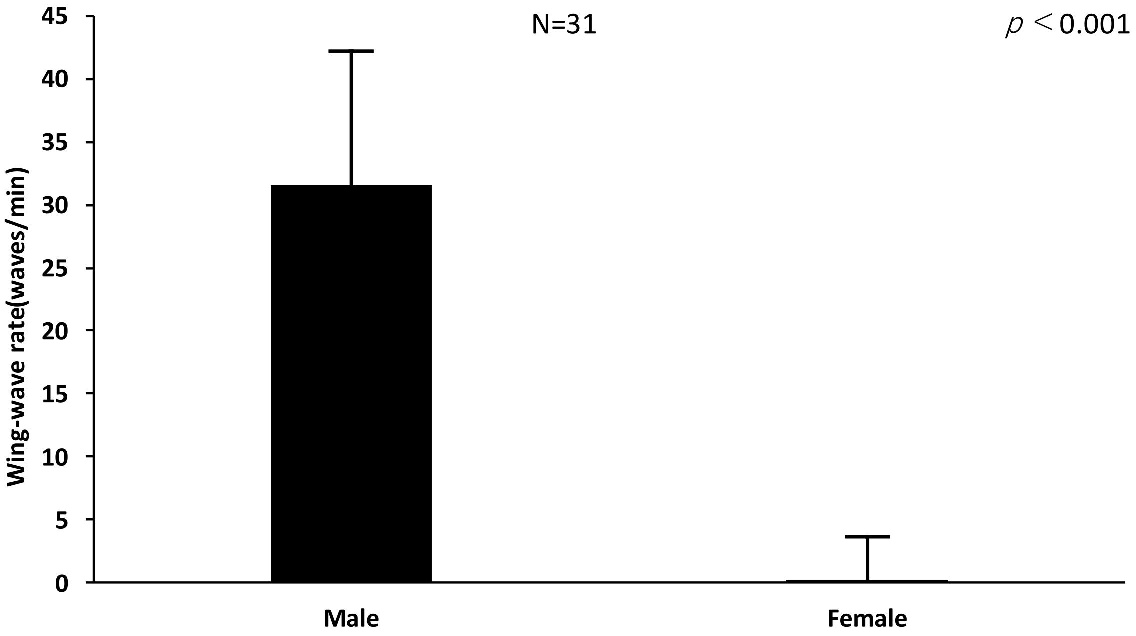


Fig. 3.5. Rates of wing-waving behavior during courtship of females by males in the laboratory (p < 0.001).

3.4.3. Field experiments

In the field experiments, 99 behaviors were observed. When I used a pinned cricket, 81 behaviors (nuptial gift; N = 26; male-male competition; N = 26; sneaking; N = 29) were observed. When a naturally dead insect was used as a food resource, a total of 18 behaviors (nuptial gift; N = 8; male-male competition; N = 5; sneaking; N = 5) were recorded.

Comparison of the frequency of wing-waving behavior between naturally dead insults and pinned crickets revealed no significant differences (Tukey's HSD test; p = 0.0914). Therefore, in this study, both results were combined.

Wing-waving behavior was observed in the following three cases: (1) nuptial gifting (as courtship behavior), (2) male-male competition, and (3) sneaking behavior (loser male's behavior after the male-male competition). In the present study, males holding food for nuptial gifting sometimes showed wing-waving behavior with attacks on houseflies (Muscidae) and ants (Formicidae) that came to the food (N = 3). In the present study, forced copulation without food during copulation (N = 1), and females finding the food before the male takes up the food (N = 2) were observed, but they were not included in the present analysis due to the small number of observations.

Fig. 3.6 shows the average inter-individual distance during nuptial gifting (courtship behaviors), male-male competition, and sneaking behavior. The wing-waving behavior was always observed when the distance between the two individuals was less than 15 cm (N = 99). Therefore, in the present study, the interactions were defined as a behavior performed with 15 cm or less between the two individuals. Comparison of average distances between the two individuals during each behavior showed no significant difference among the three cases (ANOVA; $F_{2,94} = 0.3478$; $p = 0.707$).

Since the average frequencies of wing-waving behavior did not differ at the two locations (riverbank of Asahikawa; 0.53 ± 0.02 (average ± standard error) (waves / min); Tsuneyama mountain road; 0.56 ± 0.02 (waves / min); Tukey's HSD test; $p = 0.217$), the data of both populations were calculated as one analysis.

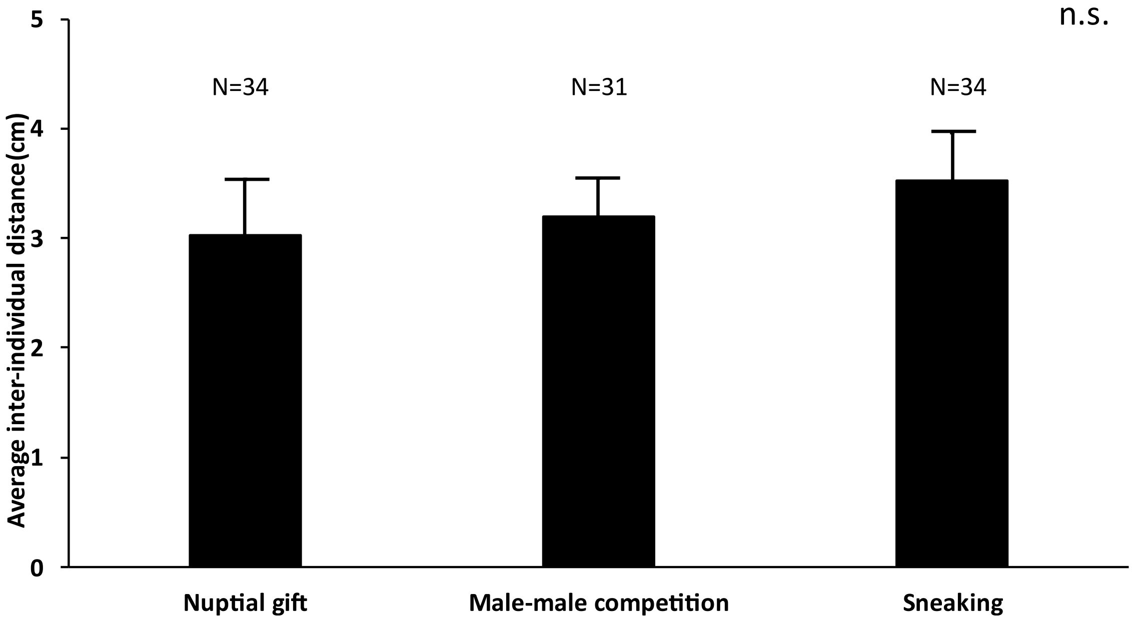


Fig. 3.6. Average distance between individuals during each behavior; left graph: male and female at nuptial gift (N =34), middle graph: male-male competition (N = 31), and right graph: sneaking and winner males (N = 34).

Fig. 3.7 shows the frequencies of wing-waving behavior for each behavior. In four of the 40 pairs, mating behavior was not observed. In the case of nuptial gifting, males performed wing-waving behavior toward females in all cases (N = 34), but only 3 out of 34 females showed wing-waving behavior toward males. Thus, the frequency of wing-waving behavior in females was significantly lower than that in males (Tukey's HSD test; $p < 0.001$; Fig. 3.7A). Comparing the results of nuptial gifting in the field and laboratory experiments, there was no significant difference in the frequency of wing-waving behavior (Tukey's HSD test; laboratory male vs. field male; $p = 0.2954$; laboratory female vs. field female; $p = 0.9954$). Before a male-male competition, both males defended their own food, and males without food attacked other males with food. In these cases, males always showed wing-waving behavior

(Fig. 3.7B). There was no significant difference in the frequencies of wing-waving behaviors between the defender and the attacker (Tukey's HSD test; $p = 0.1289$; Fig. 3.7B). Fig. 3.7C shows a comparison of wing-waving behaviors between winner and loser (sneaking) males after male-male competition. Winning males had significantly higher frequencies of wing-waving behavior than losing males (Tukey's HSD test; $p < 0.0001$; Fig. 3.7C).

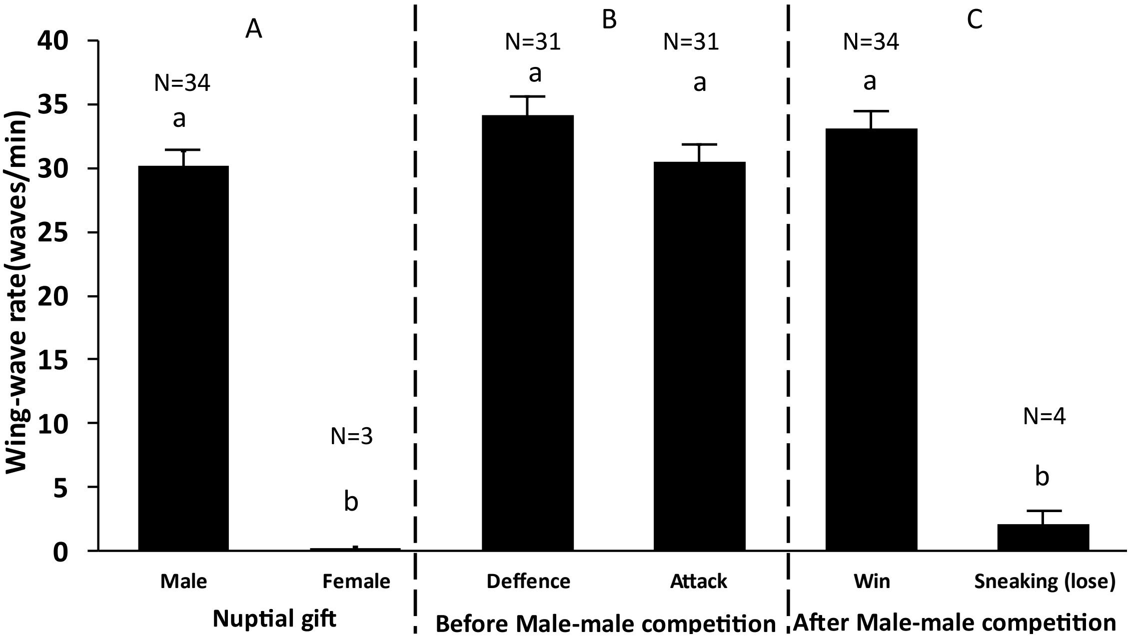


Fig. 3.7. Frequencies of wing-waving behavior during each behavior; (A) comparison between males and females during nuptial gifting, (B) comparison between attackers and defenders during male-male competition, and (C) comparison between winner and loser (sneaking) males after male-male competition. Significant differences were found in the intervals with different letters (Tukey's HSD test; $p = 0.05$).

Fig. 3.8 shows the frequencies of wing-waving behavior before each male-male competition by the winner and loser of the competition. The frequency of wing-waving behaviors by winner males was significantly higher than that by loser males (GLM; $\chi^2 = 7.039$; *d.f.* = 1; $p = 0.008$).

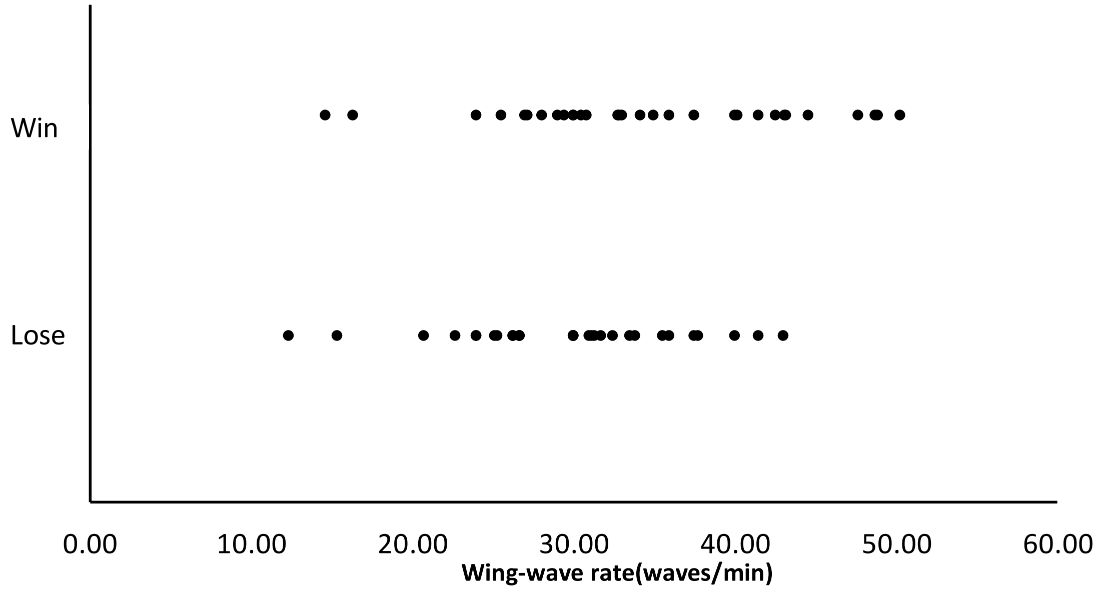


Fig. 3.8. Relationship between winner or loser males of male-male competitions and frequency of wing-waving behavior before the male-male competition.

3.5. Discussion

Many species in the genus *Panorpa* show a mating system called resource-defense polygyny (Emlen and Oring 1979). That is, males compete with other males to defend resources, and also to monopolize opportunities to mate with scorpionfly females (Byers and Thornhill 1983; Magnier and Montgomery 2017). *P. japonica* has also been confirmed to be resource-defense polygyny (Thornhill 1981; 1992).

Magnier and Montgomery (2017) suggested that the wing-waving behavior of *P. debilis*, a closely related species of *P. japonica*, relates to resource-defense polygyny, and they predicted that the frequencies of wing-waving behavior are higher in males than females in *P. debilis*. In the present study, males holding food for nuptial gifting exhibited wing-waving behavior accompanied by attacks on houseflies (Muscidae) and ants (Formicidae) that came to the food (N = 3). Thus, similar to *P. debilis* (Magnier and Montgomery 2017), I showed the possibility of using wing-waving behavior in interspecific competition over the same food resource.

In the present study, courting males always showed wing-waving behavior (Fig. 3.7B), whereas females showed almost no wing-waving behavior toward males during nuptial gifting in *P. japonica* (Fig. 3.5; Fig. 3.7A). In the blowfly *Chrysomya flavifrons*, which is known for its complex courtship behavior, differences in courtship behavior between lab and field populations have been reported (Butterworth et al. 2019).

However, the present results of the frequency of wing-waving behavior in *P. japonica* were not differed between the field and laboratory experiments. Therefore, the wing-waving behavior of *P. japonica* was not affected by the external environment. In addition, a few cases of female-to-female wing-waving behavior were observed (N = 2). These show sexual dimorphisms in the frequencies of wing-waving behavior in *P. japonica*. It is known that *P. japonica* uses three mating tactics; forced mating, nuptial gifts, and feeding mating (Byers and Thornhill 1983; Thornhill 1992a; Sato and Fujiyama 2018, see also Chapter 2). The present results show males performed wing-waving behavior toward females, but it is unclear whether females have wing-waving behavior in the three mating tactics in *P. japonica*.

Magnier and Montgomery (2017) showed that both males and females in *P. debilis* performed wing-waving behavior when the individuals of the same species approached them, and thus they considered that wing-waving behavior may be a form of communication with the same species or competition for food resources against other species. On the other hand, in *P. japonica*, the frequency of wing-waving behavior was higher in males than in females, indicating that the wing-waving behavior likely functions as a part of sexual selection, at least in the case of *P. japonica*.

In the male-male competitions of *P. japonica*, winner males performed significantly more wing-waving behavior than loser males (Fig. 3.8). In addition, winner males showed more wing-waving behavior toward sneaking (or loser) males in all cases (N = 34), and wing-waving behavior was observed by only three sneaking males. Therefore, I consider that the male wing-waving behavior of *P. japonica* is a display in male-male competition over food resources. In the future, it would be beneficial to clarify whether females of *P. japonica* show wing-waving behavior while eating food against conspecific females visiting the food.

It has been suggested that wing-waving behavior is performed by North American scorpionflies before male-male competition (Thornhill 1981). However, no studies have observed the wing-waving behavior during an incident of male-male competition. Thus, the present result is the first report to suggest that the winner of a male-male competition used wing-waving behavior as a display, probably, against the loser during male-male competition.

In resource-defense polygyny, the ability to acquire resources and defend resources from other males depends on the qualities of the male, such as strength, in male-male competition (Thornhill 1981). Studies of European Panorpidae found a significant correlation between male health and strength in male-male competition (Sauer et al. 1998), and this suggests strength in male-male competitions could be related to the larval growth environment (Thornhill and Sauer 1992). In *P. japonica*, it has been suggested that fluctuating asymmetry of forewing (FA) may increase with exposure to environmental stresses such as poor nutrition and parasites during the larval stage (Thornhill 1992). If wing-waving behavior before male-male competition acts as a signal to indicate the male's condition, males with infrequent wing-waving behavior may give up on male-male competition and escape. In the future, it would be good to show the relationships between the significance of signals in male-male competition and aspects of the larval growth environment such as nutritional status.

There was a sexual difference in the frequency of wing-waving behavior between *P. debilis* and *P. japonica*. It is known that there are interspecific differences in the area of the black portion of the wings of *Panorpa* species (e.g., Hartbauer et al. 2015), and the use of wing-waving behavior may aid in species recognition. Also, as in the case of the blowflies (e.g., Eichorn et al. 2017; Butterworth et al. 2020), there may be a sex recognition system in *P. japonica* based on the light reflected from the wings. In the future, it is necessary to verify whether there are differences between males and females in wing patterns and reflected light generated during wing-waving behavior in *P. japonica*. The mating tactics and patterns even in the same family, the Panorpidae, may vary from species to species (e.g., Byers and Thornhill 1983).

In the present study, however, I not observed satellite behaviors of males in which the loser males attempted to force mating with females. Loser males frequently tried to re-enter the feeding arena (I defined this as sneaking), the place for male-male competition. Then, the winner male attacked the sneaking male. However, the sneaking males always escaped without counter-attacking the winner male. In addition, sneaking males in the present study invaded the feeding arena and released pheromones while winner males were mating with the female. Similar pheromone-releasing behavior has been observed by Thornhill (1992a). In a study on *P. japonica* by Thornhill (1992b), loser males were satellites around the food, and they attempted forced mating with females. On the other hand, in the present study, loser males did not attempt forced mating. Why the results differ for the same species will be an interesting issue to study. Thornhill (1992b) observed mating behavior using a population of *P. japonica* in Aichi, Japan, but I used populations in Okayama in western Japan. Behavior may vary among local populations due to factors such as differences in climate and predators. In the future, it will be required to compare male mating tactics and wing-waving behavior using multiple species of Panorpidae and different regional populations.

Chapter 4

Differences in mating tactics selected by males among local populations of the Japanese scorpionfly *Panorpa japonica*.

4.1. Introduction

Sexual selection leads to the evolution of different male traits that are advantageous for male-male competition and for female mate choice in different regions (Andersson 1994). In several animal taxa, large individual variations have been confirmed in the expression of male sexual traits (e.g., Iwata et al. 2005; Kelly 2006; Ota et al. 2014).

Why such individual differences in male sexual traits occur and are maintained has received much attention in the research field of mate choice (Blum and Blum 1979; Kotiaho et al. 2008). Recent studies have shown that individual differences in sexual traits are influenced by external factors such as population density, nutritional status, and predation pressure in each individual (Hedrick and Dill 1993; Balaban-Feld and Valone 2017; Kelly 2018).

Kelly (2018) stated that a future task will be to examine how different factors, such as resource availability, predation pressure, and density among populations affect the strength and direction of sexual selection, and thus speciation.

A notable insect mating behavior is mating tactics, such as satellite or sneaking tactics. Mating tactics has been reported to be affected by other sexual traits such as body and weapon sizes (e.g., Iwata et al. 2005; Kelly 2006; Ota et al. 2014). Thus, by comparing the rate of adoption of mating tactics among different populations, it may be possible to predict the strength and direction of sexual selection. Recently, a study showed that male mating tactics vary with environmental factors (Okuzaki 2021). In addition, some studies have been reported comparing the rate of adoption of mating tactics among closely related species (e.g., Missoweit and Sauer 2007; Ota et al. 2014), but few have examined in detail geographic variation in mating tactics within the same species.

Scorpionflies (Panorpidae) are often used to study sexual selection and mating tactics (e.g., Byers and Thornhill 1983; Thornhill 1992; Missoweit and Sauer 2007; Sato and Fujiyama 2018, see also Chapter 2). Many scorpionfly species are known to use three mating tactics: (1) a nuptial gift using nutritious saliva, (2) a nuptial gift using food such as a dead insect, and (3) forced mating without a nuptial gift.

In the species using mating tactics, it has been reported that males are more likely to succeed in mating in the order of (1) > (2) > (3) in three species in North America (*Panorpa debilis*, *P. latipennis*, and *P. mirabilis*) and two species in Central America (*P. penicillata* and *Panorpa* sp.) (Thornhill 1979; Byers and Thornhill 1983). On the other hand, it has been reported that males in European species (e.g., *P. vulgaris* and *P. cognata*) do not use forced mating, but more likely succeed in mating in the order of (2) > (1) (Engqvist and Sauer 2003; Missoweit and Sauer 2007). Thus, it has been suggested that mating tactics vary among closely related species in Panorpidae. However, it is unclear whether mating tactics vary within the same species.

The Japanese scorpionfly *Panorpa japonica*, which is widely distributed in the mountains and plains of Honshu, Shikoku, and Kyushu in Japan, is known to use nuptial gifts (using food such as dead insects), forced mating, and feeding mating (Thornhill 1992a; Sato and Fujiyama 2018, see also Chapter 2). Males of *P. japonica* often perform male-male competitions (Thornhill 1992a, see also Chapter 3). Males that are defeated in male–male competition are known to adopt satellite tactics (Thornhill 1992a). Thornhill (1992a), who observed satellite tactics of *P. japonica* in the central Japan (Aichi Prefecture), reported that males that took satellite tactics (loser males) attempted to force mating by targeting females attracted by males possessing food (winner males). However, in the observations of Chapter 3 that were conducted in western Japan (Okayama Prefecture), the loser males did not adopt the satellite tactic but the sneaking tactic, even though the experimental conditions were similar to those of Thornhill (1992a) (see Chapter 3).

As to why these differences occurred, Thornhill (1992a) used a population from Aichi Prefecture, while Chapter 3 used a population from Okayama Prefecture, and thus, I hypothesized that there may be differences in the rate of adoption of mating tactics of male *P. japonica* depending on the location. In this study, I tested this hypothesis by comparing the mating tactics of males of *P. japonica* from both Okayama and Aichi populations in the same year.

4.2. Materials and Methods

4.2.1. Observation sites and insects

Field observations were conducted at the following two locations from 9 am to 3 pm based on Thornhill (1992) and Chapter 3. The locations are (1) from April 30 to May 7, 2021, at a road on Tsuneyama Mountain, Okayama City (latitude: 34 ° 6 ‘ 8 "‘ N, longitude: 133 ° 9 ‘ 3"‘ E) (hereafter; Okayama population) and (2) from May 23 to 29, 2021 at a forest on the premises of Aichi University of the Arts (latitude: 35 ° 10 ‘ 9 ″‘ N, longitude: 137 ° 4 ‘ 17 ″‘ E) (hereafter; Aichi population).

The experimental method was based on Chapter 3. Thawed last instar larvae of the field cricket (*Gryllus bimaculatus*; 2 g) were pinned to the stems or leaves of shrubs and grasses and observed by three video cameras (HDR-PJ590V, Sony, Tokyo) recording and visual observation for 6 hours from 9 a.m. to 3 p.m. In this study, I observed and recorded male-male competition behavior 25 times in Aichi and 30 times in Okayama.

4.2.2. Observation

The male-male competition in *P. japonica* begins with a characteristic wing-waving behavior, in which the males clash their wings and abdomens, and finally the males grab each other's abdomens with the grasping apparatus at the end of their abdomens (Thornhill 1992a, see also Chapter 3).

The winners and losers of male-male competitions are easy to identify; the loser males rarely engage in wing-waving behavior against the winner males (see Chapter 3). In this study, I defined a loser male as a male that waits at a distance from the feeding area where the winner male is located and does not show wing-waving behavior toward the winner male. I also recorded the behavior of each individual after the loser male lost the male-male competition.

The behaviors of loser males were defined as follows: (1) "Waiting"; waiting within 15 cm in the feeding area: the waiting behavior of loser males usually occurs within 15 cm of the winner male and the feeding area, (2) "Sneaking"; an attempt to re-enter a feeding area where a male-male competition has been held, despite the presence of a winner male, (3) "Forced mating"; a waiting loser male targets a female attracted by a winner male and attempts to mate with her, and (4) "Run-away"; a male moves 15 cm outside the feeding area and is judged to have left the feeding area. The length of time from losing the male-male competition and waiting to leaving 15 cm outside the feeding area was recorded as the waiting duration.

In addition, I measured the number of waiting males and the number of visiting females observed in a single feeding area.

4.3. Statistical analysis

All statistics were performed using JMP version 12.2 (SAS Institute Inc. 2015). The Wilcoxon signed-rank test was applied to compare the duration of male-male competition between the two populations, the waiting duration of loser males between the two populations, and the average number of waiting males and average visiting females between the two populations.

Statistical analysis using Fisher's exact test was also applied to compare the behavior of loser males after male-male competition between the two populations. The level of significance was set to $p < 0.05$.

4.4. Results

Flowcharts of the behaviors performed by loser males after a male-male competition between males at the two sites in the fields are shown in Fig. 4.1. All the first behaviors of the loser males observed in this study after defeat were waiting behaviors. Therefore, the next behavior after the waiting behavior was analyzed statistically. In the Aichi population, 25 cases of waiting males were observed. More than half of these loser males (N = 17) ran away from the feeding area and some individuals (N = 7) performed sneaking. Most of the individuals that employed sneaking in the Aichi population left the feeding area after one or two attempts of sneaking. In addition, one individual was observed to have attempted forced mating with visiting females (N = 1).

In the Okayama population, 30 cases of males with waiting behavior were observed. Most of these loser males performed sneaking behavior to the feeding area (N = 28), and a few individuals ran away from the feeding area (N = 2). In the Okayama population most of the loser males that successfully sneaked also successfully mated by giving a nuptial gift (N = 24). As a result of comparing the proportion of individuals who chose "running-away" between the two regions, there were significantly more individuals who chose "running-away" in the Aichi population than in the Okayama population (Fisher's exact test; $p < 0.001$), and there were significantly more individuals who chose "sneaking" in the Okayama population than in the Aichi population (Fisher's exact test; $p < 0.001$).

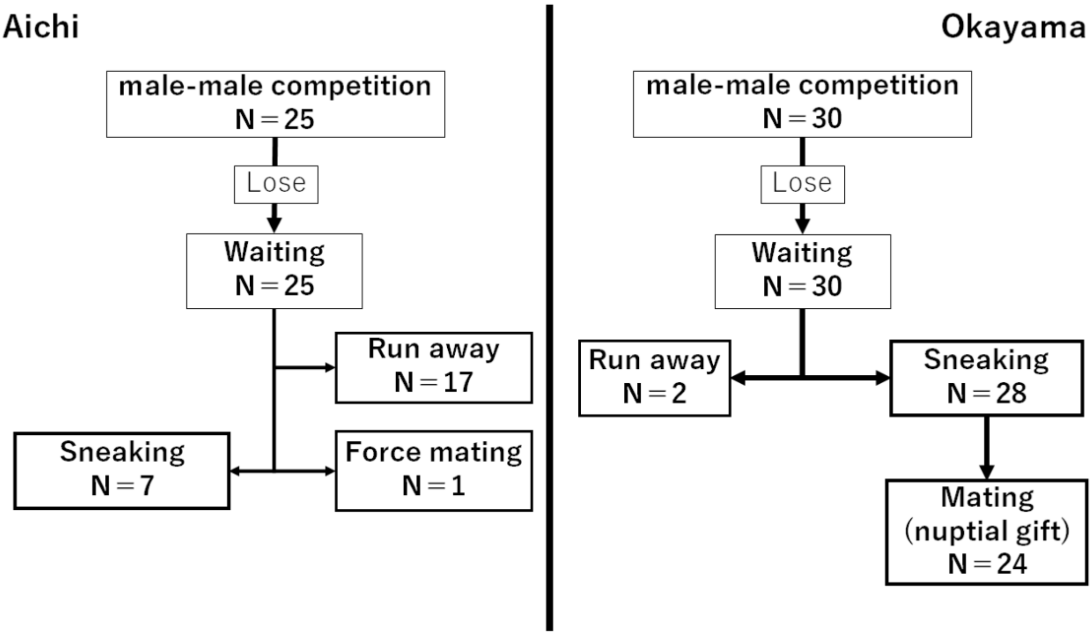


Fig. 4.1. Flowcharts of the behaviors selected by males defeated in male-male competition in the Aichi and Okayama populations.

There was no significant difference in the duration of male-male competition between the two populations (Fig. 4.2; Wilcoxon signed-rank test; p = 0.0975). On the other hand, the duration of waiting by loser males was significantly longer in the Okayama populations than in the Aichi populations (Fig. 4.3; Okayama population; 3508 ± 2479 (average ± standard error) (s); Aichi population; 1109 ± 1817 (s); Wilcoxon signed-rank test; $p < 0.001$). Many individuals in the Okayama population remained in the feeding area for more than one hour, whereas many individuals in the Aichi population flew away from the feeding area within a few minutes to several tens of minutes.

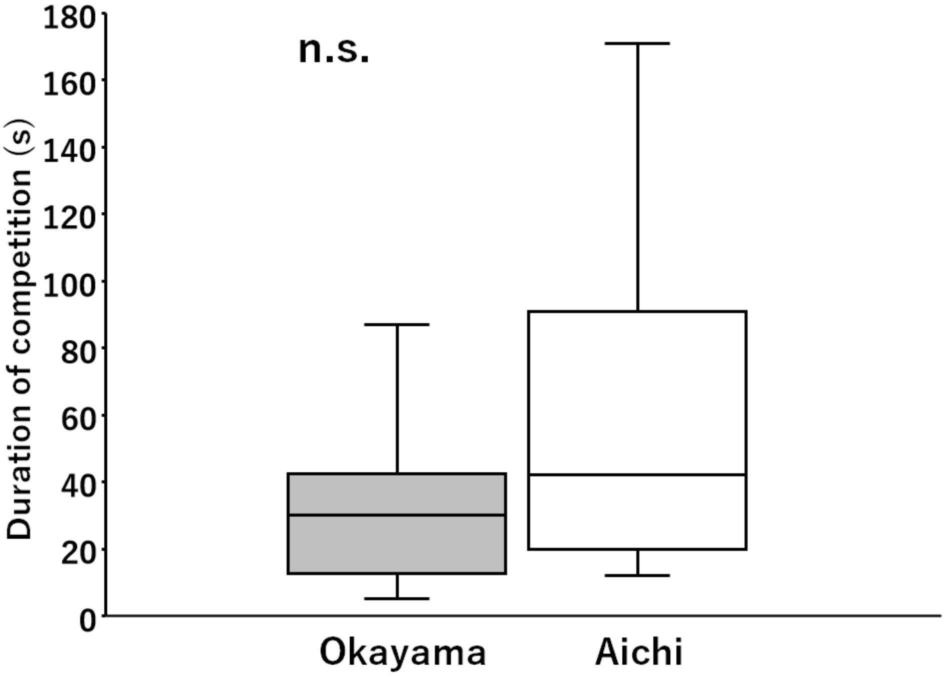


Fig. 4.2. Comparison of the duration of male-male competition between the two populations.

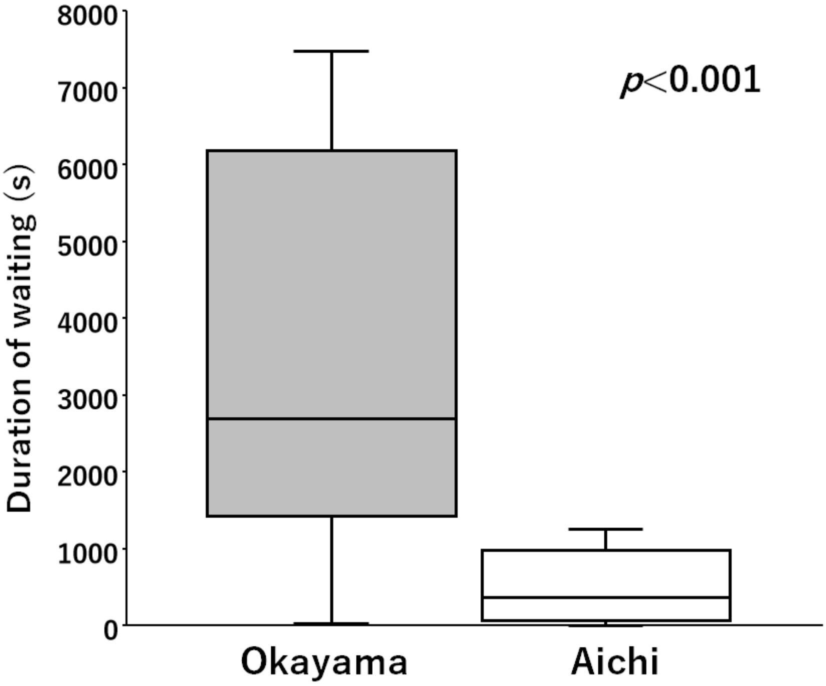


Fig. 4.3. Comparison of waiting duration of loser males between two populations.

The numbers of waiting males and of visiting females within the feeding area observed in the two local populations are shown in Fig. 4.4. The number of males and females in the feeding area differed significantly between the two populations (male population; Okayama population, median = 2.5; maximum = 6; minimum = 1; Aichi population; median = 1; maximum = 3; minimum = 1; Wilcoxon signed-rank test; $p < 0.001$; number of females; Okayama population; median = 3; maximum = 6; minimum = 1; Aichi population; median = 1; maximum = 1; minimum = 0; Wilcoxon signed-rank test; $p < 0.001$).

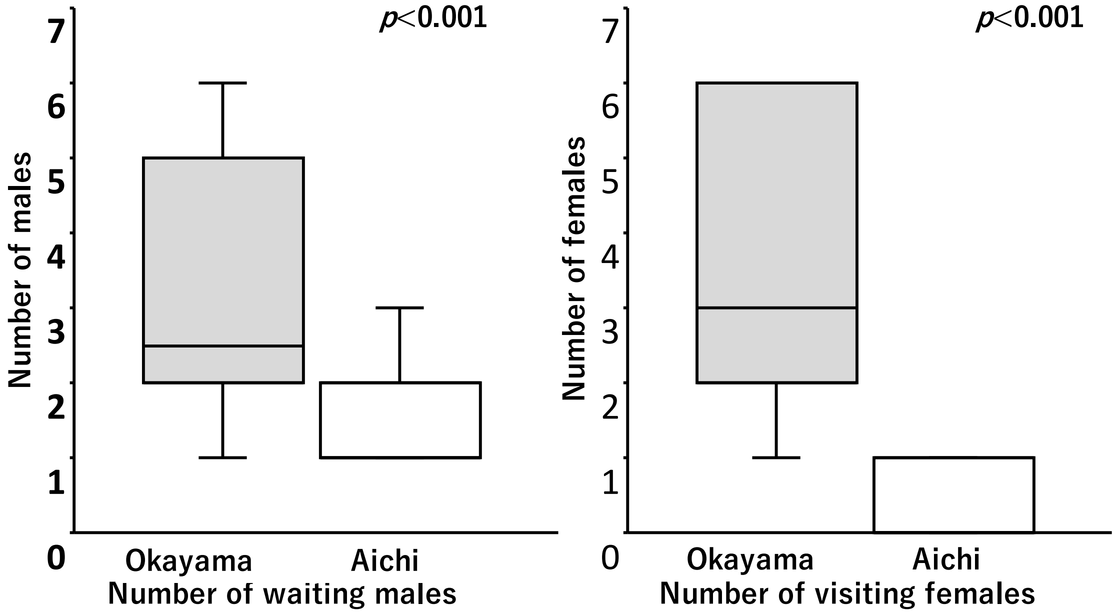


Fig. 4.4. Comparison of the maximum number of waiting males (left figure) and the number of visiting females (right figure) between the two populations.

In the Okayama population, a maximum of six individuals of both males and females were observed in a single feeding area, whereas in the Aichi population, only a maximum of three males and one female were observed. Therefore, most of the loser males in the Okayama population that successfully sneaked also successfully mated by nuptial gift (N = 24) (Fig. 4.1).

4.5. Discussion

Mating tactics in Panorpidae have been extensively studied in North American and European species, and the results suggest that there are variations in mating tactics among closely related species (Byers and Thornhill 1983; Missoweit and Sauer 2007). However, all previous studies were experiments using one regional population, and no studies have shown differences in mating tactics among regional populations within the same species. In addition, for the Panorpidae species in Japan, not only mating tactics but also behavioral and ecological knowledge is scarce (Thornhill 1992a, see also Chapter 2 and Chapter 3).

In the present study, loser male's behaviors were clearly different between Okayama and Aichi populations in *P. japonica* (Fig. 4.1; 4.3). The duration of waiting in the Aichi population was shorter than that of the Okayama population, and forced copulation was observed only in the Aichi population. In addition, the frequency of re-entering the feeding area of the Aichi population was lower than that of the Okayama population.

A direct factor influencing male mating tactics is thought to be the difference in the frequency of encounters with females in the feeding area per male. In the Aichi population, there were many cases in which no females appeared in the feeding area until the end of mating sequence. Also, the number of females visiting one feeding area was considerably larger in the Okayama than in the Aichi population (Fig. 4.4). In addition, in the Okayama population, many loser males, who adopted sneaking behavior, succeeded in mating with nuptial gifts. For these reasons, since many females gather in the feeding area, the loser males of the Okayama population may have the advantage to sneak and thus mate with new females visiting the feeding area by giving a nuptial gift. On the other hand, since new females are unlikely to visit the Aichi population, it may be advantageous for loser males to look for other feeding area or to try forced mating with the females even if sufficient mating time is not available.

However, this study was not able to clarify why differences exist in the number of females visiting to food area between the two populations. In a field cricket, *Gryllus integer*, which employs sneaking as an alternative mating tactic, the main factors for mating success with sneaking tactics are population density and the operational sex ratio, and it is clear that a high population density and female-biased sex ratio favor males adopting sneaking tactics (Rowell and Cade 1993). In *P. japonica*, population density and sex ratio may also affect mating tactics. Alternatively, differences in environmental conditions such as available food resources may influence female and male mating behavior, as in the case of the ground beetle *Carabus japonicus* (Okuzaki 2021).

It is also possible that geographical factors such as climate or amount of space in which to grow have had an impact on the difference in mating tactics. It has been suggested that adults of Panorpidae have low dispersal ability due to their weak flight ability (Byers and Thornhill 1983). The two areas surveyed this time are more than 300 km apart in a straight line, and therefore geographical reproductive isolation might be a factor in this variation in mating tactics.

Previous studies dealing with mating tactics have often compared genus-level interspecific behaviors (e.g., Missoweit and Sauer 2007; Ota et al. 2014), and in recent years there have been numerous studies comparing regional populations and male traits (e.g., del Sol et al. 2021; Okuzaki 2021). However, there were only a few studies comparing regional populations of mating tactics. For example, a report of the Japanese rhinoceros beetle *Trypoxylus dichotomus* by del Sol et al. (2021), it was considered that the mating tactics might have changed due to the number of males gathering in the feeding area, but it was not verified.

This study is the first example of Panorpidae insects showing variation in the rate of adoption of mating tactics among regional populations within the same species. By comparing the mating tactics of this species, it may be possible to evaluate how the mating tactics have evolved according to the habitat.

Thornhill (1992b) investigated the behavior of loser males in *P. ochraceopennis* and *P. nipponensis*, which are closely related to *P. japonica*, in Nagano Prefecture, and showed no difference in satellite tactics of the loser males. He concluded that there is no difference between the behavior of loser males of *P. japonica* in Aichi (Thornhill 1992a) and the behavior of loser males of two species in Nagano. In the future, it may be possible to elucidate the evolution of mating tactics of Japanese Panorpidae by investigating other species of Panorpidae in Japan.

Chapter 5

General Discussion

The mating tactics of Panorpidae and other nuptial gifting species have been studied extensively around the world for the past 50 years (e.g., Byers and Thornhill 1983; Thornhill 1981; Engqvist and Sauer 2003; Missoweit et al. 2007). However, few studies have focused on regional differences in the adoption rates of mating tactics in species with nuptial gifts. This is the first study to show that the rate of adoption of mating tactics varies between two different regions in species with nuptial gifts.

This study also clarified some of the mating behaviors of Japanese scorpionfly species, which have not been studied in many cases. The study also suggested that wing-related traits such as forewing symmetry and wing-waving behavior frequency may influence *P. japonica* choice of mating tactics.

In North American species, only males that win the male-male competition for food can perform nuptial gift using food, and loser males choose nuptial gift using salivary mass, and among loser males, males that cannot produce salivary mass due to lack of nutrition choose forced mating (Thornhill 1980). In addition, it was reported that females of North American species preferred males that selected food, especially males that used larger food as nuptial gifts, to males that selected salivary mass as nuptial gifts (Thornhill 1981). Based on observations of North American scorpionflies, Thornhill (1981) concluded that the mating system of insects in Panorpidae is resource-defense-polygyny (RDP).

However, a number of studies suggesting, that European species do not have an RDP mating system, have been reported. For example, in *P. vulgaris*, males cannot monopolize food resources, and male mating duration is longer in nuptial gift of salivary mass than in nuptial gift of food (Sauer et al. 1998; Missoweit and Sauer 2007).

From studies of European species conducted in the 1990s and 2000s, (Missoweit and Sauer 2007) concluded that the mating system of insects in Panorpidae is not species-universal RDP.

In this study, in *P. japonica*, only the winner male was able to monopolize the food, unless the winner male was unable to intercept the loser male, such as during mating (see Chapter 3), that the FA value of male forewings affected the mating tactics chosen by males (Fig. 2.5), and that the frequency of wing-waving behavior before male-male competition influenced the win or loss of the male-male competition (Fig. 3.8). Therefore, the results of this study suggest that the mating system of *P. japonica* may be RDP as in the North American example.

There are two hypotheses as to why there are differences in the rate of adoption of mating tactics among these regions. The first is that differences in available food resources may be influencing the results.

In the nine North American scorpionfly species, most (89-97 %) of the food available in the field are dead insects and the like in the wild, and only small males that lose the competition for food resources take the risk of stealing the prey of web-building spider (Thornhill 1975; 1978). While field observation experiments of European species *P. vulgaris* and *P. communis*, the majority relied on pollen, nectar, and spider-netted prey, especially individuals that used spider-netted prey, regardless of sex or male body size (Bockwinkel and Sauer 1993). This suggests that the RDP-like nuptial gifts using food system may have evolved in North America, where food resources are abundant, while the non-RDP-like nuptial gifts using salivary masses system may have evolved in Europe, where food resources are scarce, and the majority of food resources are dependent on the prey of web-building spiders.

This hypothesis needs to be tested in the future by investigating the types of food resources used under natural conditions and the frequency of food use in other regions, including Japanese species, as well as the degree of dependence of web-building spiders.

The second is the difference in the intensity of competition among related species. In North American species, the emergence of the larger and more aggressive *P. mirabilis* changes the mating tactics of the smaller *P. latipennis* males from food presentation and salivary presentation to forced mating, and females are more likely to tolerate mating (Thornhill 1987). In an observation of interspecific competition between the Japanese species *P. ochraceopennis* and *P. nipponensis*, which is closely related to *P. japonica* and has been suggested to have similar mating patterns like that of *P. japonica*, it was reported that males that lost interspecific competition with males of other species adopted the satellite tactic of waiting in the contested feeding area (Thornhill 1992c). In addition, the mating tactics of Japanese species may be more diverse than those of other regions, with some species possibly using mating styles like those of North American or European species, and others possibly using only forced mating (Sato and Fujiyama 2018). Differences in the intensity of interspecific competition may have led to this diversity in mating tactics. In the future, it is necessary to investigate mating tactics in other Japanese species in detail.

This study is the first example for variation in the rate of adoption of mating tactics among local populations of the same species. By comparing the rate of adoption of mating tactics in this species, it will be possible to identify the factors that are the initial steps in the differentiation of mating tactics in this species and to predict the behavioral evolution of this species more accurately.

In the future, it is necessary to expand the study area from Okayama and Aichi, and at the same time, to investigate how effective mating tactics are for mating success by paternity analysis using genetic markers.

The results of this study suggest that there may be differences in the rate of adoption of mating tactics among local populations of Japanese species other than *P. japonica* and insects of Panorpidae from other regions. Therefore, a comparative study of behaviors in each region may help elucidate the evolution of mating tactics in scorpionfly species.

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