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Sex ratio shifts of the solitary parasitoid wasp, *Pachycrepoideus vindemmiae* (Hymenoptera: Pteromalidae), to other foundresses

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ABSTRACT

Background: When parasitoid wasps oviposit in a limited patch, local mate competition (LMC) theories predict that, as foundress density rises, the sex ratio of offspring will be less biased towards females.

Hypothesis: Parasitoid wasp females in an experimental test should adjust their offspring sex ratio in response to the presence of other females.

Goal: Measure the sex ratio shifts of each foundress in the solitary parasitoid wasp, *Pachycrepoideus vindemmiae*.

Methods: Allow females to oviposit either alone or in a pair. Use an SSR-mark to determine which offspring belong to which female.

Results: When females oviposited alone, they produced fewer male offspring and a smaller proportion of males than when two females oviposited together. This result differed during the oviposition period. On the first day of oviposition, foundress number had no significant effect on offspring sex ratio. However, on the following days, the proportion of male offspring from two foundresses was significantly higher than that from one foundress.

Conclusion: The response of offspring sex ratio to foundress number in *P. vindemmiae* is in line theoretically with local mate competition theories.

Keywords: behaviour, *Pachycrepoideus vindemmiae*, solitary parasitoid wasp, sex ratio, local mate competition.

INTRODUCTION

Sex ratio in a local environment has been taken as a model of sex ratio theory (Hamilton, 1967; West, 2009), and a case study of adaptation in evolutionary biology (Fellowes *et al.*, 1999). In many cases, sex allocation has been extremely successful, providing qualitative and even quantitative explanations of sex ratio variation. The most popular taxa for testing sex ratio theory are the haplodiploid Hymenoptera, such as parasitoid wasps, whose primary sex

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ratio is determined by the mothers' control of fertilization at the time of oviposition. Males of most Hymenoptera are haploid and develop from unfertilized eggs, whereas females are diploid and develop from fertilized eggs. The haplodiploid genetic system, in principle, allows a foundress to control precisely her offspring sex ratio by deciding whether or not eggs are fertilized (West, 2009).

In parasitoid wasps, a small number of females reproduce within a patch, mating often occurring before female dispersal (West *et al.*, 2000; Ivens *et al.*, 2009; West, 2009). When a single female founds a patch and all mating occurs between siblings, brothers compete with each other to mate with their sisters. Local mate competition (LMC) selects for fewer male offspring. If offspring in a patch originate from two foundresses, the value of sons increases because sons have the potential to mate with the daughters of the other foundress, and a less female-biased offspring sex ratio is predicted (Hamilton, 1967, 1979; Herre, 1985, 1987; Werren, 1987). Some non-pollinating fig wasps and parasitoid wasps may have a 'partial LMC' sex ratio, because their male offspring can disperse to some extent and mate with non-native female offspring (Werren and Simbolotti, 1989; Nadel and Luck, 1992; West, 2009). 'Partial LMC' selects for a less female-biased sex ratio than 'strict LMC' (West and Herre, 1998; Fellowes *et al.*, 1999).

Previous LMC models have implicitly assumed that all foundresses contribute equally to offspring. However, research has shown that when several foundresses lay eggs in a patch, they contribute unevenly to the resultant shared broods (Molbo *et al.*, 2003). A red-eye mutant strain of *Nasonia vitripennis* was used as a marker to identify offspring in a multi-foundress patch; foundresses with only male offspring (probably unmated) or no offspring were excluded from analysis (Werren, 1980; Shuker and West, 2004; Shuker *et al.*, 2006). It has been reported that genetic markers, such as microsatellite DNA, which can help to identify the offspring of different foundresses, are necessary to distinguish the offspring sex ratio of different females, especially the first and second foundresses (Abe *et al.*, 2003). Microsatellite markers have also been used to explain sex ratio shifts of *N. vitripennis* (Burton-Chellew *et al.*, 2008; Grillenberger *et al.*, 2008), *Melittobia australica* (Abe *et al.*, 2005), and some pollinating fig wasps, such as *Lipporrhopalum tentacularis* (Moore *et al.*, 2005; Zavodna *et al.*, 2007) and *Pegoscapus* spp. (Molbo *et al.*, 2004). The number of foundresses that actually contribute to broods and the offspring sex ratio of each foundress need to be known to accurately infer sex ratio models.

Local mate competition was originally designed for gregarious species (multiple offspring per host), but some solitary parasitoids (a single offspring per host) have been demonstrated to adjust their offspring sex ratio in line with that predicted by theory (King, 1989, 2002; King and Seidl, 1993; King and Lee, 1994; Somjee *et al.*, 2010). When solitary species have clumped hosts, such as *Pachycrepoideus vindemmiae* (Pteromalidae), they are described as quasi-gregarious and LMC should apply (Nadel and Luck, 1992). However, little research on the identification of co-foundresses' offspring has focused on such quasi-gregarious parasitoids. When the oviposition patch has only one foundress, a female-biased offspring sex ratio would be predicted. But when two foundresses oviposit together, both parasitoids may have the ability to identify and adjust offspring accordingly, and a less female-biased sex ratio is predicted.

To understand the adjustment of solitary parasitoids to foundress number, we used two strains of *P. vindemmiae* females as foundresses, which were identified with a microsatellite marker, and housefly pupae as hosts. We first obtained the offspring from a single foundress of each strain, and then two females from different strains were allowed to oviposit together. Offspring were identified to determine which offspring belonged to which female. Analyses showed that offspring sex ratio adjustments were consistent with theoretical predictions.

METHODS

Pachycrepoideus vindemmiae are parasitoid wasps to the pupal stage of many fly species (Morris and Fellowes, 2002; Noyes, 2002). One offspring is produced per host, and hosts occur in clumps (Nadel and Luck, 1985). The parasitoid is an ectoparasitoid, attacking pupae and laying eggs on the surface of the pupal body within the puparium shell. Newly hatched larvae consume the host, pupation continues inside the puparium, and adults emerge from the host's puparium shell after eclosion (Tormos *et al.*, 2009).

Two strains of *P. vindemmiae* were used in this study. The Wuhu strain was obtained from the campus of Anhui Normal University, Wuhu, Anhui Province, China, and the Urumqi strain was obtained from the campus of Xinjiang University, Urumqi, Xinjiang Uygur Autonomous Region, China. Both strains were maintained with pupae of the housefly, *Musca domestica*, for about one year. Previous research has shown that the parasitoid can use freeze-preserved housefly pupae as hosts after thawing (Pickens and Miller, 1978; Rueda and Axtell, 1987; He *et al.*, 2013). With freeze-preserved pupae as hosts, no houseflies emerge and the hosts can be preserved for longer (He *et al.*, 2013). Housefly pupae obtained from Zhonghe Biotech Co. were used in a massive culture of the parasitoid. The housefly pupae were frozen at −20°C for less than one month in a refrigerated cabinet. Before being used in the culture, the pupae were removed from the cabinet and defrosted for about one hour. Parasitoid wasps were reared in glass vials (25 mm in diameter and 50 mm high) with the housefly pupae and honey-soaked cotton wool as food, in an incubator with a light/dark photoperiod of 14/10 hours. Temperature and relative humidity were 25 ± 1°C and 60 ± 5%, respectively. Before each experiment, we provided housefly pupae of similar size to obtain parasitoids that were similar in body size. Offspring that emerged within 24 hours were maintained together for about 24 hours to ensure that females were mated.

There were two treatments, one treatment with one foundress and the other with two foundresses. In the one-foundress treatment, a single mated female was introduced into a 300-mL transparent bottle, sealed with nylon mesh. Honey-soaked cotton wool was provided as food and 30 housefly pupae of similar size were provided as hosts. Fifty groups were tested using females from the Wuhu strain, and another 50 using females from the Urumqi strain. In the two-foundress treatment, one female from the Wuhu strain together with one from the Urumqi strain were introduced simultaneously into the bottle, and 30 housefly pupae of similar size were provided as hosts. The parasitized hosts were collected 24 hours later, and replaced with another 30 unparasitized housefly pupae and new honey-soaked cotton wool. Both treatments lasted for three days, and parasitized hosts were reared until emergence. All of the experiments were conducted under the photoperiod, temperature, and relative humidity conditions as described above in an incubator. About one week after emergence, all pupae were dissected and parasitoid offspring that failed to emerge were removed. All specimens were examined under a stereomicroscope for sexual identification.

Offspring from the two-foundress treatment were stored in 95% alcohol. Genomic DNA was extracted from ethanol-preserved samples using the boiling method (Kageyama *et al.*, 2006). We used the genomic DNA for polymerase chain reaction (PCR), using the primer set BL8 (BL8, F:5′-CGTTTCTGTTTGTCATCGACAG; R:5′-AGATGGTTCGGCGATAAAGA). We identified the PCR products of the Wuhu strain containing the $(AG)_{10}$ repeat, and of the Urumqi strain containing the $(AG)_{28}$ repeat. All PCR products were detected by agarose gel electrophoresis. We analysed the sequences of the PCR products using an ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

In some patches, no parasitoid wasps emerged, or only male offspring emerged (which indicated that the foundresses might be virgins); those data were not used in analyses. Proportional and count data often have non-normally distributed errors and are therefore difficult to analyse with traditional ANOVA techniques (Hardy, 2002). To avoid these problems, we analysed the sex ratio and count data using generalized linear model (GLM) analysis of deviance, assuming binomial and Poisson errors, respectively, and a logit link function in R v.2.13.0 (R Development Core Team, 2011). We assessed the appropriateness of the assumption of binomial or Poisson errors by comparing the residual deviance with the residual degrees of freedom after fitting the explanatory variables. Large relative values of the residual deviance indicate overdispersion, which may result in overestimation of significance levels, and binomial or Poisson can be replaced with quasi-binomial or quasi-Poisson in the analyses. Initially, a full model was fitted to the data, including all explanatory variables and their interactions. Terms were then removed from the full model using stepwise deletion. Whether the removal of a term caused a significant increase in deviance was assessed with a χ^2 test. The final models were tested using an F-test (Crawley, 2007).

RESULTS

Offspring from a single foundress

Total number of offspring of one foundress of the Wuhu strain was similar on all three days of oviposition (Table 1). Number of female offspring per day was also similar, but number of male offspring differed significantly and decreased during oviposition (Fig. 1A). The proportion of male offspring was 0.14 ± 0.12 ($N = 46$), 0.12 ± 0.13 ($N = 47$), and 0.09 ± 0.14 ($N = 43$) on the first, second, and third day, respectively. The sex ratio was significantly decreased during oviposition (Table 1).

Table 1. Effects of strain and oviposition duration on offspring from one foundress of *P. vindemmiae*

Parameters	Strain	Oviposition duration
Total production	Wuhu strain < Urumqi strain Day 1: $F_{1,98} = 5.76$, $P = 0.02$; Day 2: $F_{1,98} = 17.07$**; Day 3: $F_{1,98} = 13.65$**	Similar Wuhu: $F_{1,148} = 3.74$, $P = 0.06$ Urumqi: $F_{1,148} = 0.56$, $P = 0.45$
Female production	Wuhu strain < Urumqi strain Day 1: $F_{1,98} = 8.76$**; Day 2: $F_{1,98} = 22.89$**; Day 3: $F_{1,98} = 17.31$**	Similar Wuhu: $F_{1,148} = 1.94$, $P = 0.17$ Urumqi: $F_{1,148} = 0.07$, $P = 0.79$
Male production	Wuhu strain > Urumqi strain Day 1: $F_{1,98} = 3.07$, $P = 0.08$; Day 2: $F_{1,98} = 3.58$, $P = 0.06$; Day 3: $F_{1,98} = 7.57$**	Decreasing Wuhu: $F_{1,148} = 10.35$** Urumqi: $F_{1,148} = 16.94$**
Proportion male	Wuhu strain > Urumqi strain Day 1: $F_{1,84} = 10.44$**; Day 2: $F_{1,86} = 16.93$**; Day 3: $F_{1,86} = 16.37$**	Decreasing Wuhu: $F_{1,134} = 4.76$, $P = 0.03$ Urumqi: $F_{1,124} = 15.04$**

** $P < 0.01$.

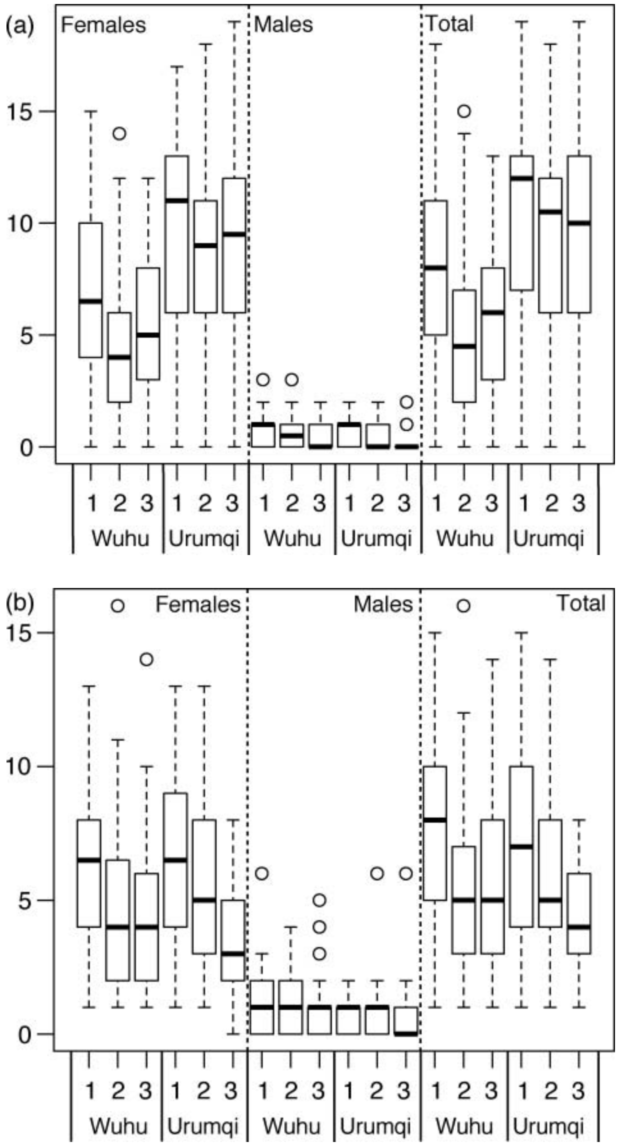

Fig. 1. Box plots showing offspring number from one foundress (a) and two foundresses (b) from the Wuhu and Urumqi strains.

Both total number of offspring and number of female offspring of one foundress of the Urumqi strain were similar during the three days of oviposition, but number of male offspring fell significantly (Table 1; Fig. 1A). The proportion of male offspring was 0.07 ± 0.05 ($N = 40$), 0.05 ± 0.10 ($N = 41$), and 0.02 ± 0.04 ($N = 45$) on the first, second, and third day, respectively. The sex ratio was also significantly decreased (Table 1).

Offspring production differed between the two strains. Compared with the Wuhu strain, females of the Urumqi strain had more offspring on all three days, and also had more female offspring on those days (Table 1). However, females of the Urumqi strain had fewer male offspring. Also, number of male offspring did not differ significantly on Days 1 or 2, but did differ significantly on Day 3. The proportion male offspring in the Urumqi strain was less than that in the Wuhu strain on all three days (Table 1).

Tibia length of male and female offspring in the Wuhu strain was 0.380 ± 0.041 mm ($N = 801$) and 0.367 ± 0.047 mm ($N = 105$), respectively. Tibia length of male and female offspring in the Urumqi strain was 0.391 ± 0.037 mm ($N = 1317$) and 0.369 ± 0.045 mm ($N = 62$), respectively. Compared with the Wuhu strain, female offspring from the Urumqi strain had significantly longer tibia ($t_{1550.72} = 6.30$, $P < 0.01$; independent samples *t*-test, equal variances not assumed); male offspring of the two strains had similar tibia length ($t_{165} = 0.28$, $P = 0.78$).

Offspring from two foundresses

When two foundresses (from the Wuhu and Urumqi strains) oviposited together for three days, 1732 offspring were obtained, of which 880 individuals were of the Wuhu strain, and 841 individuals were of the Urumqi strain. Ten female and one male offspring were not identified. Each female in the two-foundress treatment had male and female offspring, which meant that all of the foundresses had been mated.

The final GLM models for total offspring production, female offspring production, male offspring production, and offspring sex ratio, all included the variables strain and oviposition duration, but not their interaction term (Table 2). Strain had no significant effects on total offspring production or female offspring production, but had significant effects on both male offspring production and proportion male offspring (Table 2). The Wuhu strain had more male offspring and a higher proportion of males (Fig. 1B). Oviposition duration had a significant effect on total offspring production and female offspring production, with both reducing over time. However, oviposition duration had no significant effect on male offspring production or proportion male offspring (Table 2; Fig. 1B).

Sex ratio adjustment of each foundress when the number of foundresses increased from one to two

The final GLM model for sex ratio included foundress number, oviposition duration, strain, the interaction between foundress number and oviposition duration, and the interaction between foundress number and strain, but no three-way interaction (Table 3). The model showed that foundress number had a significant effect on offspring sex ratio, and when two foundresses oviposited together, there was a higher proportion of males. Also, the Wuhu

Table 2. Effects of strain and oviposition duration on offspring from two foundresses of *P. vindemmiae*

Parameters	Strain	Oviposition duration
Total production	Similar: $F_{1,287} = 0.71$, $P = 0.40$	Decreasing: $F_{1,286} = 37.54$**
Female production	Similar: $F_{1,287} = 0.06$, $P = 0.81$	Decreasing: $F_{1,286} = 39.86$**
Male production	Wuhu strain > Urumqi strain: $F_{1,287} = 10.06$**	Similar: $F_{1,286} = 0.97$, $P = 0.33$
Proportion male	Wuhu strain > Urumqi strain: $F_{1,287} = 10.44$**	Similar: $F_{1,286} = 2.70$, $P = 0.10$

** $P < 0.01$.

Table 3. Effects of foundress number, oviposition duration, and strain on offspring parameters of *P. vindemmiae*

Variable	Total offspring	Female offspring	Male offspring	Proportion male
Foundress number (FN)	$F_{1,587} = 26.67$**	$F_{1,587} = 41.22$**	$F_{1,587} = 12.97$**	$F_{1,549} = 43.59$**
Oviposition duration (OD)	$F_{1,586} = 24.18$**	$F_{1,586} = 18.97$**	$F_{1,586} = 15.16$**	$F_{1,548} = 1.98$, $P = 0.16$
Strain	$F_{1,585} = 19.68$**	$F_{1,585} = 35.03$**	$F_{1,585} = 23.06$**	$F_{1,547} = 47.15$**
FN × OD	$F_{1,584} = 7.03$**	$F_{1,584} = 11.76$**	$F_{1,584} = 8.80$**	$F_{1,546} = 17.81$**
FN × strain	$F_{1,583} = 23.20$**	$F_{1,583} = 22.22$**	—	$F_{1,545} = 5.75$, $P = 0.02$

** $P < 0.01$.

strain had a higher proportion of male offspring than the Urumqi strain. The final GLM models for total offspring, female offspring, and male offspring had similar composition (Table 3).

The interaction between foundress number and oviposition had a significant effect on offspring sex ratio (Table 3), indicating that the effects of foundress number and oviposition duration were not independent. On the one hand, the effects of oviposition duration differed with the number of foundresses. During oviposition, the proportion of male offspring decreased ($F_{1,260} = 18.37$, $P < 0.01$) when a single foundress oviposited, but the sex ratio of offspring was similar ($F_{1,287} = 3.06$, $P = 0.08$) when two foundresses oviposited (Fig. 2). On the other hand, the effect of foundress number was found to differ on oviposition days. On Day 1 of oviposition, the proportions of male offspring were similar ($F_{1,184} = 1.86$, $P = 0.17$), but on Days 2 and 3, the proportion of male offspring in the two-foundress treatment was significantly higher than that in the one-foundress treatment (Day 2: $F_{1,183} = 12.17$, $P < 0.01$; Day 3: $F_{1,178} = 34.86$, $P < 0.01$) (Fig. 3).

The interaction between foundress number and strain also had a significant effect on offspring sex ratio (Table 3), indicating independent effects of foundress number and strain. The effect of foundress number on offspring sex ratio differed between strains. In both strains, the proportion of males was higher in the two-foundress treatment than in the one-foundress treatment (Urumqi strain: $F_{1,269} = 27.76$, $P < 0.01$; Wuhu strain: $F_{1,278} = 9.72$, $P < 0.01$), but most marked in the Urumqi strain (Fig. 3).

The interaction between foundress number and strain had a significant effect on total offspring number (Table 3), indicating that the effects of foundress number on offspring differed between strains. In the Wuhu strain, the total number of offspring was similar between one-foundress and two-foundress treatments on Day 1 ($F_{1,98} = 0.04$, $P = 0.84$), Day 2 ($F_{1,96} = 0.42$, $P = 0.52$), and Day 3 ($F_{1,94} = 0.67$, $P = 0.41$) (Fig. 1). In the Urumqi strain, however, the total number of offspring was significantly lower in the two-foundress treatment on Day 1 ($F_{1,98} = 7.38$, $P < 0.01$), Day 2 ($F_{1,97} = 11.51$, $P < 0.01$), and Day 3 ($F_{1,94} = 47.31$, $P < 0.01$) compared with the one-foundress treatment (Fig. 1).

DISCUSSION

Nadel and Luck (1992) reported that males of *P. vindemmiae* do, to some extent, disperse and mating occurs elsewhere than on natal patches with non-native females. Partial LMC theories have been developed to explain the reproductive traits of such solitary parasitoids

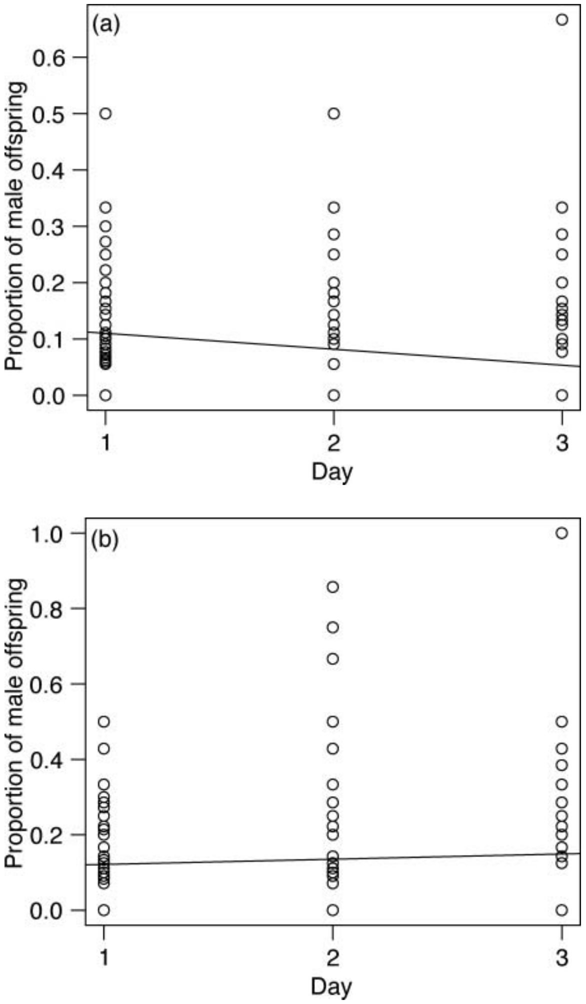

Fig. 2. Scatterplots of the relationship between offspring sex ratio and oviposition days with one foundress (a) and two foundresses (b) during oviposition.

and some non-pollinating fig wasps, and a less female-biased sex ratio than with strict LMC is expected (Werren and Simbolotti, 1989; West and Herre, 1998; Fellowes *et al.*, 1999; West, 2009). In this study, the number of foundresses that contributed to offspring in a patch was identified using a molecular marker. No virgin foundresses were found, and the offspring of each foundress in the two-foundress treatment differed. Our results show that *P. vindemmiae* can adjust their offspring sex ratio when two foundresses oviposit synchronously.

It has been shown that male offspring may be produced early during oviposition, followed by mostly females, as in some pollinating fig wasps: *Pegoscapus tristani*, *Alfonsiella fimbriata*, *Courtella gabonensis*, and *Allotriozoon heterandromorphum* (Kjellberg *et al.*, 2005), *L. tentacularis* (Kjellberg *et al.*, 2005; Moore *et al.*, 2005; Raja *et al.*, 2008), and *Ceratosolen solmsi* (Hu *et al.*, 2013). Clutch size is one explanation why more than one parasitoid wasp or fig wasp oviposits in a patch (Kjellberg *et al.*, 2005; Moore *et al.*, 2005; Raja *et al.*, 2008). When multiple foundresses oviposit

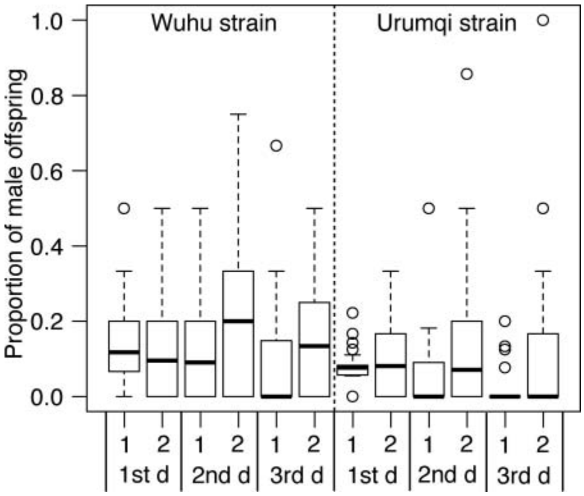

Fig. 3. Offspring sex ratio from one and two foundresses during oviposition for the Wuhu and Urumqi strains.

in a patch, there is a reduction in offspring reproduction (Moore and Greeff, 2003): limited clutch size and the oviposition order of fertilized and unfertilized eggs may lead to a less female-biased sex ratio (Kjellberg *et al.*, 2005; Moore *et al.*, 2005; Raja *et al.*, 2008). Our results show that when a single foundress lays eggs in a patch, the proportion of male offspring decreases during oviposition. However, clutch size may not be the main cue for sex ratio adjustment. In the two-foundress treatment, the hosts provided were the same as those in the one-foundress treatment; total number of offspring was similar between the two treatments for the Wuhu strain, indicating that clutch size was not limited, but there were more male offspring of the Wuhu strain in the two-foundress treatment. Therefore, foundresses may adjust their offspring sex ratios by using information about other foundresses (Hamilton, 1967, 1979; Herre, 1985, 1987; Kinoshita *et al.*, 2002; Moore *et al.*, 2002; Pereira and Prado, 2006; Herre *et al.*, 2008; Abe *et al.*, 2009; Somjee *et al.*, 2010). Furthermore, if there is more than one foundress and clutch size is limited, then both cues – clutch size and foundress number – would combine to induce a stronger sex ratio adjustment.

When two foundresses oviposit together, both can adjust their behaviour to produce more male offspring, especially on Days 2 and 3 of oviposition. A similar sex ratio adjustment has been reported for another solitary parasitoid, *Spalangia endius* (Pteromalidae) (King, 2002). Local mate competition theoretically predicts a female-biased sex ratio, but male offspring are also needed for mating (Kjellberg *et al.*, 2005). There are two explanations for such an adjustment. First, on Day 1 of oviposition, production of males would be a necessary strategy for parasitoid wasps, which might reduce the effects of LMC on offspring sex ratio adjustment. Second, a foundress would not only adjust her offspring sex ratio in relation to the number of foundresses in the patch, but also the eggs of other foundresses (Burton-Chellew *et al.*, 2008; Grillenberger *et al.*, 2008). On Day 1, relatively few eggs of other foundresses would be encountered, whereas on the following days, a foundress's accumulating information on other foundresses' eggs might enhance her adjustment of offspring sex ratio.

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