Can the sex ratio of the spiralling whitefly (*Aleurodicus dispersus*) be described by local mate competition?

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ABSTRACT

Background: Local mate competition theory predicts a female-biased sex ratio if one or a few hymenopteran foundresses, such as parasitoid wasps, oviposit in a local patch, and a less female-biased sex ratio as the number of foundresses increases. Although hemipterans, whiteflies are also haplodiploid insects, and the spatial structure of whitefly populations is similar to that of wasps.

Question: Do whitefly sex ratios match the theoretical predictions of local mate competition?

Organism: The spiralling whitefly, *Aleurodicus dispersus* Russell (Hemiptera: Aleyrodidae), a newly invasive, destructive pest on Hainan Island, China.

Methods: We investigated the effects of the number of foundresses on the sex ratio of *A. dispersus*.

Results: Offspring sex ratio was female-biased when only one foundress oviposited in a patch. Sex ratio increased with the number of foundresses. When only one foundress laid eggs in a patch, offspring sex ratio declined as the number of offspring increased. Male offspring emerged earlier than female offspring.

Conclusion: Local mate competition predicted the trends in sex ratio of the spiralling whitefly.

Keywords: behaviour, foundress, local mate competition, sex ratio, spiralling whitefly.

INTRODUCTION

Sex ratio in a local environment has been taken as a model of sex ratio theory (Hamilton, 1967; Frank, 1985; Herre, 1985, 1987; West *et al.*, 2000). Local mate competition (LMC) theory has been widely proved and well developed (Charnov, 1982), and has become a case study of adaptation in evolutionary biology (Fellowes *et al.*, 1999). In a random-mating population, natural selection

favours equal investment in both sexes and an equal sex ratio is an evolutionarily stable result (Fisher, 1930). However, if individuals mate within a highly structured population, such as mating only between full siblings, the evolutionarily stable strategy favours a female-biased sex ratio (Hamilton, 1967; Maynard Smith, 1976). In haplodiploid hymenoptera, males and females are often haploid and diploid, respectively. Relatedness differs between parents and both male and female offspring, with asymmetric relatedness such that female offspring are more related to their mother than are male offspring, and inbreeding strengthens the asymmetry (Hamilton, 1964; Sheldon and West, 2002). When a patch is founded by a single female and all mating occurs between siblings, brothers may compete with each other for mating with their sisters. Local mate competition selects for less male offspring, and a female-biased sex ratio benefits the foundress (Sheldon and West, 2002). 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 preferred (Hamilton, 1967, 1979; Herre, 1985, 1987; Werren, 1987).

The most popular taxa for testing LMC theory are the haplodiploid hymenoptera, such as parasitoid wasps, whose primary sex ratio is determined by mothers' control of fertilization at the time of oviposition (Charnov, 1982). In parasitoid wasps, a small number of females reproduce within a patch, and mating takes place before females disperse (Anstett *et al.*, 1997; West *et al.*, 2000).

In addition to wasps, the offspring sex ratio of a haplodiploid ambrosia beetle, *Xylosandrus germanus*, increases with number of foundresses in line with the expected LMC sex ratio (Peer and Taborsky, 2004). A haplodiploid mite, *Tetranychus urticae*, can also control the offspring sex ratio, producing highly female-biased sex ratio when the offspring are from a single foundress (Roeder *et al.*, 1996). Whitefly (Hemiptera: Aleyrodidae) are to some extent similar to the above taxa. They are haplodiploid insects (Byrne and Bellows, 1991; Byrne and Devonshire, 1996). Their dispersal ability is relatively poor, and dispersal occurs under deteriorating conditions or when needing to search for better feeding and oviposition sites (Byrne and Bellows, 1991). Mating always occurs soon after eclosion (Byrne and Bellows, 1991; Luan *et al.*, 2008). Such a structured population also results in inbreeding. It is unknown whether the sex ratio of whiteflies can be described by LMC theory. In the present study, therefore, we investigated the sex ratio of a spiralling whitefly, *Aleurodicus dispersus* Russell. This whitefly is a destructive invasive pest and was first observed in Hainan Island, China in 2006 (Yu *et al.*, 2007). The female whiteflies produce a white wax to cover the eggs when ovipositing. A spiral oviposition trail (wax trail) is formed by the female whitefly as she oviposits. Thus a spiral trail always indicates an egg pod of the foundress, and several spiral trails on a leaf might indicate several foundresses since females often change oviposition sites (personal observation). We found that: (a) offspring sex ratio was female-biased with a single foundress; (b) offspring sex ratio increased when more foundresses oviposited together, in line with the expected LMC sex ratio; and (c) when the brood size was small, the sex ratio of a single foundress was less female-biased. Male offspring emerged earlier than female offspring in an egg pod.

METHODS

Experiments with *A. dispersus* were conducted in the Chinese Academy of Tropical Agricultural Sciences (CATAS), Danzhou, Hainan province (Hainan Island), China (19°30.410′N, 109°29.340′E). The whitefly has spread throughout Hainan Island since 2006. The shortest durations at 28°C are: egg, 7.15 days; first instar nymph, 4.0 days; second instar

nymph, 3.83 days; third instar nymph, 4.09 days; fourth instar nymph, 7.56 days; and one complete generation, 26.63 days (Han *et al.*, 2009). The longest life span of female adults is 36 days at 18°C (Han *et al.*, 2009). The host plant used was *Bauhinia blakeana* Dunn (Leguminosae), an arbor tree about 6–10 m in height with drooping branches. Leaves on the plant are almost circular, 5–12 mm in diameter, with petioles about 5 mm in length. Hainan Island is located south of the China mainland separated by the Qiongzhou Strait, which is about 40 km wide with well-defined dry (November–April) and rainy (May–October) seasons. The annual mean temperature is 24.3°C, with the lowest mean temperature in February (18.2°C) and the highest in July (29.6°C).

To investigate the effect of foundress number on offspring sex ratio, we first introduced the newly emerged male and female whiteflies to a new leaf free from whitefly eggs, near leaves on which whiteflies oviposited naturally, on the same *B. blakeana* tree. But after more than 100 attempts, only five whiteflies had oviposited with few eggs ($n = 0$–7); the tiny insect (less than 2 mm) is hard to introduce artificially to a new leaf. Thus alternative projects were conducted. Spiral trails soon after oviposition were used to estimate foundress number. One trail on a leaf indicates the egg pod of one foundress, while several trails could indicate several foundresses because females often change oviposition sites. New egg pods on *B. blakeana* leaves were marked.

Three groups of egg pod types were chosen: one egg pod on a leaf, 3–6 egg pods on a leaf (more than 6 egg pods were difficult to identify), and leaves with innumerable egg pods. There are often too many egg pods on a leaf. Therefore, 3–6 egg pods were taken as a single group, due to the limited sample size for each. The leaves were protected with specially made nylon bags. The experiments continued until all offspring emerged. The male and female offspring were identified under a microscope (Han *et al.*, 2009). Male and female whiteflies emerging each day were recorded. The experiments were conducted on 24 *B. blakeana* trees from July to December 2009.

Proportionally distributed data often have non-normally distributed errors and are thus difficult to analyse with traditional analyses of variance (ANOVA). To overcome this problem, we analysed the sex ratio data using generalized linear model (GLM) analysis of deviance, assuming binomial errors, and a logit link function in R2.6.1 (Ihaka and Gentleman, 1996). The number of male whiteflies was used as the response variable, and the total offspring number from a leaf was used as the binomial denominator. When analysing the effects of foundress number on offspring sex ratio, egg pod types were used as the explanatory variable. When analysing the data for single egg pods (one foundress), offspring numbers on a leaf and duration of emergence since the first whitefly emerged were used as the explanatory variable, respectively. The models were tested using an *F*-test (Crawley, 2007). The ratios of the residual deviance to the degrees of freedom were all more than 1 but less than 2, and a test for a quasi-binomial distribution was used in the analysis (Crawley, 2007).

RESULTS

The mean offspring number from a single egg pod was 5.089 ± 4.954 (range 1–31, $n = 123$), from 3–6 egg pods it was 16.278 ± 10.604 (range 3–41, $n = 18$), and from innumerable egg pods it was 25.034 ± 24.100 (range 1–125, $n = 59$).

The sex ratio differed among the three groups of egg pods ($F_{1,198} = 35.928$, $P = 9.536 \times 10^{-9}$). When only one egg pod was laid on a leaf, the offspring sex ratio was 0.376 ± 0.316. In the 123 egg pods with only one oviposition trail, 30 egg pods saw the

emergence of only one whitefly (13 males and 17 females). With 3–6 egg pods on a leaf, the offspring sex ratio reached 0.409 ± 0.143. On leaves with innumerable egg pods, the offspring sex ratio from one leaf was 0.507 ± 0.149. Offspring sex ratio increased with more egg pods.

On the leaves with only one egg pod, the offspring sex ratio decreased with offspring number ($F_{1,121} = 5.654$, $P = 0.019$) (Fig. 1). The proportion of male offspring was higher when the offspring number was small. Sex ratio decreased with the duration of emergence ($F_{1,410} = 7.560$, $P = 0.006$) (Fig. 2).
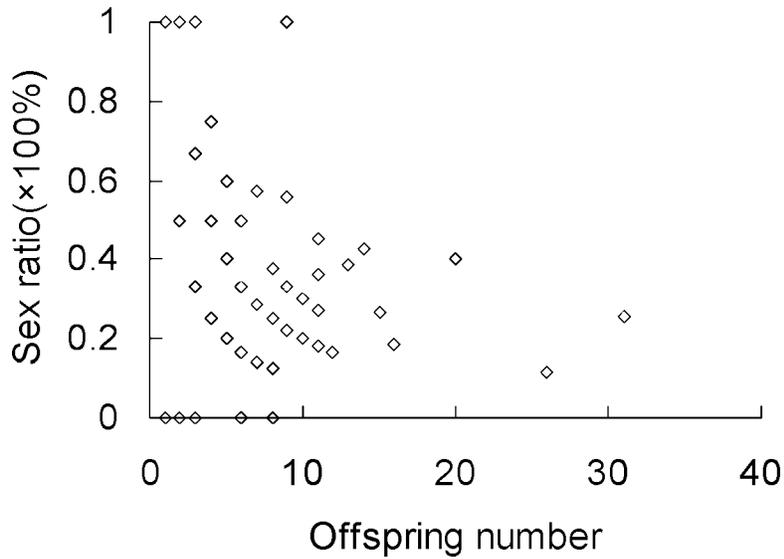

Fig. 1. Relationship between *A. dispersus* offspring sex ratio and offspring number.
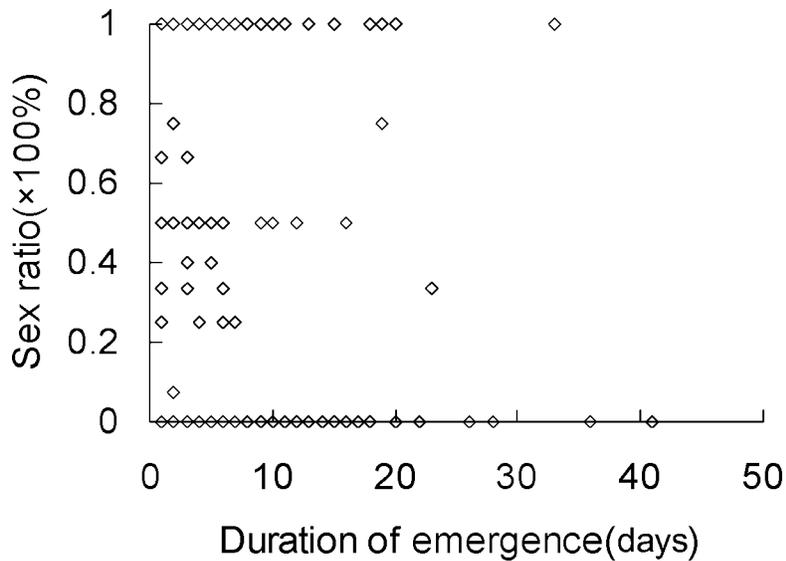

Fig. 2. Offspring sex ratio of *A. dispersus* during the emergence period.

DISCUSSION

A female-biased sex ratio was observed when there was only one egg pod on a leaf. But our results also show that the sex ratio on a leaf crowded with eggs is near 1:1. When there is only one egg pod on a leaf, the sex ratio decreases with offspring number, and male offspring emerge earlier than females (Figs. 1 and 2).

In populations of haplodiploid parasitoid wasps, mating often takes place before female dispersal and a small number of females reproduce within a patch (Anstett *et al.*, 1997; West *et al.*, 2000). Local mate competition between male offspring, and asymmetry relatedness between a foundress and two sexual offspring select for a female-biased sex ratio in such spatially structured populations, and inbreeding strengthens it further (Hamilton, 1964; Herre, 1987; Greeff, 1996; West and Herre, 1998; Sheldon and West, 2002). When only one foundress oviposits in a patch, a highly female-biased sex ratio is expected, whereas a less female-biased sex ratio is anticipated with an increasing number of foundresses (Hamilton, 1967, 1979; Charnov, 1982; Herre, 1985, 1987; Werren, 1987; West and Herre, 1998; Hu *et al.*, 2010). Some parasitoid wasps may have a 'partial LMC' sex ratio, since their male offspring can to some extent disperse and mate non-native female offspring (Werren and Simbolotti, 1989). In addition to wasps, the offspring sex ratio of the haplodiploid ambrosia beetle, *X. germanus*, and mite, *T. urticae*, are both in line with the theoretical predictions of LMC theory (Roeder *et al.*, 1996; Peer and Taborsky, 2004). Our results show that sex ratio adjustment of the haplodiploid spiralling whitefly is 'partial local mate competition'. When just one spiralling whitefly oviposits on a leaf (one egg pod), the offspring sex ratio is female-biased. When more whiteflies oviposit (more egg pods), a less female-biased offspring sex ratio is observed. Whitefly population structure seems to be like that of haplodiploid parasitoid wasps. Female whiteflies reproduce within a patch, mating often takes place between siblings and before the females disperse. Male whiteflies have limited dispersal ability and can mate several times with emerged females in their natal patch (personal observation). Males emerge earlier than females (Fig. 2). Therefore, foundresses may gain more fitness with a female-biased sex ratio. When more foundresses oviposit on the same patch, the value of male offspring increases due to their chances to mate with non-sibling females, and a less female-biased sex ratio is selected.

Local mate competition theory predicts a negative relationship between offspring number and sex ratio in, for example, parasitoid wasps, when only one foundress lays eggs in a patch (Kinoshita *et al.*, 1998, 2002; Moore *et al.*, 2002, 2005; Kjellberg *et al.*, 2005; Raja *et al.*, 2008). Some researchers have argued that brood size and the oviposition order of fertilized and unfertilized eggs may be the 'cue' for LMC sex ratio when more foundresses oviposit in a limited patch (Kjellberg *et al.*, 2005; Moore *et al.*, 2005; Raja *et al.*, 2008). In the present study, the negative relationship between sex ratio and offspring number in broods from one foundress may also be an important reason for the increasing sex ratio when more foundresses oviposit on a leaf.

Some other whiteflies also have a female-biased sex ratio (Byrne and Bellows, 1991). *Bemisia tabaci* has a sex ratio of 2.7 females to 1 male (Salas and Mendoza, 1995). The sex ratio of *Trialeurodes vaporariorum* is also female-biased (Elhag and Horn, 1984). It has been reported that the sex ratio of whiteflies varies in different circumstances, such as under different temperatures (Enkegaard, 1993; Sohani *et al.*, 2007), on different host plants (McKenzie *et al.*, 2004), and with different mate frequencies (Horowitz and Gerling, 1992). Some authors have argued that favourable conditions lead to high female-biased sex ratios, while adverse conditions lead to less female-biased sex ratios (Van Giessen *et al.*, 1995). But the factor affecting whitefly sex ratio in those studies was the proportion of virgin foundresses. More virgin foundresses lead to a

high offspring sex ratio, since virgin whiteflies can only produce male progeny (Van Giessen *et al.*, 1995). We argue that sex ratio adjustment of the fertilized females may also affect their sex ratio. In this article, both male and female offspring emerged from most egg pods, which indicates the foundresses were fertilized. Sex ratio adjustment of those fertilized foundresses is affected by brood size and foundress number, which indicates a link to the adjustment that local mate competition predicts.

ACKNOWLEDGEMENTS

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