

The effects of relatedness on offspring sex ratio in pollinating fig wasps

Hao-Yuan Hu^{1,2}, Guang-Chang Ma², Li-Ming Niu^{1,3}, Yue-Guan Fu³,
Zheng-Qiang Peng³ and Da-Wei Huang^{1,4}

¹Key Laboratory of Zoological Systematics and Evolution,
Institute of Zoology, Chinese Academy of Sciences, Beijing,

²Key Laboratory of Biotic Environment and Ecological Safety in Anhui Province,
College of Life Sciences, Anhui Normal University, Wuhu, Anhui,

³Key Laboratory of Monitoring and Control of Tropical Agricultural and Forest Invasive Alien Pests,
Ministry of Agriculture, Environment and Plant Protection Institute,

Chinese Academy of Tropical Agricultural Sciences, Danzhou, Hainan and

⁴Plant Protection College, Shandong Agricultural University, Tai'an, Shandong, China

ABSTRACT

Background: Sex ratio theory predicts a more female-biased sex ratio if the haplodiploid foundresses of pollinating fig wasps are siblings when those foundresses oviposit in a local patch.

Question: Do pollinating fig wasps adjust their offspring sex ratio in response to their relatedness to other nearby ovipositing fig wasps?

Organisms: *Ceratosolen solmsi* Mayr, an obligate pollinator of the functionally dioecious fig tree, *Ficus hispida* Linn.

Methods: From May to October 2007, we reared pollinators from a single foundress through five generations. We collected male syconia before they matured and placed them in a fine-mesh bag. We used female pollinators that emerged. Our protocol ensured that females were sibs at each stage. We counted female and male wasps, galls, bladders (inflated, hollow ovules), and total female flowers in 25% of each syconium. More than 10 syconia were obtained in each generation. For comparison, we ran a non-sibling foundress treatment in July and August 2007. We analysed the data using a generalized linear model

Results: Offspring sex ratio was similar among the five sibling mating generations in our experiments, and the offspring sex ratio in the local mate competition models with sibling foundresses and non-sibling foundresses was also similar. The offspring sex ratio increased in the presence of more foundresses.

Conclusion: *Ceratosolen solmsi* does not adjust its offspring sex ratio according to sibling relatedness.

Keywords: behaviour, co-evolution, inbreeding, pollinating fig wasp, relatedness, sex ratio, sibling.

Correspondence: D.W. Huang, Institute of Zoology, Chinese Academy of Sciences, Datunlu, Chaoyang, Beijing 100101, People's Republic of China. e-mail: huangdw@ioz.ac.cn

Consult the copyright statement on the inside front cover for non-commercial copying policies.

INTRODUCTION

Fig trees (*Ficus*) and their obligate pollinating wasps (Chalcidoidea: Agaonidae) are a classic example of a co-evolved mutualism (Cook and Rasplus, 2003). This mutualism originated about 90 million years ago (Machado *et al.*, 2001; Cook and Rasplus, 2003; Cook and West, 2005). Highly specific fig pollinators pollinate fig trees while fig trees provide food and nurseries for the fig pollinator offspring. Short-lived (usually 1–3 days) female fig pollinators fly several kilometres to find receptive syconia using chemical signals released by figs. There they enter the closed inflorescences (Ware *et al.*, 1993; Compton *et al.*, 2000; Proffitt *et al.*, 2008). They lay eggs in the ovules before they die and simultaneously pollinate the syconia. Their larvae develop in the ovules. When syconia are ready to ripen, their female offspring emerge, mate with wingless males, and leave the syconia to begin a new generation.

All mating of pollinating fig wasps occurs in a local patch, the narrow zone of the syconial cavity (Anstett *et al.*, 1997; West *et al.*, 2000). Fig wasps are haplodiploid, with haploid males (from unfertilized eggs) and diploid females (from fertilized eggs), and the primary sex ratio is determined by mothers' control of fertilization at the time of oviposition (Charnov, 1982). Sex ratio of pollinators in the local environment has been taken as a model of sex ratio theory (Hamilton, 1967; Frank, 1985b; Herre, 1985, 1987; West *et al.*, 2000). Among those, the theory of local mate competition (LMC) 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 the sexes and an equal sex ratio is an evolutionarily stable result (Fisher, 1930). But, if individuals mate within a highly structured population, such as mating only with full siblings, the evolutionarily stable strategy (ESS) favours a female-biased sex ratio (Hamilton, 1967; Maynard Smith, 1976). In addition, when patches are founded by a single female and all mating occurs between siblings, brothers may compete with each other to mate with their sister. Local mate competition selects for a female-biased sex ratio. Relatedness differs between parents and both sexes of the offspring of haplodiploid hymenoptera. Female offspring are more related to their mother than are males, and inbreeding strengthens the asymmetry (Hamilton, 1964; Sheldon and West, 2002). So a female-biased sex ratio is preferred (Sheldon and West, 2002). If the 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. In that case, a less female-biased sex ratio is preferred (Hamilton, 1967, 1979; Herre, 1985, 1987; Werren, 1987).

Theories predict that sex ratio may be more female-biased if the haplodiploid foundresses are siblings when they oviposit in a local patch (Herre, 1985, 1987; Greeff, 1996; Roeder *et al.*, 1996; Greeff and Taylor, 1997). However, Reece *et al.* (2004) tested the theories in a parasitoid wasp, *Nasonia vitripennis*, and found that the offspring sex ratio laid by a foundress was not influenced by whether she mated with a sibling or not, and foundresses may not be able to assess relatedness. Shuker *et al.* (2004) also confirmed the results of Reece *et al.* (2004). Adjustment of the offspring sex ratio with the cue of sibling relatedness may illuminate kin discrimination among the foundresses (Greeff, 1996; Roeder *et al.*, 1996; Greeff and Taylor, 1997; Reece *et al.*, 2004; Shuker *et al.*, 2004). Whether pollinating fig wasps can adjust their sex ratio in response to sibling relatedness is unknown. In this study, we investigated the effect of relatedness on the sex ratio adjustment of *Ceratosolen solmsi* Mayr, an obligate pollinator of a functionally dioecious fig, *Ficus hispida* Linn. We found that the offspring sex ratio was similar among the five sibling mating generations, and the offspring sex ratio in the LMC models with sibling foundresses and non-sibling foundresses are also similar.

METHODS

Experiments with the *Ficus hispida*–*Ceratosolen solmsi* mutualism 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). *Ficus hispida* is a functionally dioecious and free-standing tree (Corlett, 2006), distributed widely in Hainan Island. Hainan Island is located south of the China mainland across the Qiongzhou Strait, which is about 40 km wide. Hainan has 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 sibling mating on the offspring sex ratio, we reared the pollinators from a single foundress, generation by generation. We collected the male syconia before they were mature and placed them in a fine-mesh bag. Female pollinators that emerged were immediately used in our experiments. The receptive syconia were protected from entry by pollinators by means of secured nylon bags before the receptive phase. When the syconia reached receptivity, female pollinators were introduced with a soft brush. A single pollinator was allowed to enter each syconium, and then the syconia were re-bagged, individually. When the syconia matured, female wasps from one of the syconia were immediately introduced into protected receptive syconia on another fig tree, with a single foundress in each syconium. After the syconia matured, the first generation of the foundress was finished, and female pollinators from one of the syconia were introduced to receptive syconia on the other fig tree, with a single foundress in each syconium. This protocol was repeated for five wasp generations. We counted female and male wasps, galls, bladders (inflated, hollow ovules), and total female flowers in each syconium. Total female flowers were counted from 25% of each syconium. More than 10 syconia were obtained in each generation. The experiments were conducted from May to October 2007.

To examine the effect of sibling relatedness on the offspring sex ratio, we compared the offspring sex ratio in the LMC models with sibling and non-sibling foundresses. The following experiments were performed in July and August 2007. We collected female wasps from a single one-foundress syconium of the third generation in the above experiments, and introduced the wasps into protected receptive syconia. Five treatments were made, with 1, 2, 3, 4, and 5 foundresses respectively. Each treatment had more than 10 syconia. On the same fig crop, the control treatments were also carried out with the above five treatments. Pollinators introduced into the control treatments were from naturally mature syconia, and wasps introduced to multiple foundress syconia were from mature syconia on different fig trees to ensure that the wasps were non-siblings. In the multiple foundress syconia, foundresses were allowed to enter each syconium at 20-min intervals so that the earlier entrants could finish crawling through most of the ostiolar bracts. The syconia were re-bagged individually. After the syconia had matured, female and male wasps, galls, bladders, and total female flowers in each syconium were counted as above.

Proportionally distributed data often have non-normally distributed errors and are thus difficult to analyse with traditional ANOVA techniques. To avoid these problems, we analysed the sex ratio data using generalized linear model (GLM) analysis of variance, assuming binomial errors, and a logit link function in S-plus 8.0 (Crawley, 2002). The number of male wasps in a syconium was used as the response variable, and the total number of offspring in a syconium was used as the binomial denominator.

Initially, a full model was fitted to the data, including all explanatory variables and their interactions. Terms were then removed from the full model by stepwise deletion. Whether the removal of a term caused a significant increase in variance was assessed with a chi-square test. We checked the appropriateness of the assumption of binomial errors by comparing the residual variance with the residual degrees of freedom after fitting the explanatory variable. The minimal adequate model (including generations, gall and floret number) was tested using an *F*-test (Crawley, 2002). When we compared the offspring sex ratio from sibling and non-sibling foundresses in the full model, the ratio of the residual variance to the degrees of freedom was very high (> 11). The sex ratio was angular transformed, and a standard ANOVA was used (West *et al.*, 1997; Flanagan *et al.*, 1998). A power analysis was conducted in G*Power 3.1.0, with alpha held at a uniform value of 0.05 (Faul *et al.*, 2007).

RESULTS

We recorded the sex ratio of offspring from a single foundress in the five sibling mating generations (Fig. 1). The female floret number was similar in the syconia of the five generations ($F_{4,38} = 0.259$, $P = 0.903$, power = 0.199 at medium effect size). The mean ($\pm$ s.d.) female floret number was 1867 ± 250 . Gall number differed among generations ($F_{4,38} = 8.079$, $P < 0.0001$), and in the fifth generation the number of galls was significantly

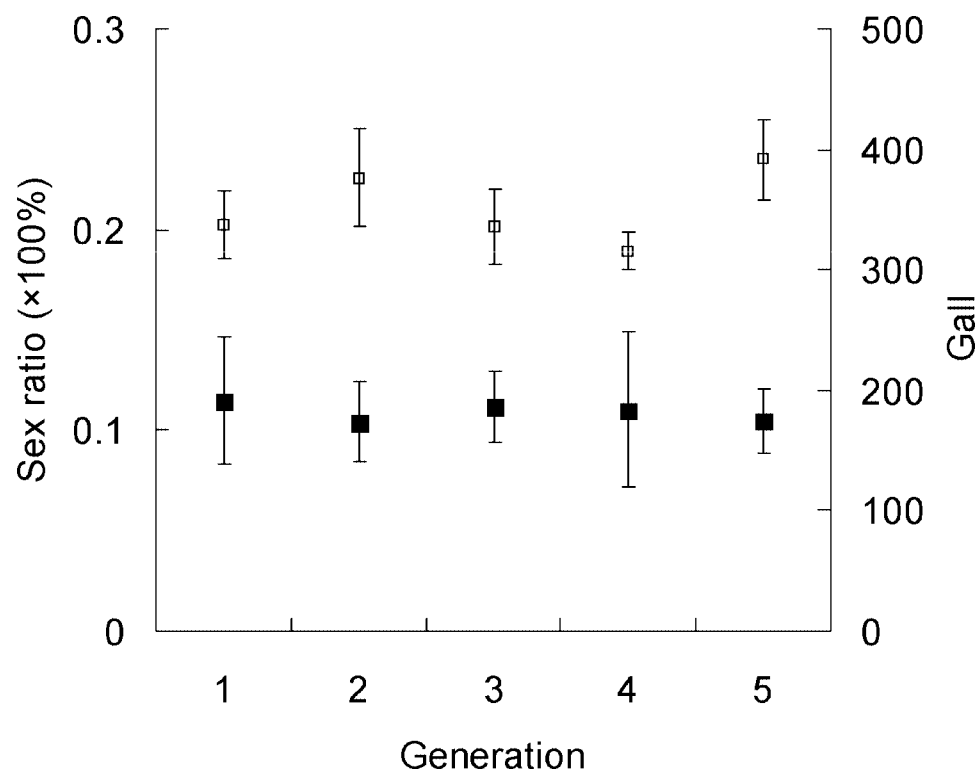


Fig. 1. Offspring sex ratio in the five sibling-mating generations. $\square$, number of galls; $\blacksquare$, sex ratio.

higher than that in the third and fourth generation. But gall number was not significantly related to generation (Pearson's correlation coefficient, $r = 0.232$, $P = 0.134$). The number of offspring differed among generations ($F_{4,38} = 5.646$, $P = 0.001$), but bladder number was similar among generations ($F_{4,38} = 1.554$, $P = 0.206$). The offspring sex ratio was similar in the five sibling mating generations ($F_{4,38} = 0.226$, $P = 0.924$), and neither gall number nor floret number had a significant effect on sex ratio ($F_{1,37} = 1.116$, $P = 0.291$; $F_{1,36} = 0.022$, $P = 0.883$, respectively).

We compared sex ratio in the LMC models with sibling and non-sibling foundresses (Fig. 2). The female florets did not differ between the sibling and non-sibling treatments ($F_{1,77} = 2.692$, $P = 0.105$), or among the five treatments ($F_{4,77} = 0.465$, $P = 0.761$). Galls in syconia increased with the number of foundresses (Pearson's correlation coefficient, $r = 0.869$, $P < 0.0001$; $r = 0.908$, $P < 0.0001$ for the sibling and non-sibling foundress treatments, respectively). Sex ratio increased significantly in the presence of more foundresses (sex ratio was angular transformed, $F_{4,77} = 14.067$, $P < 0.0001$). The offspring sex ratio in the LMC model with sibling foundresses was similar to that with non-sibling foundresses (sex ratio was angular transformed, $F_{1,77} = 0.514$, $P = 0.476$, power = 0.635 at medium effect size).

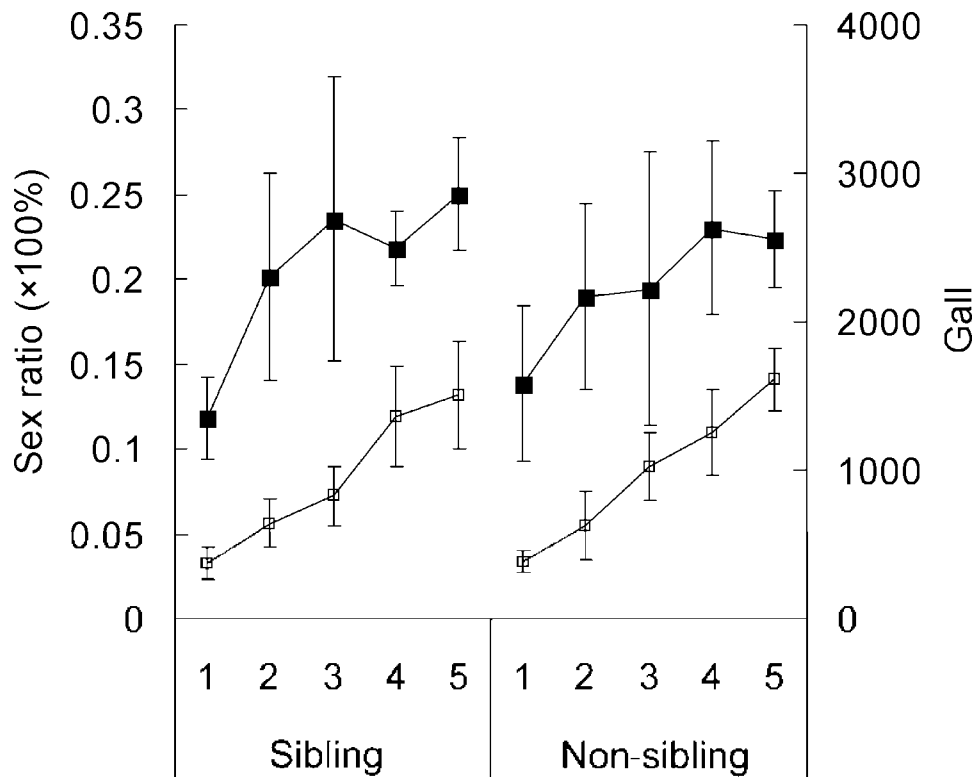


Fig. 2. Offspring sex ratio with sibling foundresses and non-sibling foundresses. The offspring sex ratio in the LMC models with sibling foundresses was similar to that with non-sibling foundresses. □, number of galls; ■, sex ratio.

DISCUSSION

The similar sex ratio in the five sibling mating generations shows that sex ratio may not depend on whether foundresses mate with siblings generation after generation. Gall number was not significantly related to generation, although it differed among generations. In both the sibling and non-sibling foundress treatments, the offspring sex ratio was less female-biased when more foundresses oviposited in the syconial cavity. The offspring sex ratio in the LMC models with the sibling and non-sibling foundresses was similar.

Foundresses may adjust their offspring sex ratio based on interactions between relatives, such as LMC and so on (West *et al.*, 2003). Local mate competition selects for a female-biased sex ratio, especially in species where foundresses have control over offspring sex. Previous reports have shown that a female-biased sex ratio is expected when one foundress oviposits in the syconial cavity, and a less female-biased sex ratio when more than one foundress does so (Hamilton, 1967, 1979; Charnov, 1982; Herre, 1985, 1987; Werren, 1987; West and Herre, 1998). In line with this, our results also show that when only one foundress oviposited in a syconium, the offspring sex ratio was female-biased. When more foundresses oviposited, a less female-biased offspring sex ratio occurred.

Inbreeding is one of the key factors affecting the female-biased sex ratio in pollinating fig wasps, and given more inbreeding, a more female-biased offspring sex ratio is expected (Herre, 1987; Werren, 1987; West and Herre, 1998; West *et al.*, 2000). Theories predict that the offspring sex ratio may be more female-biased if the foundresses are siblings when they oviposit in a local patch (Herre, 1985, 1987; Greeff, 1996; Greeff and Taylor, 1997). Greeff (1996) showed theoretically that individuals can be selected to adjust their sex ratio facultatively in response to whether they mate with a sibling or not, with sibling-mated foundresses producing a more female-biased sex ratio than foundresses who do not mate with siblings. Roeder *et al.* (1996) demonstrated that the spider mite, *Tetranychus urticae*, may be capable of discriminating between kin and non-kin, because there is a more female-biased sex ratio when the foundresses are siblings. But the offspring sex ratio of a parasitoid wasp, *Nasonia vitripennis*, laid by a foundress, was not influenced by whether she mated with a sibling or non-relative, and foundresses of this species may not be able to assess sibling relatedness (Reece *et al.*, 2004; Shuker *et al.*, 2004).

Our results show that sex ratios from a single foundress are similar among the five continuously sibling-mating generations. The offspring sex ratio in the LMC model with sibling foundresses does not differ significantly from that with non-sibling foundresses. Pollinating fig wasps may not adjust their offspring sex ratio according to the cue of siblings or non-siblings.

Recent studies have demonstrated that inbreeding is higher than expected in pollinating fig wasps (Greeff, 2002; Molbo *et al.*, 2003, 2004), and sibling mating may be very common in nature. Molbo *et al.* (2004) found no genetic differentiation among the pollinator populations of *F. obtusifolia* sampled from different trees across 20 km. It may be hard to differentiate siblings, and pollinating fig wasps may instead have to adjust their offspring sex ratio in response to the average level of inbreeding in their population (Reece *et al.*, 2004).

ACKNOWLEDGEMENTS

We thank the anonymous reviewers for many useful comments. This project was supported by the National Natural Science Foundation of China (NSFC grants #30770302, #30570970), in part by a Program of the Ministry of Science and Technology of the Republic of China (2006FY110500),

and by the National Science Fund for Fostering Talents in Basic Research ('Special Subjects in Animal Taxonomy', NSFC-J0630964/J0109). All experiments reported here comply with the current laws of China.

REFERENCES

- Anstett, M.C., Hossaert-Mckey, M. and McKey, D. 1997. Modeling the persistence of small populations of strongly interdependent species: figs and fig wasps. *Conserv. Biol.*, **11**: 204–213.
- Charnov, E.L. 1982. *The Theory of Sex Allocation*. Princeton, NJ: Princeton University Press.
- Compton, S.G., Ellwood, M.D.F., Davis, A.J. and Welch, K. 2000. The flight heights of chalcid wasps (Hymenoptera, Chalcidoidea) in a lowland Bornean rain forest: fig wasps are the high fliers. *Biotropica*, **32**: 515–522.
- Cook, J.M. and Rasplus, J.-Y. 2003. Mutualists with attitude: coevolving fig wasps and figs. *Trends Ecol. Evol.*, **18**: 241–248.
- Cook, J.M. and West, S.A. 2005. Figs and fig wasps. *Curr. Biol.*, **15**: R978–R980.
- Corlett, R.T. 2006. Figs (*Ficus*, Moraceae) in Urban Hong Kong, South China. *Biotropica*, **38**: 116–121.
- Crawley, M.J. 2002. *Statistical Computing: An Introduction to Data Analysis Using S-Plus*. Chichester, UK: Wiley.
- Faul, F., Erdfelder, E., Lang, A. and Buchner, A. 2007. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods*, **39**: 175–191.
- Fellowes, M.D.E., Compton, S.G. and Cook, J.M. 1999. Sex allocation and local mate competition in Old World non-pollinating fig wasps. *Behav. Ecol. Sociobiol.*, **46**: 95–102.
- Fisher, R.A. 1930. *The Genetical Theory of Natural Selection*. Oxford: Clarendon Press.
- Flanagan, K.E., West, S.A. and Godfray, H.C.J. 1998. Local mate competition, variable fecundity and information use in a parasitoid. *Anim. Behav.*, **56**: 191–198.
- Frank, S.A. 1985a. Are mating and mate competition by the fig wasp *Pegoscapus assuetis* (Agaonidae) random within a fig? *Biotropica*, **17**: 170–172.
- Frank, S.A. 1985b. Hierarchical selection theory and sex ratios. II. On applying the theory, and a test with fig wasps. *Evolution*, **39**: 949–964.
- Greeff, J.M. 1996. Alternative mating strategies, partial sibmating and split sex ratios in haplodiploid species. *J. Evol. Biol.*, **9**: 855–869.
- Greeff, J.M. 2002. Mating system and sex ratios of a pollinating fig wasp with dispersing males. *Proc. R. Soc. Lond. B*, **269**: 2317–2323.
- Greeff, J.M. and Taylor, P.D. 1997. Effects of inbreeding depression on relatedness and optimal sex ratios. *Evol. Ecol.*, **11**: 245–247.
- Hamilton, W.D. 1964. The genetical evolution of social behaviour. II. *J. Theor. Biol.*, **7**: 17–52.
- Hamilton, W.D. 1967. Extraordinary sex ratios. *Science*, **156**: 477–488.
- Hamilton, W.D. 1979. Wingless and fighting males in fig wasps and other insects. In *Reproductive Competition, Mate Choice and Sexual Selection in Insects* (M.S. Blum and N.A. Blum, eds.), pp. 167–220. New York: Academic Press.
- Herre, E.A. 1985. Sex ratio adjustment in fig wasps. *Science*, **228**: 896–898.
- Herre, E.A. 1987. Optimality, plasticity and selective regime in fig wasp sex ratios. *Nature*, **329**: 627–629.
- Herre, E.A. and West, S.A. 1997. Conflict of interest in a mutualism: documenting the elusive fig wasp–seed trade-off. *Proc. R. Soc. Lond. B*, **264**: 1501–1507.
- Machado, C.A., Jousselin, E., Kjellberg, F., Compton, S.G. and Herre, E.A. 2001. Phylogenetic relationships, historical biogeography and character evolution of fig-pollinating wasps. *Proc. R. Soc. Lond. B*, **268**: 685–694.
- Maynard Smith, J. 1976. Evolution and the theory of games. *Am. Sci.*, **64**: 41–45.

- Molbo, D., Machado, C.A., Sevenster, J.G., Keller, L. and Herre, E.A. 2003. Cryptic species of fig-pollinating wasps: implications for the evolution of the fig-wasp mutualism, sex allocation, and precision of adaptation. *Proc. Natl. Acad. Sci. USA*, **100**: 5867–5872.
- Molbo, D., Machado, C.A., Herre, E.A. and Keller, L. 2004. Inbreeding and population structure in two pairs of cryptic fig wasp species. *Mol. Ecol.*, **13**: 1613–1623.
- Proffitt, M., Schatz, B., Bessiere, J.M., Chen, C., Soler, C. and Hossaert-McKey, M. 2008. Signalling receptivity: comparison of the emission of volatile compounds by figs of *Ficus hispida* before, during and after the phase of receptivity to pollinators. *Symbiosis*, **45**: 15–24.
- Reece, S.E., Shuker, D.M., Pen, I., Duncan, A.B., Choudhary, A., Batchelor, C.M. *et al.* 2004. Kin discrimination and sex ratios in a parasitoid wasp. *J. Evol. Biol.*, **17**: 208–216.
- Roeder, C., Harmsen, R. and Mouldey, S. 1996. The effects of relatedness on progeny sex ratio in spider mites. *J. Evol. Biol.*, **9**: 143–151.
- Sheldon, B.C. and West, S.A. 2002. Sex ratios. In *Encyclopedia of Evolution* (M. Pagel, ed.), pp. 1040–1044. Oxford: Oxford University Press.
- Shuker, D.M., Reece, S.E., Whitehorn, P.R. and West, S.A. 2004. Sib-mating does not lead to facultative sex ratio adjustment in the parasitoid wasp, *Nasonia vitripennis*. *Evol. Ecol. Res.*, **6**: 473–480.
- Ware, A.B., Kaye, P.T., Compton, S.G. and van Noort, S. 1993. Fig volatiles: their role in attracting pollinators and maintaining pollinator specificity. *Plant Syst. Evol.*, **186**: 147–156.
- Werren, J.H. 1987. Labile sex ratios in wasps and bees. *BioScience*, **37**: 498–506.
- West, S.A. and Herre, E.A. 1998. Stabilizing selection and variance in fig wasp sex ratios. *Evolution*, **52**: 475–485.
- West, S.A., Herre, E.A., Compton, S.G., Godfray, H.C.J. and Cook, J.M. 1997. A comparative study of virginity in fig wasps. *Anim. Behav.*, **54**: 437–450.
- West, S.A., Herre, E.A. and Sheldon, B.C. 2000. The benefits of allocating sex. *Science*, **290**: 288–290.
- West, S.A., Shuker, D.M. and Sheldon, B.C. 2003. Sex-ratio adjustment when relatives interact: a test of constraints on adaptation. *Evolution*, **59**: 1211–1228.