# Trade-off between reciprocal mutualists: local resource availability-oriented interaction in fig/fig wasp mutualism

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# Summary

- 1. The mechanisms that prevent competition (conflict) between the recipient and co-operative actor in co-operative systems remain one of the greatest problems for evolutionary biology. Previous hypotheses suggest that self-restraint, dispersal or spatial constraints can prevent direct competition for local resources or any other common resources, thereby maintaining stable co-operation interactions. In this study, we use the obligate fig-fig-wasp mutualism to examine whether the above mechanisms can maintain stable co-operation sufficiently between figs and fig wasps.
- **2.** Our data on obligate co-operation between figs (*Ficus racemosa* Linn.) and fig wasps (*Ceratoslen fusciceps* Mayr) show that the number of viable seeds of figs is positively correlated with the number of pollinator offspring when the number of vacant female flowers is high while the foundress number is low (two foundresses). Meanwhile, they are negatively correlated when the number of vacant female flowers is low and the number of foundresses is increased manually (eight foundresses). The correlation coefficient between viable seeds and wasp offspring (galls) depends on vacant female flower availability.
- 3. Our data suggest that the interaction between figs and fig wasps is conditional, and that they co-operate when local resource availability is plentiful but are in conflict when local resource availability is limited. The self-restraint, dispersal and spatial heterogeneity previously hypothesized in maintaining stable co-operation cannot sufficiently prevent the symbionts from utilizing more local resources at the expense of the recipients. The conflict, which can disrupt the co-operation interaction, exists after the local resource is saturated with symbionts. The repression of symbiont increase, therefore repressing the utilization of local resources in the conflict period, is crucial in the maintenance and evolution of co-operation.

**Key-words:** conflict, co-operation, fig, mutualism, repression, trade-off

# Introduction

In interspecific mutualistic systems, symbionts (co-operative individuals) provide services or goods for the recipients. These goods and services are costly in some systems, while the recipients reward the symbionts with direct or indirect benefits. Therefore in co-operation systems, both species will bilaterally increase their fitness. However, the conflict for local resources (or any other common resources) can be increased through augmentation of the symbiont if the available resource is limited, potentially disrupting the co-operation system (Williams 1966; Taylor 1992; Wilson, Pollock & Dugatkin 1992;

West, Pen & Griffin 2002a). What mechanisms prevent co-operative partners from over-utilizing local resources at the expense of the recipient, and therefore maintain a stable co-operation interaction, remains a heated argument in the scientific community (Clutton-Brock 2002; West *et al.* 2002b).

Classical co-operation theories emphasize that the genetic relatedness resulting from viscosity of the individuals involved can increase the inclusive or direct fitness of involved partners (Hamilton 1972; Mitteldorf & Wilson 2000). However, the cost of co-operative behaviour can cancel out the benefits with increasing numbers of actors (co-operative individuals), because the benefits received from the recipient may decrease disproportionately with the augmentation of actors, while the cost of co-operative behaviour is a relatively constant value

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(Williams 1966; Taylor 1992; Wilson et al. 1992). Genetic relatedness (between the original donor and recipient) in reciprocity selection, or kin relatedness in kin selection (Hamilton 1964; Frank 1994), will be unimportant in the evolution of co-operation (Williams 1966), and the co-operation interaction should proceed to extinction with the augmentation of actors if there is no mechanism that can sufficiently prevent the competition resulting from an augmentation of actors (Hamilton 1972; Taylor 1992; Wilson et al. 1992).

Spatial models of the evolution of altruism hypothesized that self-restraint (Hamilton 1972; Axelrod & Hamilton 1981), dispersal of the altruists (Greenwood-Lee & Taylor 2001), or spatial constraints (Nowak, Bonhoeffer & May 1994; Doebeli & Knowlton 1998) might play a role in preventing extra actors or cheating individuals from over-exploiting the commons at the expense of the recipient, and therefore might maintain stable co-operation. However, the above mechanisms cannot explain sufficiently why the cheating or less co-operative individuals do not invade the co-operation system, especially in cases where individuals cannot disperse to another colony or cannot live alone (for a critique of self-restraint mechanisms through recognition see Doebeli & Knowlton 1998; Clutton-Brock 2002; for a critique of spatial constraints see Hauert & Doebeli 2004).

Essentially, the evolution of co-operation is determined by whether a positive correlation coefficient of inclusive or direct fitness between the recipient and co-operative actors can be created (Price 1970; Hamilton 1972; Queller 1992a). If the spatial structure created by genetic restraint can maintain the stable co-operation sufficiently, a positive correlation coefficient should be observed, because the cheating individual of co-operative actors or extra co-operative actors cannot utilize the commons at the expense of the recipient (Price 1971; Hamilton 1972; Doebeli & Knowlton 1998), and the direct conflict (the negative correlation coefficient of inclusive or direct fitness between the recipient and co-operative actors) should not be observed. Unfortunately, most arguments and evidence for the above theories are conceptual or based on indirect evidence; little evidence using correlation analysis of inclusive or direct fitness exists due to the difficulties in collecting inclusive or direct fitness in most model systems for the evolution of co-operation.

In this study, we use fig-fig-wasp mutualism as a model to examine whether the positive correlation of the direct fitness between figs and fig wasps could be maintained, and then analyse what mechanisms might maintain the stable cooperation. Fig-fig-wasp mutualism, in which the direct fitness of both plant (viable seeds) and its obligate pollinator (offspring number) can be collected, is one of the most famous reciprocal mutualisms. In this system, fig wasps carry pollen for the figs, while the pollen is useless for fig wasps (foundresses), and the foundresses cannot obtain any immediate reward from the figs. The foundresses altruistically benefit the figs in the short term (Bronstein 1988; Bronstein & Hossaert-McKey 1996; West, Griffin & Gardner 2007). The figs provide some of their female flowers for oviposition by the pollinator wasps, and the development of wasp offspring is at the expense of the

female flowers. Therefore both partners benefit from each other's services (Bronstein 2001). However, monoecious Ficus species must allocate their female flowers for viable seeds and for the development of pollinator offspring that can then disperse their pollen to other figs (Anstett, Hossasert-Mckey & Kjellberg 1997; Kjellberg et al. 2001). In contrast, the only benefits to the wasps are the available female flowers that can be consumed by their offspring (Anstett et al. 1997; Herre & West 1997). Because the galls (female flowers that are consumed by wasp larvae) can be developed from viable seeds, a negative relationship can be expected between seed and gall production (Herre & West 1997; Herre et al. 1999).

In the fig-fig-wasp system, the fitness of both figs and fig wasps must be at the expense of the female flowers, while the total number of female flowers is limited, therefore the efficiency of pollen dispersion (contribution of fig wasps to figs) of augmented fig wasps will decrease even to zero as a function of unutilized, commonly vacant female flowers, which is called the marginal effect (Taylor 1992; West et al. 2002a). We can thereby examine whether the contribution of foundresses to the fitness of figs varies according to the availability of unutilized female flowers (space) or the frequency of altruists. It is then possible to analyse the trade-off between figs and fig wasps, and what mechanism can maintain stable co-operation.

### SAMPLE SITES AND MATERIAL

Data collection was carried out in Xishuangbanna, Yunnan, China. Xishuangbanna is in the south of Yunnan province (21°41′ N, 101°25′ E), at an altitude of ≈600 m. The climate presents a rainy season and a dry season. The rainy season is from May to October, and 79-82% of total yearly rain falls during this season. The temperature begins to increase in March, and reaches its peak in May with an average of 25.6 °C. Temperature in the region is relatively stable, with a yearly mean of 21.8 °C. From November to January the temperature drops, and January is the coldest month with an average temperature of 15.6 °C.

The monoecious fig *Ficus racemosa* Linn. (*F. sycomorus*) is distributed from India to Australia (Corner 1965). Ficus racemosa is a large tree that can reach 30 m high and bears cauliflorous fruits synchronously within the tree in very large numbers. It grows mainly in moist valleys or along rivers. Trees of F. racemosa usually grow in groups of five to 10. Ficus racemosa is pollinated by the wasp species Ceratoslen fusciceps Mayr (Agaonidae). The foundress number per receptive fruit of F. racemosa is usually five to 30, but can sometimes reach more than 70.

## Methods

### DATA COLLECTION

Our experiments were carried out from November 2003 to January 2004. Sample fruits were taken from the same crop, ensuring that fruit size and the environmental conditions of the experimental fruits were the same. In order to exclude the interference of non-pollinators, which can independently utilize female flowers and influence the trade-off between figs and their pollinator wasps, we enclosed the treated fruit in nylon bags. When the fruit developed into the receptive period, we introduced foundresses manually to the receptive fruit using three treatments. In this experiment we introduced either two, five or eight foundresses to receptive fruits (n = 13, 25 and 21 fruits, respectively). After introducing the foundresses, we enclosed the fruit again to prevent oviposition by non-pollinators. Because of different foundress abundance, resulting in different utilization of female flowers, we can describe utilization levels of female flowers as a function of foundress abundance. In the premature period, when the remains of foundresses can be distinguished, and the viable seeds and wasp off-spring (galls) are well developed, we collected the treated fruits and counted the number of viable seeds, galls and vacant female flowers.

We also collected 192 fruits from their natural environment over a year. Oviposition by non-pollinators will not obviously affect the quantitative characteristics of both fig and pollinator fitness (West & Herre 1994), especially in primary forest and clustering fig trees (Wang et al. 2005a), because the non-pollinators tend to oviposit in female flowers already galled by pollinators (West & Herre 1994; Wang et al. 2005a; Wang & Zheng 2008). However, one nonpollinator species (Apocryptophagus testacea) that can oviposit before the pollinators is found in much greater numbers in isolated fig trees than in primary forest and clustering fig trees (Wang et al. 2005a; R.W.Wang and B.F. Sun, unpublished data). We therefore collected sampled fruits only from clustering trees to negate the non-pollinator's interference in this data analysis. For each fruit, we counted the remains of foundresses, viable seeds, galls and vacant female flowers. Foundress abundance was different for each fruit, thus the variation in number of vacant female flowers (unutilized female flowers) was available.

# MODELLING EXERCISE

Because the total number of female flowers per fruit limits the numbers of both viable fig seeds and wasp offspring, an exponential function can be used to simulate the distribution of viable seed and wasp offspring production as a function of foundress number (Doebeli & Knowlton 1998; Holland, DeAngelis & Bronstein 2002). We conducted a simulation exercise in which fig seed production was simulated to reach the asymptote close to the number of total female flowers per fruit, because almost all the female flowers can develop viable seeds. While wasp offspring production was simulated to reach an asymptote of proportion of total female flowers, the existence of spatial restraints can prevent foundresses from ovipositing on some female flowers. The details of the modelling process are presented in Appendix S1 (see Supplementary material).

# STATISTICAL ANALYSIS

In this study, we analysed the correlation of the direct fitness of mutualists, the correlation between the number of viable seeds of figs and the number of offspring of pollinators, to examine the trade-off at different levels of altruist abundance. In fig-fig-wasp mutualism, the total number of female flowers of each fruit is limited, while the fitness of both figs and fig wasps must be at the expense of female flowers. Therefore viable seed and wasp offspring production will depend on the availability of unutilized female flowers, and the correlation coefficient between viable seeds and wasp offspring might be a function of unutilized female flowers or the frequency of foundresses (Wilson *et al.* 1992; West *et al.* 2002a). As a nonlinear process, we can separate unutilized female flowers into

different levels, calculating the correlation coefficients at different levels of unutilized female flowers (for background knowledge of correlation analysis see Appendix S2).

The data distribution pattern satisfies the parametric assumption. We therefore conducted a Pearson correlation analysis with the manipulative experimental data treated separately. The correlation coefficient between viable seeds and wasp offspring numbers was calculated at different levels of symbiont (foundress) abundance. To analyse the data from 192 field observations over a year, we divided these 192 samples into 14 groups sequentially according to the level of the unutilized resource (vacant female flowers). In the first 13 groups, each group has 13 observations, and the last group has  $192 - 13 \times 13 = 23$  observations. For each group the correlation coefficient is calculated, denoted by r(i); the average number of vacant female flowers is denoted by z(i), i = 1, 2, ..., 14. Using this method, we could observe whether the correlation coefficient between viable seeds and pollinator offspring depends on the number of vacant female flowers. The results using this method are independent of grouping if the appropriate sample size of each group is satisfied. MATLAB ver. 6.1 was used for all statistical analyses.

Because oviposition and pollination efficiency of foundresses differ greatly among crops due to changes in living condition (Wang et al. 2005b), we used observations collected under similar conditions (November–January) to describe the viable seed-production pattern as a function of foundress number (N = 115). If the extra foundresses decrease viable seed production after the commons are saturated, the seed production will be an inverse U-shape. We used a quadric function to test whether the empirical data fitted a U-shaped function.

### Results

The total number of female flowers per *F. racemosa* fruit varied between 4332 and 6905 in our treated sample. Viable seeds and wasp offspring were positively correlated when the foundress number was low and vacant (unutilized) female flowers were plentiful (Table 1), indicating that higher production of wasp offspring resulted in higher production of viable seeds and that, when unutilized female flowers were plentiful, wasp offspring production was not at the expense of viable seeds. However, when the foundress number was high and the number of vacant female flowers was low, wasp offspring negatively correlated with viable seeds, and higher wasp offspring production was at the expense of viable seeds (Table 1).

The total number of female flowers per fruit under natural conditions varied from 5494 to 11234 in the 192 sample fruits. The number of vacant female flowers per fruit varied greatly from 858 to 6478, and the proportion of vacant female flowers varied from 9 to 73%. When we grouped the vacant female flowers according to abundance, the correlation coefficient between viable seeds and wasp offspring was strongly positively dependent on the number of vacant female flowers (Fig. 1).

When the number of vacant female flowers was low, more foundresses resulted in lower seed production of figs within the same crop (Fig. 2). The extra pollinators in the receptive fruits are harmful to fig fitness when the number of unutilized female flowers is limited, while it can increase fig fitness when the number of vacant female flowers is high. These results indicate that the spatial constraint of female flowers, or the evolutionary constraint of pollinators, cannot completely

Table 1. Pearson's correlation analysis between viable fig seeds and wasp offspring of pollinators at different levels of vacant female flowers (different levels of foundress number) with manipulative experiment data in *Ficus racemosa* 

Parameter	Number of foundresses introduced per fruit		
	Two $(N = 13)$	Five $(N = 25)$	Eight $(N=21)$
Mean gall percentage per fruit	50·6 ± 7·0	55·4 ± 6·4	$48.4 \pm 8.8$
Mean seed percentage per fruit	$13.8 \pm 3.4$	$22.8 \pm 4.8$	$26.8 \pm 10.0$
Mean vacant female flower percentage	$35.5 \pm 8.9$	$21.8 \pm 7.0$	$24.8 \pm 3.6$
Total female flowers per fruit	$5449 \pm 723$	$6072 \pm 780$	$5556 \pm 553$
Correlation (r) between viable seeds and galls	0.74**	0·09 (ns)	-0.75***

<sup>\*\*,</sup> P < 0.01; \*\*\*, P < 0.001; ns, not significant at P = 0.05; N, sampled fruit number. Averaged value, mean  $\pm$  SD.

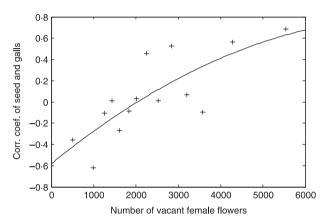


Fig. 1. Fitting curve of correlation coefficients between viable seeds and wasp offspring (galls) as a function of number of vacant female flowers in Ficus racemosa. The curve indicates that the correlation coefficient (r) between viable seeds and wasp offspring depends strongly on the number of vacant female flowers (F-test significant, P = 0.0012). These 192 samples are from four crops over a year.

prevent pollinators from ovipositing more eggs and utilizing more female flowers at the expense of viable seeds.

## **Discussion**

Hamilton (1972) hypothesized that limited dispersal of interacting individuals would increase their genetic relatedness in interspecific mutualism, and so would be an important factor favouring evolution of co-operation (see also Frank 1994). Limited dispersal keeps individuals of both species in close proximity, and thereby benefit given from one to the other is likely to be returned to the original donor or its immediate neighbours (Trivers 1971; Hamilton 1972; Frank 1994). However, limited dispersal of interacting individuals will also result in competition with an increase in co-operative individuals, which can disrupt the co-operation interaction in both intra- and interspecific co-operation systems (Queller 1992b; Taylor 1992; Wilson et al. 1992; West et al. 2002a). Although the hypotheses argue that dispersal, self-restraint of competition or spatial constraint can regulate competition resulting from the augmentation of co-operative individuals, we observe that the positive correlation between fig and figwasp fitness becomes a negative correlation with the decrease in local resource availability resulting from an augmentation

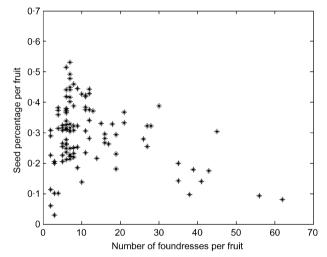


Fig. 2. Variation of seed production as a function of foundress number per fruit in Ficus racemosa. The data indicate that more foundresses in the fruit cavities can increase fig fitness when foundresses are scarce; however, when there are too many foundresses in a fruit cavity, more foundresses do not increase (and can even be harmful to) fig fitness (U-shaped, *F*-test = 10.3420, P = 0.00008 < 0.05). All samples were from the cold months (November-January) of clustering trees (N = 115), ensuring that environmental conditions for the pollination of foundresses are similar.

of foundresses in the fig-fig-wasp mutualism. This result indicates that self-restraint, dispersal of foundresses or spatial constraints cannot balance the competition resulting from the augmentation of foundresses. Our data suggest that repression of co-operative individual augmentation is required to repress competition and maintain stable co-operation in fig-fig-wasp mutualism.

Although dispersal to other colonies or self-restraint of competition can balance the competition resulting from the augmentation of co-operative individuals, these mechanisms are not likely to regulate competition in the fig-fig-wasp mutualism. Similarly to many intragroup co-operation systems (bees, ants, mole rodents), the cost of dispersal to another colony is much higher than competing with local individuals, and it is impossible to disperse to another colony in the fig-fig-wasp mutualism. After entering the fruit cavity, foundresses seldom exit again except in very rare cases (Moore et al. 2002), and foundresses have no chance of re-entering

other fruit cavities to reproduce (S. G. Compton, personal communication). Dispersal to other fruits for reproduction cannot balance out the augmentation of foundresses, and therefore cannot prevent competition in the fig-fig-wasp mutualism.

Self-restraint of competition is also unlikely to exist in the fig-fig-wasp system, because self-restraint can only be rewarded in the future in a repeated game. The game in the fig-fig-wasp mutualism, however, is a one-shot game, and the wasps cannot be rewarded from co-operation in the next generation. Direct reciprocity requires an immediate reward from the recipient. Furthermore, because competition for local resources will be increased with the increase in foundresses, the cost of self-restraint will also increase, and no immediate reward will be obtained from the recipient if the foundresses increase indefinitely. Therefore the self-restraint of wasps is highly unlikely in fig-fig-wasp mutualism. The empirical data and direct observations on fig-fig-wasp mutualism further demonstrate that there is no self-restraint of fig wasps to reduce competition with figs (Anstett et al. 1997; Herre & West 1997; Yu et al. 2004).

Another recently developed model, using senita-senita moth as a model system, argued that abortion might play a role in preventing direct conflict between reciprocal mutualists (Holland et al. 2002). However, abortion occurs only when viable seeds or galled flowers are very few, which is observed in the yucca-yucca-moth (Pellmyr & Huth 1994; Huth & Pellmyr 2000) and fig-fig-wasp mutualisms (personal observations); or when the plant can selectively abort the fruits that are only galled but not pollinated (Huth & Pellmyr 2000). The abortion mechanism might be important in preventing cheating individuals from invading the reciprocal mutualism. The direct conflict between the fitness of recipient and cooperative actors, however, can also result from an increase in co-operative actors, as analysed for the fig-fig-wasp mutualism. The abortion rate cannot increase as a function of the density of co-operative actor density, and therefore is not likely to play a role in preventing the conflict resulting from augmentation of the co-operative actors.

Spatial constraint, which is closely related to self-restraint, is considered to be another important factor that can prevent competition between co-operative partners and thereby maintain stable co-operation (Doebeli & Knowlton 1998; Le Galliard, Ferriere & Dieckmann 2003). However, spatial constraint theories meet difficulty in explaining why mutants that can overcome the spatial constraint cannot be copied into the next generation, as competitive individuals that overcome the spatial constraint can increase their fitness advantage when the space is saturated. Instead of promoting the evolution of co-operation, the spatial constraint will often inhibit the evolution of co-operation (competition among altruists will be increased if spatial constraints create local space for the altruists, and the less altruistic individuals will have higher fitness; Hauert & Doebeli 2004). In the fig-fig-wasp mutualism, which lacks a recognition mechanism, the local resource (female flowers) can be saturated with an increased number of foundresses (Nefdt & Compton 1996; Yu et al. 2004), therefore

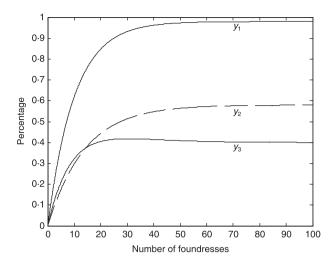


Fig. 3. Theoretical curve of seed percentage without oviposition of pollinators, and theoretical oviposition profile of pollinators and seed percentage after oviposition of pollinators where there are spatial constraints that prevent oviposition in some female flowers.  $y_1$ , theoretical seed percentage without oviposition;  $y_2$  and  $y_3$ , oviposition profile and seed percentage after oviposition by pollinators, respectively, in the case of spatial restraints. Curves are simulated using the spatial constraint model of Doebeli & Knowlton (1998). For an explanation of curve modelling see Appendix S1.

pollinators that can overcome the constraint of female flowers obviously have a fitness advantage in the process of evolution.

In the fig-fig-wasp mutualism, there are several hypotheses suggesting that style length (Ganeshaiah *et al.* 1995), pedicel length (Anstett 2001), or any other structural limitation (possibly including chemical composition) of female fig flowers (West & Herre 1994; Yu *et al.* 2004) can restrain the production of wasp offspring, due to their short ovipositors and other evolutionary constraints. However, quantitative measurements of these spatial constraints show that the suggested mechanisms cannot sufficiently prevent pollinators from utilizing female flowers at the expense of viable seeds (Bronstein 1988; Kathuria *et al.* 1995; Bronstein & Hossaert-McKey 1996; Nefdt & Compton 1996; Yu *et al.* 2004).

If there are any spatial constraints that can prevent pollinators from utilizing viable seeds, wasp offspring should reach an asymptote that will parallel the production of viable seeds (Nefdt & Compton 1996; Fig. 3), and a negative correlation cannot be expected after the female flowers are saturated. Our study, using direct fitness rather than conceptual analysis, shows that the spatial constraints suggested in the previous hypotheses cannot sufficiently prevent competition between figs and fig wasps, and that the negative correlation can directly exist between figs and fig wasps when the local resource is saturated. This is the first direct evidence that the correlation coefficient between recipient and altruist is a function of common space availability or frequency of co-operative individuals, and that conflict will exist in the co-operation system with the augmentation of co-operative individuals or a decrease in the available commons (Taylor 1992; Wilson et al. 1992; West et al. 2002a).

Spatial co-operation models have shown that co-operation can evolve only in cases where space can be kept unsaturated; otherwise the co-operation will go to extinction, which is also implied in our data (Taylor 1992; Wilson 1992; Mitteldorf & Wilson 2000; Le Galliard et al. 2003). In the fig-fig-wasp mutualism, the spatial constraints of female flowers, reemergence of foundresses and self-restraint are unlikely to play a role in keeping female flowers unsaturated. Our data suggest that repression or policing of co-operative individual (symbiont) augmentation in cases where space or other commons are limited will be critical for the evolution and maintenance of co-operation; otherwise the co-operation will go to extinction because of the competition resulting from augmentation of the co-operative individuals. These results indicate that not only is policing or repression of cheating required in the evolution and maintenance of co-operation (Clutton-Brock & Parker 1995; Frank 1995, 1996, 2003; Clutton-Brock 2002; West et al. 2002b; Jousselin et al. 2003), but policing or repression of co-operative individual augmentation is also required. In fig-fig-wasp mutualism, active regulation of the number of foundresses in fruit cavities has been observed (Khadari et al. 1995b; Anstett, Kjellberg, & Bronstein 1996), and figs can repress an increase in foundress numbers to prevent too many pollinators from entering fruit cavities (Wang et al. 2005b; unpublished data). The repression of augmentation of foundresses is critical in co-operation maintenance between figs and fig wasps.

It is worth emphasizing that it is essentially the limited local resources, rather than symbiont (actor) abundance, that lead to competition between co-operative individuals, although the effect of actor (altruist or symbiont) abundance could be treated as equal to local resource availability in theoretical models (Taylor 1992). However, whether the local resource can be saturated will be affected by the total quantity of the local resource and how many resources have already been utilized (Herre & West 1997; Avilès 1999; Pepper & Smuts 2002). Utilization of the local resource will be strongly affected by the utilization effectiveness of each individual, which could be greatly affected by environmental or ecological factors, not just by the abundance of involved individuals.

Figure 2 and Anstett, Bronstein & Hossaert-McKey (1996b) show that foundress abundance is the main factor in seed production, wasp offspring production and availability of vacant female flowers within the same crop in the fig-fig-wasp mutualism. However, more foundresses in the fruit cavities will not definitely result in a negative correlation between wasp offspring and viable seeds, as more foundresses in the fruit cavities cannot maximize utilization of the local resource in many cases. In warm months, even though the number of foundresses per fruit is very high  $(n = 27.1 \pm 11.5)$ , the number of viable seeds is only weakly correlated with wasp offspring (n = 15, r = -0.2, P > 0.05), and the proportion of vacant female flowers remains high  $(44.1 \pm 13.6\%)$ . Interference between foundresses and their short life span might prevent oviposition and pollen dispersion in the warm months, therefore the female flowers cannot be saturated (Yu et al. 2004; unpublished data). However, in cold months, because of the

longer lifespan of the foundresses, the utilization of female flowers can be maximized, and a negative correlation will exist between viable seeds and wasp offspring with increasing numbers of foundresses (Wang et al. 2005b). Whether reciprocal mutualists co-operate or conflict depends on local resource availability, and the interactions are strongly affected by environmental and ecological factors.

An argument exists about whether or not conflict can exist directly in fig-fig-wasp mutualism. In classical theoretical correlation analysis of evolution of co-operation, the correlation coefficient was treated as an average value (Hamilton 1970; Price 1970; Frank 1994), which assumed that the correlation coefficient was independent of local resource availability or other non-genetic factors (Queller 1992a). The correlation analysis of Bronstein (1992); Bronstein & Hossaert-McKey (1996) on the fig-fig-wasp mutualism did not consider that local resource availability might affect the correlation coefficient between viable seeds and wasp offspring, therefore the correlation coefficient should be averaged, similarly to the classical model of the evolution of co-operation (Hamilton 1970; Queller 1992a). The average correlation coefficient between viable seeds and wasp offspring is positive (Bronstein 1992; Bronstein & Hossaert-McKey 1996). This might be because local female flowers are seldom saturated, therefore viable seeds are positively correlated with wasp offspring rather than negatively correlated, as seen in most cases from the rest of the year (Table 1; Wang et al. 2005b; unpublished data). In general, a positive correlation can ensure the spread of co-operative interactions (Hamilton 1970, 1972; Price 1970; Frank 1994), and is a powerful conceptual tool in the understanding the evolution of co-operative interactions (Hamilton 1970; Queller 1992a).

The negative correlation existing between figs and fig wasps in Herre & West's (1997) statistical model basically argued that negative correlation can exist only in cases where female flowers are saturated. Because Herre and West used multivariate linear regression, which held the covariates as constant values, this method was based on a cryptic assumption that female flowers are saturated, when the total number of female flowers and the proportion of developed female flowers were included as covariates. If the total number of female flowers and the proportion of developed female flowers are held as covariates (are held constant), this method essentially holds the total number of viable seeds and wasp offspring as constant, which will definitely lead to a negative correlation between viable seeds and wasp offspring. The correlation method of Herre and West is essentially a regression at the point where the total number of viable seeds and wasp offspring is a constant value, and assumes that unutilized (vacant) female flowers cannot either develop viable seed or be utilized by wasp offspring. In the fig-fig-wasp mutualism, all the female flowers can either develop viable seeds or be utilized by the wasp offspring, and the development of viable seeds and wasp offspring will be at the expense of vacant female flowers if vacant female flowers are available. Therefore only when the female flowers are saturated can the total number of viable seeds and wasp offspring be held constant (Appendix S2).

In the fig-fig-wasp mutualism, the total number of female flowers of each fruit is limited, while the fitness of both figs and fig wasps must be at the expense of female flowers, therefore viable seed and wasp offspring production will depend on the availability of female flowers (unutilized female flowers) or foundress frequency. In such a situation, the correlation coefficient between viable seeds and wasp offspring is a function of unutilized female flowers or foundress frequency (Table 1; Fig. 2), which is theoretically demonstrated in previous models of the evolution of co-operation (Taylor 1992; West *et al.* 2002a).

Our analyses suggest that it is necessary to consider the bilateral variation of interactions of the co-operative partners in different situations when we discuss the exact trade-off between co-operative individuals, rather than relying on conceptual definitions of whether whole systems are cooperation or conflict systems. Only when the direct fitness of co-operative individuals is a linear function of the local resource or other uncertain factors will the correlation coefficient between the individuals involved be a constant value (Hamilton 1964, 1972; Queller 1992a). However, if the direct fitness of individuals is not a linear function of local resource availability, such as when a marginal effect exists, the correlation coefficient will be a function of the related factors (Queller 1992b; Taylor 1992; West et al. 2002a; Le Galliard et al. 2003). Therefore the correlation coefficient between individuals will vary with the variation of related factors, which can easily be overlooked in empirical data and theoretical analysis. Our data show that the correlation coefficient will depend on the variation of local resources or foundress frequency. Theoretically, it is also possible that the correlation coefficient between recipients and altruists may vary with other factors, such as space or public service. This study suggests that it is necessary to consider the effects of environmental factors in co-operation theories.

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# Supplementary material

The following supplementary material is available for this article.

Appendix S1. Theoretical curve of the seed and wasp offspring production existing with spatial constraints.

This material is available as part of the online article from http://www.blackwell-synergy.com/doi/full/10.1111/ j.1365-2656.2008.01359.x

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