

Factors influencing populations of the fig pollinator (*Liporrhopalum tentacularis*) and its parasitoid (*Sycoscapter* sp.)

By

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Submitted in accordance with the requirements for the degree of PhD.

**The University of Leeds
Institute of Integrative and Comparative Biology
September 2006**

The candidate confirms that the work submitted is her own and that appropriate credit has been given where reference has been made to the work of others.

Acknowledgements

I would like to thank my supervisor, Dr Steve Compton for introducing me to the world of figs and for his encouragement and time so generously given throughout this study. I am also thankful to Dr Steve Sait my co-supervisor and Dr Yan Wong for their help, as their suggestions and constructive comments lead to substantial improvements in this thesis.

Many sincere thanks are also due to Martin Lappage whose help in the glasshouse was invaluable. Thanks are also extended to my friend Nazia for her moral support. I am also grateful to my family for all the support and love they have given me. But I am very much thankful to the most important person, who always motivated me whenever I was very low my father (be his soul rest in peace) who is no more to see the fruit of his efforts.

Finally, I am grateful to the Ministry of Education, Government of Pakistan and Higher Education Commission Pakistan for financial support during my study at Leeds.

Assistance Provided

I am very thankful to David Pilbeam for his input in the writing of chapter two “The responses of male and female fig trees to variation in soil nutrition and the consequences for pollinators, parasitoids and seed dispersers”. His comments and notes on plant-soil interactions are much appreciated.

I am also indebted to my colleague Nazia Suleman for allowing me to use her data on foundresses that spent different lengths of time in male figs (Chapter four) and for the experiment where varying numbers winged and wingless foundresses were used (Chapter six).

I am also thankful to Yan Wong for doing the analysis for my chapter “Parasitoids do not change the realized sex ratio of a fig wasp that pollinates a dioecious fig tree” (Chapter eight).

Abstract

In this thesis, I investigate factors affecting the ecology and evolution of the dioecious fig *Ficus montana*, its pollinator *Liporrhopalum tentacularis* and the non-pollinating fig wasp *Sycoscapter* sp. *F. montana* grows naturally in soils of varying nutrient quality. Under experimental conditions, with enhanced soil nutrition, both female and male plants responded in the same way, producing more leaves, stems and many more figs, but in female plants the figs were also larger, and produced more seeds. In male plants the size of the figs remained unaltered. Female figs that contained more flowers produced more seeds, but male figs that produced more flowers did not produce more female pollinators. Although the female pollinators laid more eggs in figs with more flowers, the number of female pollinators was differentially reduced by *Sycoscapter* and other factors, cancelling out any male fig size effect.

The timing and frequency of foundress re-emergence from male and female figs was similar. Foundresses first started to re-emerge from figs of both sexes after about one hour and after an over-night halt, keep on emerging through to the next day. Using a novel poisoning technique the rates of oviposition and pollination were found to be rapid when the wasps first entered, but declined rapidly in both male and female figs. The likelihood of foundresses re-emerging from male figs was not influenced by wasp age, flower number, or the timing of entry into the figs, but re-emergence was more frequent from older figs. Foundresses laid most of their male eggs early in an oviposition bout. The responses of winged and wingless foundresses to varying foundress numbers were different, because the wingless foundresses laid smaller clutches that were independent of foundress number because competition for oviposition sites was reduced. Consequently, wingless foundresses did not adjust their sex ratio when the density of foundresses increased in a fig. *Sycoscapter* sp. oviposits two-three weeks after pollinator entry. There was no negative correlation present between the number of pollinators and *Sycoscapter* sp. in the figs, suggesting that the latter might not be a parasitoid. However, in experiments where the numbers of pollinators entering a fig was controlled, *Sycoscapter* sp. significantly reduced the number of pollinators. It is suggested that *Sycoscapter* sp. is a parasitoid, or inquiline. It also did not affect the sex ratio of its host.

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General Introduction

Mutualism, where both participants benefit from a relationship, has been viewed as a rare type of ecological interaction (May, 1973). However, it occurs in a wide variety of taxa from viruses to mammals, and ranges from highly specialized obligate mutualism to facultative interactions. Earlier it was believed that such relationships are the result of altruistic processes where partners are selected to benefit each other (Brown, 1975) but in contrast, current thinking proposes that mutualisms are characterized as reciprocal exploitations that provide net benefits to each of the involved species (Leigh and Rowell, 1995). Obligate mutualisms are those that are essential to the life of one or both associates, such as association between fungi and algae in lichens, where the algae gain the protection of a fungal thallus and fungus derives nutrition from the photosynthetic algae (Herre *et al.*, 1999). Facultative mutualisms are beneficial, but not essential for the survival and reproduction of either party (Keeler, 1981). The root-fouling sponge and the red mangrove are an example. Sponges growing on the roots of mangroves increase the growth of mangrove roots two-to four fold. The sponges increase in growth rates, up to ten times faster than sponges not associated with mangroves. The sponges gain carbon from the mangrove and the mangrove gets dissolved inorganic nitrogen from the sponges (Ellison *et al.*, 1996).

Pollination is a common mutualistic interaction. The fig/fig wasp and yucca/yucca moth pollination mutualisms are examples where the typical flower reward of pollen or nectar are replaced by 'brood places' where insect larvae can develop. In both cases pollinators lays eggs when pollinating, and the larvae develop at the expense of the plant (Weibes, 1979). These mutualisms have led to co-adaptations of life cycles and high levels of specialization.

Fig trees (*Ficus* spp.) are one of the most conspicuous components of tropical and subtropical vegetation. There are approximately 750 species of fig trees. The genus *Ficus* is recognized by its production of a characteristic inflorescence, called a fig or syconium. The fig is in the form of a hollow ball lined on its inner surface with hundreds of tiny flowers (Berg, 1989). Figs of different species are produced from below ground level up to more than fifty metres above the ground in rain forests.

Fig trees are probably the single most important source of food for fruit-feeding vertebrates in tropical and sub tropical regions, with for example around 1000 species of birds recorded as feeding upon them (Shanahan *et al.*, 2001). Various factors contribute to this 'keystone' role, but their commonly all-year-round fruiting pattern (linked to the preservation of their pollinator populations) may be particularly significant (Gautier and Michaloud, 1989).

Another important characteristic feature of this genus is their mutualistic relationship with wasp species of the family Agaonidae (Chalcidoidea: Hymenoptera) (Hill, 1967; Janzen, 1979; Weibes, 1979). With few exceptions (Machado *et al.*, 1996) most *Ficus* species rely on species specific wasps for pollination and oviposition. Figs and agaonid wasps are totally dependent on one another for sexual reproduction. The wasps pollinate the flowers while the figs provide them with their flowers for feeding sites for wasp larval development (Ramirez, 1974, Weibes, 1979). An adult female wasp (called a foundress) locates a receptive fig and enters through the 'entrance gate' ostiole, often losing her wings as she squeezes inside (Kathuria *et al.*, 1999; Bronstein *et al.*, 1989). Usually the foundress pollinates and oviposits, then dies inside the fig, but in some species she can leave a fig and enter another (Gibernau *et al.*, 1996; Moore *et al.*, 2003a). The wasp progeny develop and then emerge from galled ovules. Mating

occurs inside the fig, wingless males chew an exit hole and the females leave to seek out receptive figs (Kerdelhue, 2000).

The short-lived adult female pollinating wasps have been shown to disperse over large distances, sometimes using winds above the canopy of forests (Compton *et al.*, 2000). It is hypothesized that this behaviour has been selected for because of the ephemeral nature of receptive figs, which are on trees that are often highly dispersed (Nefdt and Compton, 1996).

Each species of fig is also host to a number of non-pollinating wasps and according to one study by Compton and Hawkins (1992), up to 29 species have been recorded from one tree. They include gallers and parasitoids. Most non-pollinating wasp species do not enter the fig fruit to oviposit, but they use their long ovipositors to penetrate the fig wall from outside. The morphologies and mating strategies of non-pollinators are quite variable and very little knowledge is available of their impact on the mutualism (Hamilton, 1979; Bronstein, 1991; Compton and Hawkins, 1992; West and Herre, 1994). Some of these species have wingless males, some have winged males and some have both winged and wingless males (Hamilton, 1979; Cook *et al.*, 1997). Such male dimorphism has repercussions for their sex ratios and sexual behaviour (Cook *et al.*, 1997). Non-pollinating wasps are likely to affect the mutualism between pollinating wasps and fig plants. Although it is a rare case, Patel (1998) found a dioecious fig species where 90% of the figs contained dead pollinating and non-pollinating wasps that had been trapped inside, presumably because too many non-pollinating wasps had reduced the number of pollinator males to the extent that none survived to chew an exit hole. The effects of the non-pollinating wasps are likely to vary as they can negatively

affect pollinators and seed production, as well as the population of other non-pollinators.

Figs fall into two functional/reproductive categories, monoecious and dioecious, although the later are anatomically gynodioecious (Bronstein, 1988). Monoecious figs have one type of tree where each fig has male and female flowers. The male flowers produce pollen and the female flowers develop into seeds or become galled by wasps. Male flowers develop later, the female at the same rate as the wasp progeny, so that female wasps are supplied with pollen when they emerge to transfer to another fig (Kerdelhue *et al.*, 2000). In contrast, dioecious fig trees have both male and female trees. Female figs have only female flowers with styles that are of a specific shape and size that prevents the wasps from laying eggs, thus a wasp entering a female fig will most likely die without reproduction as she loses her wings on entry. The male trees are hermaphrodite with figs that have both male and female flowers. The female flowers of the male fig are used almost exclusively for wasp oviposition and development and only very rarely produce seeds (Patel, 1998).

Because the early stages of the life cycle of fig wasps take place in a closed environment, with often known numbers of foundresses present, it is easy to study their sex ratios. If a fig has only one foundress, mating takes place between siblings and brothers are competing against each other for mates (Greeff, 1997). This is called Local Mate Competition. Female wasps can control the gender of the eggs they are laying (Kinoshita *et al.*, 1998). A single foundress is expected to lay mostly female eggs and just enough males to mate with all the females. When a second female enters a fig, her sons will have to compete with the first female's sons, therefore she should lay eggs with a higher male biased sex ratio than the first female did (Kathuria, 1999).

1.1. Study species

Ficus montana is a small pioneer shrub found along rivers or in disturbed forest in lower Myanmar, the Malaya Peninsula, Sumatra, Java and Borneo and is placed in subgenus *Sycidium* section *Sycidium* by Berg and Corner (2005) (Fig 1.1). This species is functionally dioecious with male trees bearing figs having both male and female flowers, but producing only pollen and pollinators and with female trees bearing figs containing only female flowers that produce seeds. Like many other dioecious figs, this species has asynchronous fruiting within plants (Moore, 2001).

The development stages of figs are generally subdivided according to the terminology devised by Galil and Eisikowitch (1968) (Fig 1.2), for monoecious species and modified by Valdeyron and Lloyd (1979) for dioecious figs. The stage before the wasp's entry is called the A or "pre-receptive" phase and this phase may take some three weeks. In the "receptive" or B phase the wasps enter the fig through the ostiole. This phase may last for 3-6 days and if no wasps enter the fruit, it may extend longer. The subsequent phase is the C or "inter-floral" phase, in which the wasps develop in the male fruits and seeds develop in the female figs. The C phase can last from two to six or up to ten weeks, depending on the species. In male figs at the D or "emission" phase the next generation of wasps emerges, the anthers open and the female wasps leave the figs, loaded with pollen. This phase may take 3-4 days (Galil and Eiskowitch, 1968; Ramirez, 1974). Female fig plants lack D phase and have only one extended post receptive phase E, during which the seeds and fig mature, become soft and fleshy and ready to be eaten. In male figs the last phase is E or "post-floral" phase, when the wasps have already left and the fig withers before abscission. In some species it is reported that male figs develop and ripen in two-thirds the time required by female figs (Corner, 1952).

The pollinator of *F. montana* is *Liporrhopalum tentacularis* Grandi (Fig 1.3). Pollinating females of *L. tentacularis* lose their wings and most of their antennae upon entry into a receptive fig and have pollen pockets on their thorax. The males are apterous. The mating of adults takes place only inside the galls of the females. Foundresses of *L. tentacularis* have been shown to routinely re-emerge from the figs after oviposition and successful attempts to enter figs within walking distance have been reported (Moore *et al.*, 2003a). It was also shown that *L. tentacularis* females are unable to discriminate between the sexes of *F. montana*, probably because of selection for inter-sexual fig mimicry (Moore *et al.*, 2003b). Zavonda *et al.* (2005a), showed by using micro-satellite markers and by observing the behaviour of *L. tentacularis* wasps that single paternity clutches are the norm in this species (females usually mate only with one male). By using micro-satellite markers it was also shown that more offspring of *L. tentacularis* foundresses developed in the central part of the fig, as compared to the ostiolar and basal parts, irrespective of foundress number. The sons of second foundresses were positioned at similar minimum distances to both sibling and non-sibling females, whereas the sons of the first foundress were closer to their sibling females than to non-sibling females (Zavonda *et al.*, 2005b). Moore *et al.* (2005), used maternal analysis involving micro-satellite markers to show that *L. tentacularis* foundresses contribute equally to multifoundress broods and that their sex ratios are determined by their clutch sizes, but not foundress numbers per se.

The most common and widespread non-pollinating fig wasps species associated with *F. montana* is a *Sycosapter* species (Sycorytinae:Agaonidae) (S. Compton, Pers. Comm.) (Fig 1.4). It is an undescribed species (Rasplus JY Pers. Comm). The winged female wasp oviposits externally through the walls of the fig fruit. Males are also

apterous and like the pollinators mate inside the galls of the females. Studies of other *Sycoscapter* spp. indicate that they are probably parasites of pollinator fig wasps (Cook and Power, 1996; Kerdelhue and Rasplus, 1996).

1.2. Study site

The plant and insect populations were maintained in a glasshouse at the Experimental Gardens of the University of Leeds, which represents the only example of an artificial fig tree- fig wasp system in the world today. The plants originated from seed collected at CIFOR HQ, Bogor, Indonesia and from the Krakatau islands, Indonesia in 1995 (Moore *et al.*, 2002). The insects were collected from the same locations the following year. Most of the work done so far on fig plants has been in their natural habitats. Under natural conditions male and female plants may be growing under slightly different environmental conditions. Laboratory studies also help to control conditions, as it is possible to design to eliminate, or at least minimize, effects from unwanted influences. Laboratory studies have advantages with regard to design, setting, and measurement, the advantages primarily relate to precision, control, internal validity and the environmental factors that can be eliminated or controlled.

1.3. Aims and outline of the thesis

An extensive body of literature is present on many aspects of fig trees and their pollinator wasps (pollinator attraction, mode of pollination, sex ratio evolution and sexual selection), but considerable variation exists in their ecology and evolution. Most of the work done has been on monoecious species of fig trees and relatively little is known about dioecious species. In this thesis I mainly use an experimental approach to study different factors which affect the biology of the dioecious *F. montana* and its

pollinator *L. tentacularis* and their interaction with the non pollinator fig wasp *Sycoscapter* sp.

Soil nutrients influence plant growth, and herbivores in turn often respond to changes in host plant size and resource quality, as may their associated natural enemies. In nature *F. montana* grows on both fertile volcanic and nutrient-poor limestone soils (Corner, 1952; S. Compton, Pers. Comm). I utilized the laboratory culture of fig trees and fig wasps to compare the vegetative and reproductive responses of male and female plants of *F. montana* exposed to contrasting levels of soil nutrients and in chapter two we describe the consequences of nutrient manipulation for the reproductive success of male and female plants and how it influences the value of female figs to vertebrate seed dispersers and of male figs to their pollinators and their parasitoids

One aspect of plant responses to nutrition is inflorescence size (the numbers of flowers present). In chapter three I compare the relationship between inflorescence size and reproductive success in male and female figs of *F. montana* in the presence of the tree's pollinator and its main parasitoid. I determine whether fitness gains associated with larger inflorescence sizes were present in both female and male figs, and examine the causes of any sexual differences in response.

Until recently it has been assumed that fig wasp foundresses rarely re-emerge after entering into a fig. *L. tentacularis* foundresses, in contrast, routinely re-emerge (Moore *et al.*, 2003 a). The behaviour of this species can therefore be more flexible than has previously been assumed for fig wasps in general. Why do foundresses that are capable of re-emergence choose not to do so immediately, once they discover that

they are in female figs? To examine this question the fourth chapter compares the frequency and timing of re-emergence of *L. tentacularis* foundresses from male and female figs, and, as a measure of how active they are inside the figs, we measured the rates at which they pollinate or oviposit during different time intervals after they enter.

In the fifth chapter we investigate the re-emergence behaviour of foundresses from male figs of *F. montana*. We determine the factors that influence re-emergence, including female flower number, fig and wasp age and the entry of a second pollinator.

In the sixth chapter we directly recorded the oviposition sequence of *L. tentacularis* foundresses by terminating oviposition after varying lengths of time by injecting poison through the ostioles. We also tested whether sex ratio adjustment is generated by a combination of laying male eggs first and oviposition site limitation, as has been hypothesised, by comparing the responses to foundress numbers of females entering their first fig (winged foundresses, with larger egg loads) and entering their second figs (wingless foundresses, with fewer eggs to lay).

Non-pollinating wasps probably have extremely diverse trophic relationships (gallers, kleptoparasites, inquilines and parasitoids) but little is known about the details of their biology and oviposition sites. In chapter seven we examined the biology of *Sycoscapter* sp. specifically, we observed the timing of oviposition of *Sycoscapter* sp. We also examined its impact on the population of pollinating wasps, in an attempt to confirm whether or not it is a galler or parasitoid.

Parasitoids are a very important component of fig wasp communities and have been shown to be capable of changing the sex ratios of their hosts (Periera and Prado, 2005).

In chapter eight, we examine the effect of *Sycoscapter* sp. on the realized (secondary) sex ratio of *L. tentacularis*. We compare pollinator sex ratios in the presence and absence of parasitoids in two situations where we also varied putative primary sex ratios by varying the number of foundresses contributing to combined broods and by varying the length of time that a pollinator had available to oviposit.

Finally, in chapter nine we briefly summarize the key findings of the study and then discuss their implications for the ecology and evolution of this mutualism.



Fig. 1.1 University of Leeds *Ficus montana* and *Liporrhopalum tentacularis* populations.

Fig. 1.2 Developmental stages of male *Ficus montana*.

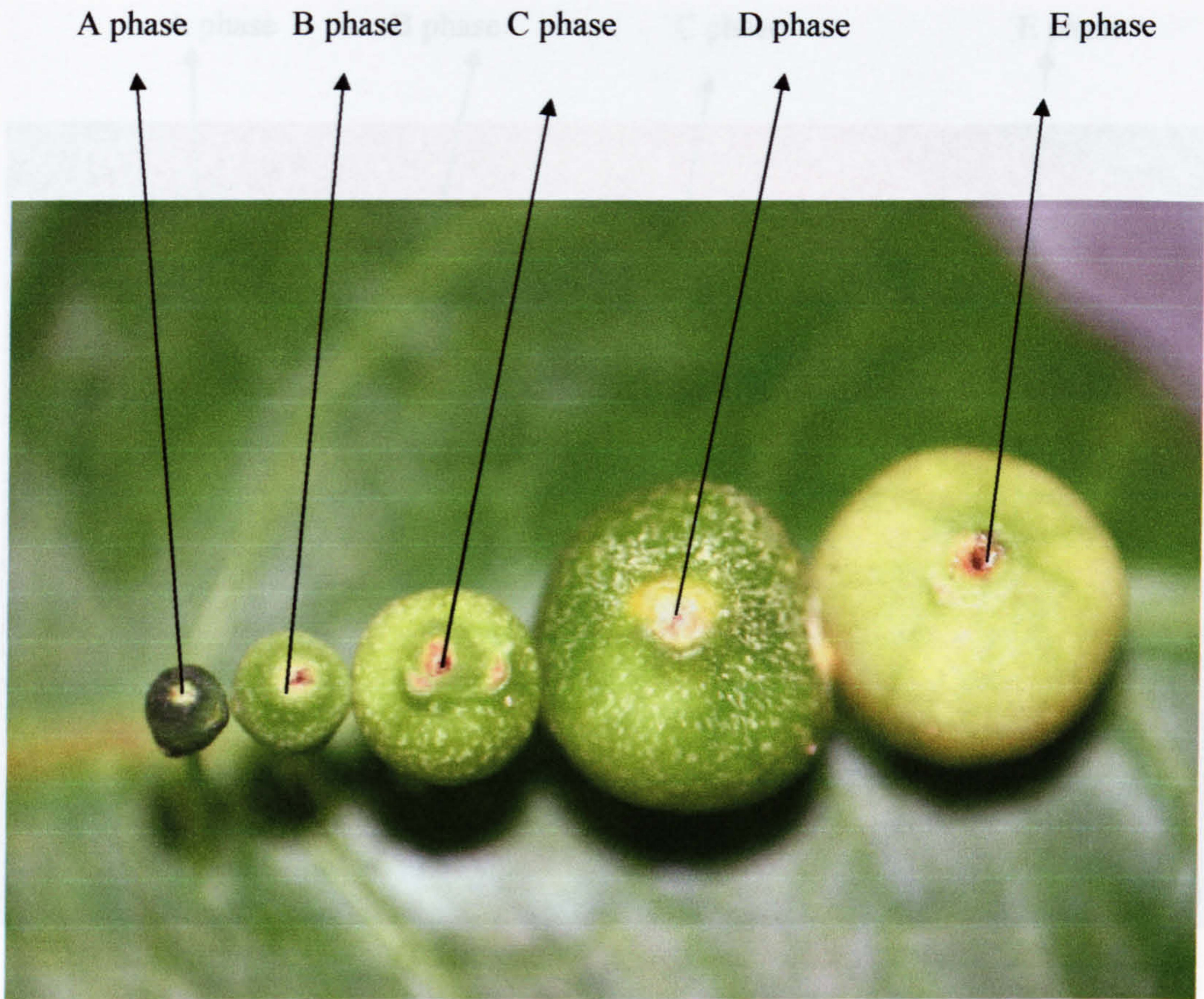


Fig. 1.3 Developmental stages of female *Ficus* species

Fig. 1.2 Developmental stages of male *Ficus montana*.

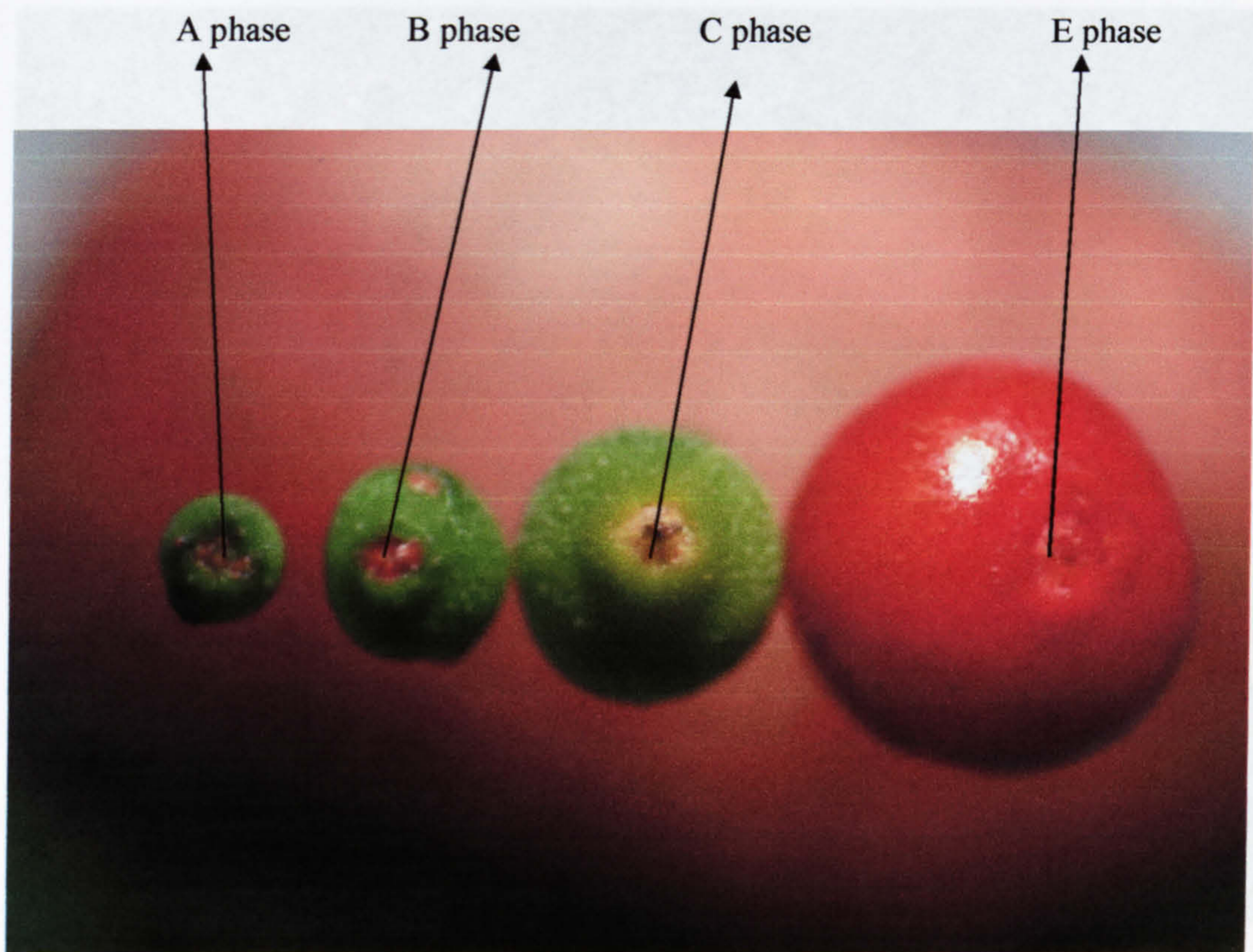


Fig. 1.4 Female *Levinsia* *montana* entering into a receptive male *Ficus montana*
Fig. 1.3 Developmental stages of female *Ficus montana*



Fig. 1.4 Female *Liporrhopalum tentacularis* entering into a receptive male *Ficus montana* fig.

2. The responses of male and female fig trees to variation in soil nutrition and the consequences for pollinators, parasitoids and seed dispersers



Fig. 1.5 The *Sycoscapter* sp. Adult female is trying to oviposit from outside the fig wall.

2. The responses of male and female fig trees to variation in soil nutrition and the consequences for pollinators, parasitoids and seed dispersers

2.1. Abstract

Soil nutrients influence plant growth, and herbivores feeding on them often respond to related changes in host plant size and resource quality, as may their associated natural enemies. We utilized a laboratory culture of fig trees *Ficus montana*, its pollinator fig wasp *Liporrhopalum tentacularis* and the parasitoid *Sycoscapter* sp. indesc. to compare the vegetative and reproductive responses of male and female plants of a functionally dioecious fig tree exposed to contrasting levels of soil nutrients. We describe the consequences of nutrient manipulation for the reproductive success of male and female plants and how nutrient enhancement influences the value of female figs to vertebrate seed dispersers and of male figs to its pollinators and their parasitoids. We found that:

1. In terms of vegetative growth, male and female *F. montana* responded in similar ways to supplemental nutrients.
2. With enhanced nutrition, both female and male plants produced many more figs, but whereas in female plants the figs were also larger, with more flowers, in male plants the size of the figs was unaltered.
3. High nutrition female *F. montana* plants produced figs that contained more seeds, but the seeds were smaller in size.
4. There was no corresponding increase in total pollinator progeny numbers from figs on high nutrition male plants, due largely to the effects of the parasitoids.

These results illustrate the complex relationship between plant nutrition and reproductive success in fig trees and show that in dioecious species the sexes may differ in their responses to soil conditions.

2.2. Introduction

Soil nutrients limit plant growth and reproduction in most natural and artificial environments (Mengel and Kirkby, 2001). Consequently, they also have a profound influence on the growth and reproduction of the plant-feeding animals that depend upon them, and their natural enemies (Forkner and Hunter, 2000; Awmack and Leather, 2002). In turn, the complex responses taking place at these higher trophic levels mediate the extent to which plants can benefit from improved soil nutrition (Awmack and Leather, 2002). Plants growing in better quality soils can be more suitable for particular herbivores (with rapid plant growth associated with higher water and nitrogen content and reduced toughness), or less suitable if the plants are able to invest more heavily in defenses (Scriber, 1984; White, 1984; Matson, 1987; and Janson *et al.*, 1991). Herbivores respond to both resource quality and quantity (Root, 1973; Stiling and Moon, 2005) and increased growth can also influence herbivory rates by making plants easier to find by herbivores, and their associated natural enemies (Faeth, 1985; Hunter and Schultz, 1995).

Increased vegetative growth in response to good quality soils does not necessarily lead to increased production of flowers and seeds, and supplementary nutrition can even favour vegetative growth while depressing sexual reproduction, at least in the short term (Muñoz *et al.*, 2005). Plants nonetheless often show spectacular increases in female reproductive output in response to nutrient enhancement. Campbell and Halama (1993), for example, found that fertilizing *Ipomopsis aggregata* (Polemoniaceae)

increased the number of flowers and seeds by 89% and 30% respectively. Bateman's principle suggests that male reproductive success via pollen donation may be less responsive to nutritional effects, but will still occur (Burd, 1994; De Jong and Klinkhamer, 1994).

In dioecious plants, females generally allocate more resources to reproduction than males, because seeds and fruits require larger inputs than pollen (Lloyd and Webb, 1977; Cipollini and Stiles, 1991; Nicotra, 1999; Obeso, 2002; Pickering and Arthur, 2003). As reproduction in female plants is considered to be more prone to nutrient limitation, they should show more frequent and greater responsiveness to variation in nutrition than male plants (Gehring and Linhart, 1993). Ecological consequences of this difference have frequently been reported (Dawson and Ehleringer, 1993), but are inconsistent between species. They include examples of slower growth (Boecklen *et al.*, 1990) and lower survival in female plants (Lloyd, 1974), along with a reduced ability to compensate for herbivory (Turcotte and Houle, 2001). Female plants can also be more prone to disease (Ahman, 1997), but be more resistant to herbivores (Boecklen *et al.*, 1994; Elmqvist and Gardfjell, 1988), reflecting sexual differences in defensive investment (Coley *et al.*, 1985).

Supplementary plant nutrition can influence the size, abundance and sex ratios of the insects that feed on them (Mopper and Whitham, 1992; Awmack and Leather, 2002). It can also influence their parasitoids and other natural enemies, through preferential searching of larger plants or leaves (Loader and Damman, 1991; Stiling and Moon, 2005) or aggregation on plants where their host populations are more abundant (Virtanen *et al.*, 1999; Forkner and Hunter, 2000; Awmack and Leather, 2002; Strengbom *et al.*, 2005). Such top-down effects can negate any benefits for plant-

feeding insects with potential benefits for their host plants. Parasitoid fecundity can also be influenced by plant and insect host quality (Kaneshiro and Johnson, 1996), as can their sex ratios because larger hosts are often favoured for female progeny (Strand, 1988; Fox *et al.*, 1990; Schulthess *et al.*, 1997).

Most insects that act as pollinators do so as an accidental by-product of their foraging for pollen, nectar and other floral rewards. Similarly, seed dispersal in those plants that produce fleshy fruits is typically achieved as a by-product of dispersers that accidentally consume the seeds. Where soil nutrient enhancement leads to more or larger flowers and/or more rewards per flower, pollinator attraction is likely to be enhanced, and they can also potentially gain through improved foraging efficiency (Levin and Kerster, 1969). Greater apparency and fruit quality may also make fruits more attractive to birds and other dispersal agents, and potentially offer similar benefits to them, at least in situations where seeds are limiting.

Some specialized plant-pollinator systems are atypical in that the insects often actively pollinate their hosts, then produce larvae that destroy some of the seeds they have produced. Their responses to host plant quality may therefore be closer to those of herbivores than to more usual pollinators. The obligate mutualism between fig trees (*Ficus* spp., Moraceae) and their associated pollinating wasps (Hymenoptera, Chalcidoidea, Agaonidae) is one of the most extensively studied of plant-animal interactions (Janzen, 1979; Wiebes, 1979). The 'brood place' pollination systems of fig trees results in atypical relative costs of male and female reproduction in these plants because male reproductive function depends on both pollen production and the commitment of resources to allow the feeding and successful development of pollinator larvae. Consequently, the costs associated with male reproductive effort are

likely to be much more than those associated with more typical pollination systems involving alternative rewards such as nectar.

Over 750 species of fig trees have been described, mostly from the tropics and subtropics, and each species has one (or sometimes more, Machado *et al.*, 1996) species of pollinating fig wasp, associated with that one species of tree. Fig trees have a distinctive and unique inflorescence (the fig) that dictates the nature of the relationship with their pollinators. It is formed like a hollow ball, lined on the inside with many tiny flowers. Because each fig is protogynous, pollination can only be achieved by pollen that is carried into each fig, via a narrow bract-lined slit, called the ostiole. Adult female fig wasps (foundresses) seek out and enter figs in order to lay their eggs in the ovules there. Pollination and galling of the ovules occurs at the same time. Foundresses usually lose their wings and part of their antennae on entry through the ostiole, and are often unable to re-emerge again. Consequently they often pollinate only one fig, where all their eggs are laid. Mating takes place within natal figs, and as only one or a small number of foundresses will have laid their eggs there, this leads to frequent mating between siblings and inbreeding. Foundresses respond to this 'local mate competition' (Hamilton, 1979) by adjusting the sex ratios of their progeny and producing progressively less female-biased sex ratios as the number of foundresses increases (Moore *et al.*, 2002). In some species at least this is because their clutch sizes decline due to competition for oviposition sites and most eggs laid later in a sequence are females (Moore *et al.*, 2005; S. Raja in prep.). As only female fig wasp progeny can eventually transport pollen, sex ratio adjustment has the effect for the plant of increasing the cost of male reproduction in figs as foundress numbers increase.

Around half the species of fig trees are monoecious and half are functionally dioecious (though anatomically gynodioecious). Monoecious species have one type of tree where each fig has male and female flowers (Verkerke, 1989). The male flowers produce pollen and the female flowers develop into seeds or become galled by the fig wasps. When the adult female wasps emerge they actively or passively collect pollen which is then transferred once they successfully reach another fig that is at an earlier stage of development. Dioecious species have distinct male and female trees (Verkerke, 1989). Female trees have figs that prevent any pollinators that enter from laying eggs, so they only produce seeds. In contrast, male figs permit oviposition, but are typically unable to produce any seeds, only (pollen-carrying) fig wasps. Foundress re-emergence, from both male and female figs, appears to be more widespread amongst pollinators of dioecious than monoecious figs (Moore *et al.*, 2003a). When female figs (and monoecious figs) ripen, latex is withdrawn, the fig wall is sweetened and the color and smell change to make them more attractive to vertebrate seed dispersers. Male figs eventually shrivel and fall off several days after the pollinators have mated and left, allowing time for some resources to be recovered by this sex (Ashman, 1994).

Each species of fig tree is also host to a number of non-pollinating fig wasps, most of which lay their eggs into the ovules from the outside of the figs (Compton and Hawkins, 1992). Their biology is diverse and relatively poorly known, but they include ovule-gallers, inquilines and parasitoids (Bronstein, 1991; Compton and van Noort, 1992; West and Herre, 1994). More species of non-pollinators tend to be associated with monoecious than dioecious host plants, with very few associated with female figs.

Fig trees are probably the single most important source of food for fruit-feeding vertebrates in tropical and sub tropical regions, with for example around 1000 species

of birds recorded as feeding upon them (Shanahan *et al.*, 2001). Various factors contribute to this 'keystone' role, but their commonly all-year-round fruiting pattern (linked to the preservation of their pollinator populations) may be particularly significant. In dioecious species it is the seed-containing figs of female plants that become attractive to vertebrates, although occasional feeding on male figs has been observed.

Here we utilize a laboratory culture of fig trees and fig wasps to compare the vegetative and reproductive responses of male and female plants of a dioecious fig tree exposed to contrasting levels of soil nutrients. We describe the consequences of nutrient manipulation for the reproductive success of male and female plants and how it influences the value of female figs to vertebrate seed dispersers and of male figs to its pollinators and their parasitoids.

2.3. Natural History

F. montana Burm.f is a functionally dioecious species of fig tree, which despite its name is found in lowland forest edges, disturbed areas and similar habitats in South East Asia (Corner, 1952). It has a shrubby growth form, rarely reaching two metres in height under natural conditions, and through vegetative spread it can form loose clumps 10 m or more in diameter. Male figs are yellow to green in colour throughout their development while female figs are deep red when mature. Mature figs of both sexes are 8-12 mm in diameter. The figs are borne in the leaf axils and also on older wood, arising directly from the stems. Fruiting within plants is typically asynchronous, with all stages of fig development often present on the same plant.

The pollinator of *F. montana* is the diurnally active fig wasp *Liporrhopalum tentacularis* Grandi. Adult female *L. tentacularis* that enter figs (foundresses) lose their wings and most of their antennae while passing through the ostiole, but are nonetheless capable of re-emerging. Consequently they can pollinate and lay their eggs in several figs on one plant. One larva develops in each galled ovule where successful oviposition takes place (on male plants only). The sex ratios amongst their progeny vary according to how many eggs they lay, which is influenced by how many other females share the figs with them (Moore *et al.*, 2002). *Sycoscapter* sp. is an undescribed solitary non-pollinating fig wasp that oviposits into male figs from the outside. It destroys the larvae of *L. tentacularis* (S. Raja in prep.), but may also feed on the remaining plant material inside the ovules. In the plant's natural range it is the most common non-pollinator fig wasp associated with *F. montana*, and in many crops is the only non-pollinator present.

2.4. Methods

2.4.1. Glasshouse conditions

The plant and insect populations were maintained in a glasshouse at the Experimental Gardens of the University of Leeds. The plants originated from seed collected at CIFOR HQ, Bogor, Indonesia and from the Krakatau islands, Indonesia in 1995 (Moore, 2001). The insects were collected from the same locations the following year.

Twenty male and twenty female plants were selected, from each of which six cuttings (100 mm long woody shoots with heels) were taken in November 2003. The cuttings were dipped in hormone rooting powder (50:50 mix of 0.1 % IBA and 0.4 % NAA +

2.1 % Captan fungicide), inserted into expanded jiffy No 7 peat pellets and then placed in propagating trays over benches heated with soil warming cables.

After establishment they were shifted to 15 cm pots and then finally into 20 cm pots in March 2004. A month later one pair of clones from each plant was selected, based on their similar size and vigour. This provided 20 male and 20 female experimental plants, each represented by two clones. Contrasting nutrient treatments were started at the end of April 2004. Each pot was provided with 45 cm³ of liquid fertilizer (Peter's Excel water-soluble fertilizer in crystalline form) every week. The high nutrient ('H') group received 1.4 g/litre (N = 0.01134 g, P = 0.0063 g and K = 0.01134 g) and the low nutrient group ('L') received 0.28 g/litre (N = 0.00227 g, P = 0.00126 g and K = 0.0027 g). To allow for nutrient distribution within the soil, for different rates of absorption due to different moisture levels of soils, and for variation in root numbers, saucers were placed under the pots so that no feed would be lost as runoff. The pots were placed into equally spaced rows with alternate high and low nutrient plants. The pots were rotated fortnightly to minimize any long-term variation in light levels. The experiment was started on 23rd April and completed on 18th October. The day length at the start was 13 hrs and at the end of experiment was 10 hrs and 20 minutes. The mean maximum temperature for the experimental period was 28.8 °C and mean minimum temperature was 19.8 °C.

2.4.2. Plant vegetative and reproductive characters

Half of each population of 40 male (20 H: 20 L) plants and 40 female (20 H: 20 L) were selected at random at monthly intervals. On each of the 40 sampled plants the numbers of young, mature and unhealthy or senescing leaves were counted. The

number of stems present was also recorded, and the lengths of the main and any additional stems were measured.

The numbers of figs on the plants were also counted and divided into the developmental phases A+B, C, D and E as recognized by Galil and Eisikowitch (1968). Pollinators enter in phase B, with pollinator emergence at phase D. Female figs lack a D phase.

Twenty mature (E phase) figs from female plants (10 H and 10 L) and 20 mature (D phase) figs from male plants (10 H and 10 L) were also collected each month. Normally one fig was collected from each plant, but up to three figs were collected from the same plant when they were in short supply. Female figs were dissected under a binocular microscope to give counts of total seeds and un-pollinated flowers. Male figs were placed in plastic containers covered tightly with a fine mesh. They were left for 24 hrs to let the wasps emerge naturally for counting, sexing and identification. The figs were then split open with fine forceps and any wasps remaining within the lumen were also counted, along with adult insects still present in their galled ovules. Along with ovules with wasp exit holes, the remaining female flowers were scored as either 'bladders' (developed galls that had not produced wasps) or un-pollinated. The numbers of male flowers were also counted.

The weights of female figs were examined in October 2004, after the plants had been provided with high or low nutrition for six months. Three mature figs were chosen at random from H and L clones of ten plants (60 figs in total). Their fresh weights were recorded, and the seeds were then counted, dried at room temperature and weighed. The difference in weights between whole figs and the seeds was taken as the pulp

weight and the seed burden (sensu Snow and Snow 1988) taken as the seed weight expressed as a percentage of fig weight.

The seeds selected for weighing were further used for a germination trial. For this, 10 seeds from each fig were placed on filter papers, in petri dishes in the glasshouse. The filter papers were regularly moistened, the seeds were observed daily and any germinated seeds were taken out of the petri dishes. The experiment was stopped on day 25, by which time most seeds had germinated. The mean maximum and mean minimum temperatures during this experiment were 26.1⁰C and 20.5⁰C. Single figs from H and L clones of three different male plants were selected. The wasps present were reared as above, and then dried in an oven at 40⁰C for 30 hrs before being weighed individually.

2.4.3. Seed and wasp production in figs with a single foundress

After nutritional treatments had been continuing for six months, single foundresses, allowed to emerge naturally from their figs, were introduced into the ostioles of B phase (receptive) male and female figs with a moist paint brush and the figs were then bagged to prevent entry of further foundresses. Two groups of ten plants were selected (10H, 10L) and two figs from each plant. After three days the bags were removed from female figs and half the male figs. The bags on the remaining male figs prevented oviposition by the parasitoid. When the experimental figs reached maturity (after five to seven weeks), they were dissected to reveal their contents.

2.5. Results

2.5.1. Vegetative growth

Female plants grew rapidly during the six month trial period, resulting in significant variation between months in all the vegetative characters other than the number of senescing leaves and the length of the main stems (Table 1, Figs 2.1a-2.1c, N = 120 plants). Plants with supplementary nutrition (H plants) supported almost 50 % more leaves across this period and their total stem length was about 50% greater than that of the L nutrition plants, but they did not differ in the length of their main stems, nor in their number of stems (Table 2.1, Figs 2.1a-2.1c).

Male plants showed broadly the same vegetative responses to supplementary nutrition as female plants (Table 2.2, Figs 2.1d- 2.1f, N = 120 plants), although in this sex the number of stems and main stem length were also significantly greater in H plants.

2.5.2. Reproductive output – female plants

Female plants showed a clear and rapid elevation in reproductive effort in response to supplementary nutrition. Within two months H plants were producing more figs than L plants and at six months they were producing around five times as many (Table 2.1, Fig 2.2a). H female plants did not only produce more figs. From the first month of the experiment, their figs generally contained a significantly greater total number of flowers, and usually produced a greater numbers of seeds (Table 2.3, Figs 2.3a and 2.4a, N = 120 plants). The ‘efficiency’ of pollination (the proportion of flowers that produced seeds) was nonetheless almost identical across treatments (77.5 % v 77.7 %),

resulting in H plants also producing figs with greater numbers of flowers that remained un-pollinated.

Despite the considerably greater numbers of seeds present in figs produced by H female plants, the wet weight of their mature figs (as measured at the end of the experiment) was only marginally higher than those of L plants (ANOVA, $F(1, 59) = 4.028$, $P = 0.049$) (Mean \pm SE = $0.519 \text{ g} \pm 0.213$ and = $0.460 \text{ g} \pm 0.202$ respectively). Reflecting this disparity, the weight of seeds in H plants was significantly lower than in L plants (Paired t test, $df = 9$, $P = 0.002$). The weight of H seeds (Mean \pm SE) was $0.40 \text{ mg} \pm 0.0056$, compared with $0.46 \text{ mg} \pm 0.0048$ from L plants.

Despite their smaller average size, there was no evidence from the germination trial that the smaller seeds from figs on H plants were less likely to be viable than those from L plants (Fig 2.5). The first seeds from H plants germinated on day five, a day earlier than the first seeds from L plants. Germination success was high in both groups (83 % in seeds from H plants and 77 % from L plants, $N = 2 \times 300$) and the speed of germination did not differ noticeably (ANOVA, $F(1, 55) = 0.057$, $P = 0.813$).

Although they were producing lighter seeds, the total weight of seeds in figs on H plants was nevertheless significantly higher than on L plants, because so many more seeds were being produced (ANOVA, $F(1, 59) = 68.62$, $P = 0.006$; Mean total seed weight \pm SE = $0.036 \text{ g} \pm 0.02$, compared with $0.030 \text{ g} \pm 0.002$). Pulp weights of the figs were not significantly different however (ANOVA, $F(1, 59) = 3.26$, $P = 0.076$; mean pulp weights \pm SE were $0.48 \text{ g} \pm 0.02$ (H figs) and $0.43 \text{ g} \pm 0.01$ (L figs)). Despite this, seed burdens (total seed weight as a percentage of total fig weight) of high nutrition figs, although higher, were not significantly different (ANOVA, $F(1, 59) = 1.46$, $P =$

0.46; Mean seed burdens \pm SE were 7.49 % \pm 0.56 (H figs) and 6.59% \pm 0.40 (L figs)).

From the perspective of the birds that feed on and disperse the figs of this species, improved nutrition therefore resulted in more figs being available, but the likely nutritional value of individual figs was unchanged and the dispersers would have to process a greater volume of seeds through their guts to obtain this reward.

2.5.3. Reproductive output – male plants

Fig production by male plants responded to supplementary nutrition in a similar way to female plants (Table 2.2, Fig 2.2b), and by the end of the experiment H plants were producing about four times as many figs as L plants. The response at the level of individual figs was different to that seen in female plants, however, with male figs on both H and L plants showing a decline in the numbers of flowers per fig as the experiment progressed, and no significant differences between them in the numbers of female (or male) flowers they contained (Table 2.4, Fig 2.3b, N = 120 plants). Figs on H plants therefore did not contain more potential oviposition sites for their pollinators.

There was no evidence that fig wasp survivorship was enhanced in figs on H plants as they did not produce significantly greater numbers of total pollinators, nor of female pollinators (Table 2.4). The number of galled, empty flowers (bladders) was also unaffected (Table 2.4). Pollinator sex ratios also did not vary between treatments, although figs on H plants did contain more of the (relatively rare) males (Table 2.4, Fig 2.4b, 2.4c). They also contained significantly more parasitoids (Fig 2.4d), resulting in the production of significantly more fig wasps overall, averaging about 20 % more through the course of the experiment (Fig 2.4e). As a pollinator is destroyed to support

each parasitoid, this means that the pollinators had laid more eggs in the H figs, but this increase had been negated by generally higher levels of parasitoid attack there (Fig 2.4f).

Figs on H plants had failed to produce more pollinators, and (from figs collected at the end of the six month period) these fig wasps were not significantly heavier than those reared from figs on L plants. The mean dry weights (\pm SE) of female pollinators were 0.0915 g \pm 0.026 (H plants) and 0.084 g \pm 0.003 (L plants) (ANOVA, $F_{(1, 59)} = 0.081$, $P = 0.78$). Male pollinators also did not vary significantly in weight (0.115 g \pm 0.031 on H plants and 0.081 g \pm 0.001 on L plants (ANOVA, $F_{(1, 59)} = 2.63$, $P = 0.11$). Female parasitoid weight again did not vary significantly between treatments (0.054 g \pm 0.001 on H plants and 0.059 g \pm 0.004 on L plants (ANOVA, $F_{(1, 59)} = 0.039$, $P = 0.845$). Male parasitoids were similarly unaffected by nutrition treatment (0.052 g \pm 0.014 on H plants and 0.055 g \pm 0.013 on L plants, ANOVA, $F_{(1, 59)} = 0.194$, $P = 0.661$).

2.5.4. Reproductive output when foundress numbers were controlled

In the female figs where only a single foundress was allowed to enter, there were again more female flowers in the figs from H plants (Table 2.5, H plants $N = 14$, L plants $N = 13$). The lone foundresses pollinated significantly more flowers and these produced on average almost 20 more seeds than in figs on L plants (Table 2.5, Fig 2.6a). Pollination efficiency (the proportion of flowers pollinated) was not lower, despite each foundress having more flowers to pollinate (86 % H and 81 % L).

There were no significant differences in the numbers of female or male flowers in figs from H and L plants amongst the male figs where a single foundress was introduced (Tables 6 and 7). In those figs where pollinators were shielded from the attacks of parasitoids, the single foundresses nonetheless produced significantly more progeny if they had entered figs from H plants (Table 2.6, Fig 2.6b, H plants N = 12, L plants N = 13). Their sex ratios were not significantly different nor the numbers of un-pollinated flowers and bladders in the figs (Table 2.6).

Amongst the lone-foundress figs that were not shielded from parasitoids, about 50 % more wasps (of both species combined) emerged from figs originating from H plants (Table 2.7, H plants N = 9, L plants N = 13). Both more pollinators and more parasitoids emerged from these figs (Figs 2.7a to 2.7c). The numbers of female flowers, male flowers, un-pollinated flowers and total flowers did not differ significantly between the treatments (Table 2.7). Pollinator sex ratios were also the same. In contrast to the results when parasitoids were excluded, significantly more bladders were present in figs from L plants when parasitoids had access (Figs 2.8a and 2.8b). This suggests that larvae of the parasitoid, but not of the pollinator, were more likely to die during larval development in these figs, though parasitism rates based on counts of emerging adult wasps were very similar (32.4 % H and 31.5 % L).

2.6. Discussion

In terms of their vegetative growth, male and female *F. montana* responded in similar ways to supplemental nutrients, with both sexes producing longer stems and more leaves. Such increases in leaf number are typical of many plants (e.g. Bellaloui and Pilbeam, 1990). A parallel response in reproductive output is also common (Eckhart

and Chapin, 1997; Nagy and Proctor, 1997; Ewert and Honermeier, 1999; Muñoz *et al.*, 2005) and was present in *F. montana*, but here the responses of the sexes showed some differences. With enhanced nutrition, both female and male plants produced more than three times as many figs, but whereas in female plants the figs were also larger, with more flowers, in male plants the size of the figs was unaltered. Each high nutrition female fig could therefore potentially produce more seeds, given adequate pollination. This potential was realized, with more seeds in the figs on high nutrition plants, because the proportion of flowers that were pollinated remained constant between treatments. The combination of more figs, and that each fig contained more seeds, led to high-nutrient plants producing an average of 4.4 times more seeds over the six month period.

A combination of factors is likely to contribute to the higher number of seeds produced in the larger, high-nutrition figs. These include extrinsic effects, not linked to the individual figs themselves. The higher densities of figs on high nutrition plants had the effect of reducing the distances between figs, making it easier for the flightless females emerging after pollination of one fig to find another to enter. Consequently, these figs are likely to have been entered by larger numbers of foundress *L. tentacularis*, bringing with them more pollen. However, the results of the experiments where single foundress females were introduced into female figs were consistent with the longer-term observations, and show that more seeds are produced by figs on high nutrition plants, independent of whether or not more pollinators enter them. This might result from individual pollinators spending longer in the larger figs, or might reflect a relaxation of spatial constraints on the movements of pollinators inside larger figs, allowing more flowers to be pollinated (flowers from different regions of a fig vary in their likelihood of being utilized, Zavodna *et al.*, 2005b). Independent of pollinator

behavior, increased seed production could have reflected an increase in the proportion of pollinated flowers that produced seeds, but this seems unlikely as it was very rare to find a flower with evidence of development that did not go on to produce a seed.

High nutrition female *F. montana* plants produced figs that contained more seeds, but the seeds (sampled after six months of supplementary nutrition) were smaller in size. This could be indicative of the photosynthetic capacity of the parent plants being saturated, but it could alternatively be due to restricted space for seed development. Although it is commonly stated that average seed size does not vary much in relation to plant nutrition, there can be large differences between sizes of ovaries in different positions within inflorescences (Milthorpe and Moorby, 1974). In wheat (*Triticum aestivum*, Poaceae) for example, there are differences in grain weight depending on where in a spikelet each floret develops and the position of the spikelet on the plant (Milthorpe and Moorby, 1974; Simmons and Moss, 1978; Herzog and Stamp, 1983; Calderini and Ortiz-Monasterio, 2003). There is also abundant evidence that an increased number of developing grains in wheat gives rise to smaller grains. For example, Bingham (1967) showed that removal of some developing grains in an ear gave higher masses amongst the remaining grains. Such compensation in the distribution of assimilates could be a common feature of plant species that form inflorescences, including fig trees. There is also a common pattern that earlier-developing fruit dominate. This could be because they synthesize plant growth regulators that suppress the development of later fruits, because there is limitation in the development of vascular tissue to the later fruits or because they act as stronger sinks and take a higher proportion of the available resources (Casper and Neisenbaum, 1993). However, the evidence that competition for resources amongst developing fruits and seeds leads to smaller structures when there are more of them is not conclusive,

even in species as extensively studied as wheat. There are also numerous examples of supplementary nutrition leading to the production of larger seeds. In experiments on the potassium and nitrogen fertilization of wheat, Forster (1973) showed increased yields with supply of higher concentrations of these elements, and that yield increases were comprised of both larger grains and more grains per plant. Similarly, in *Geranium sylvaticum* (Geraniaceae) an increase in the number of seeds per plant with fertilization was also associated with the seeds having a higher mass (Asikainen and Mutikainen, 2005).

Irrespective of whether or not the decrease in size of the seeds observed with *F. montana* offered supplemental nutrition is a common response, it did not appear to be disadvantageous in so far as it had no adverse effects on their germination rate. If anything, the seeds from the high nutrition plants also germinated slightly quicker. Post-germination success, particularly in relation to competitive ability, is nonetheless often seen as being linked to seed size (Leishman *et al.*, 2000) and the smaller size of seeds from high nutrition plants might be detrimental to them in the longer term.

Mature female figs of *F. montana* ripen to a bright red in order that attracts birds such as bulbuls to eat them (L. Shilton, Pers. Com.). Under natural conditions, because crop sizes are small, and fruiting is asynchronous, seed dispersal is likely to be effected by small numbers of birds that regularly visit the plants over long periods, rather than the large, diverse flocks that briefly descend on the better-known large, synchronized crops of monoecious fig trees such as stranglers (Shanahan and Compton, 2001). From a frugivore's perspective, female plants provided with supplementary nutrition produced mature figs more frequently, and at higher densities, but each contained a greater mass of seeds than those produced by low-nutrition plants. Consequently, *F.*

montana growing in high nutrition situations can provide seed dispersers with more resources (figs), but the quality of the resources is not improved. The increase in rewards available to frugivores has the potential to be translated into an additional benefit for female plants growing under high nutrient conditions, through an increase in rates of frugivory, and hence improved seed dispersal away from parent plants.

Reproductive success in male *Ficus* is related to how many pollen-carrying female fig wasps a plant releases, which is determined by how many figs they mature and how many female pollinators are contained in each. The number of pollinator progeny emerging from a fig depends on a number of factors, including the numbers of available oviposition sites (ovules), the numbers of foundresses entering a fig, whether they have previously laid eggs in other figs, and the survivorship of their offspring (many of which may be destroyed by parasitoids such as *Sycosapter* sp.). Male *F. montana* plants provided with supplementary nutrition produced more figs, but they did not produce figs that contained more female (or male) flowers. Their individual figs therefore offered similar numbers of oviposition sites, but as their figs were more numerous, there were nonetheless more opportunities for flightless foundresses to find and utilise second or subsequent figs.

Pollinator fig wasps, as long as they have mated, produce more female than male progeny, but the extent of this bias is not fixed. Female progeny are relatively more abundant when individual foundresses manage to lay larger clutches, which tends to be inversely related to how many foundresses enter and lay (Moore *et al.*, 2005). Consistent with this, there were significantly more male progeny produced in figs on the high-nutrition male plants, where the number of foundresses per fig was expected to be higher because the figs were closer together, making walking between figs easier.

However, there was no significant increase in total pollinator progeny numbers from figs on high nutrition plants, nor was there a fall in the number of female progeny. Consequently, from a male plant's perspective, individual figs on the high and low nutrition plants were equally successful at producing pollen-carrying wasps. The former simply produced many more figs.

As mentioned above, the number of pollinator progeny that successfully emerge from a fig can be greatly reduced through the actions of parasitoids. Parasitoid responses to plant nutrition can therefore influence the costs and benefits of any plant responses to soil nutrition. In *F. montana*, each *Sycosapter* develops at the expense of a single pollinator larva (only a very small number of additional pollinators appear to be destroyed by the probing actions of adult parasitoids, at least in high nutrition figs, S. Raja unpublished). The total wasps emerging from figs therefore provides an indication of how many pollinators were originally present, although it is a minimum estimate because some wasps may die early in their development, increasing the total of bladders (galled flowers which do not provide adult wasps). Figs on high nutrition plants contained significantly more parasitoids, contributing to an overall increase in wasps emerging from these figs and showing that pollinators had laid more eggs in each of these figs.

Parasitoid aggregation on high nutrition male plants (where more figs were present), or on figs that contained more pollinator larvae, therefore appears to have prevented high nutrition male plants from producing more pollinators per fig. The increased pollinator production that would have been achieved in the absence of pollinators was demonstrated in the experiments where parasitoids were excluded. They also showed that the increased numbers of pollinator progeny emerging from high nutrition figs was

not only a reflection of more entries by foundresses, as it was evident when only a single foundress was allowed to enter. Unlike the seeds, there was no trade-off with pollinator size, as the fig wasps emerging from high nutrition figs were not significantly smaller, and were therefore not likely to be less fecund.

These results illustrate the complex relationship between plant nutrition and reproductive success in fig trees and show that in dioecious species the sexes may differ in their responses to better soil conditions. Female plants in general are expected to respond more dramatically to differences in nutrition than male plants, because seeds are more expensive to produce than pollen (Ashman, 1994), and if our studies had been carried out in the absence of parasitoids then we might have concluded that the increased inflorescence size seen only in female plants reflected greater nutrient limitation in this sex. However, our inclusion of a third trophic level has provided an alternative explanation, that there is no advantage to male plants in producing figs with more flowers, because parasitoids eliminate any potential gains.

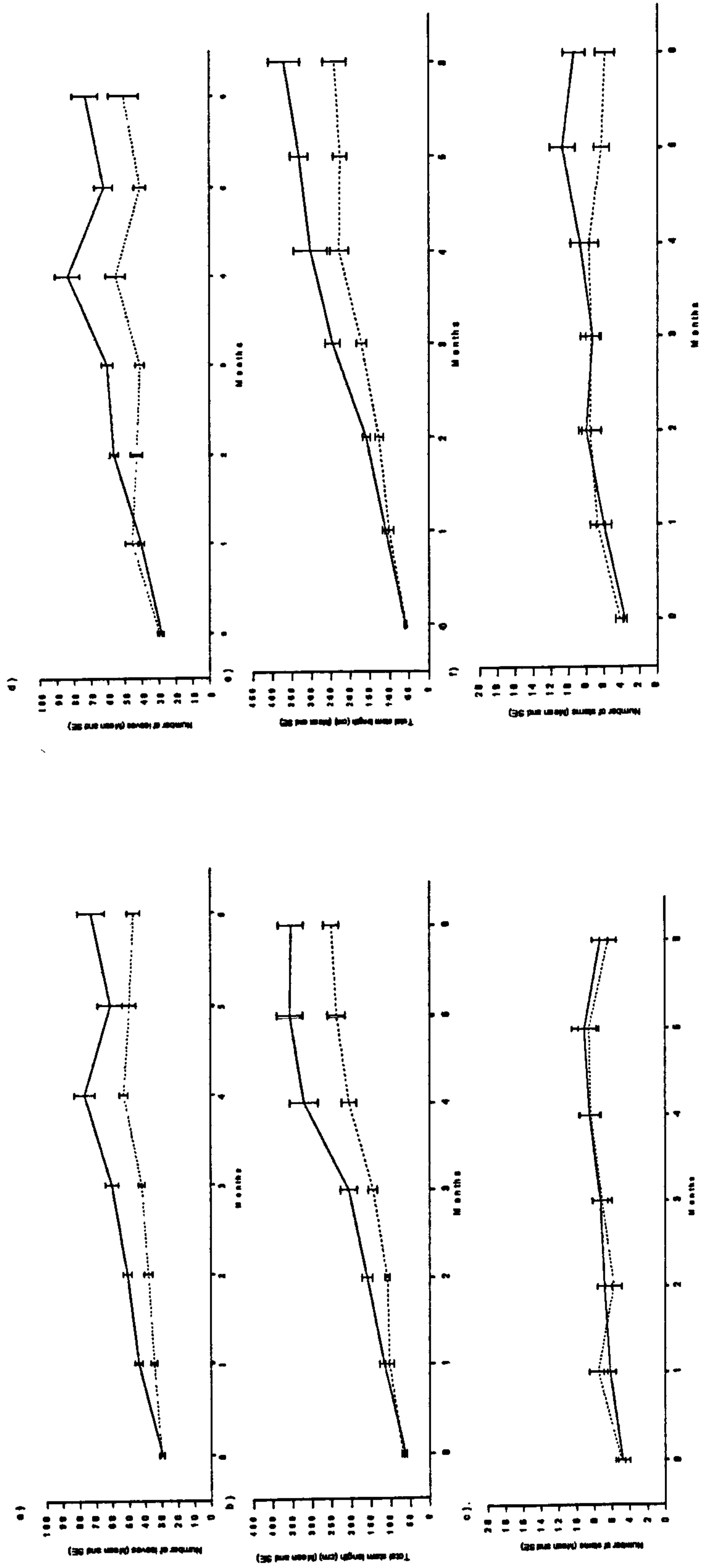


Fig. 2.1 Vegetative responses of *Ficus montana* to contrasting nutrient conditions (high nutrition solid line, low nutrition dashed line). a). Total Leaf numbers (female plants), b). Total stem length (female plants), c). Number of stems (female plants), d). Total Leaf numbers (male plants), e). Total stem length (male plants), f). Number of stems (male plants).

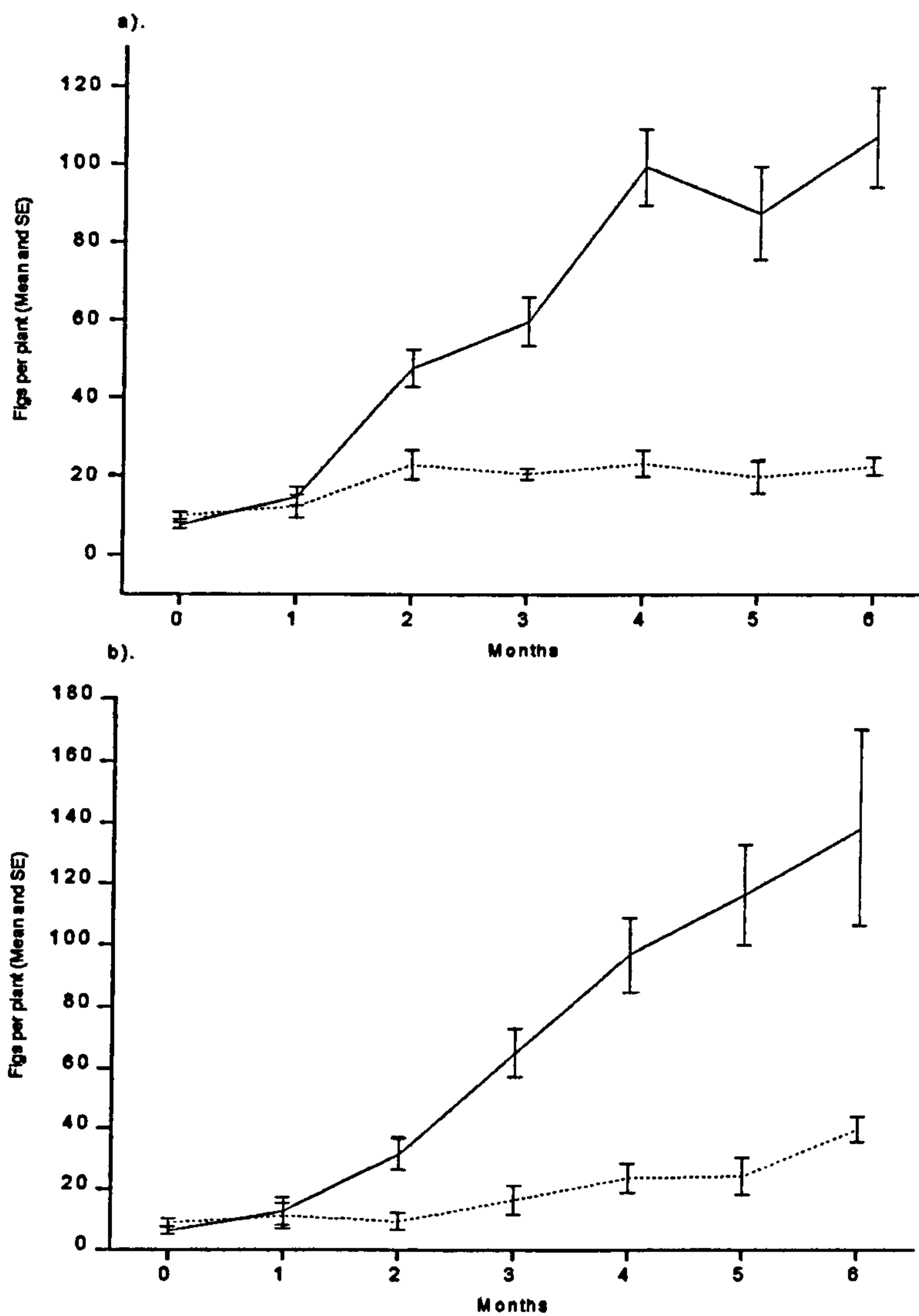


Fig. 2.2 The numbers of figs on plants grown for six months under high (solid line) and low (dashed line) nutrient conditions.
a). Female plants, b). Male plants.

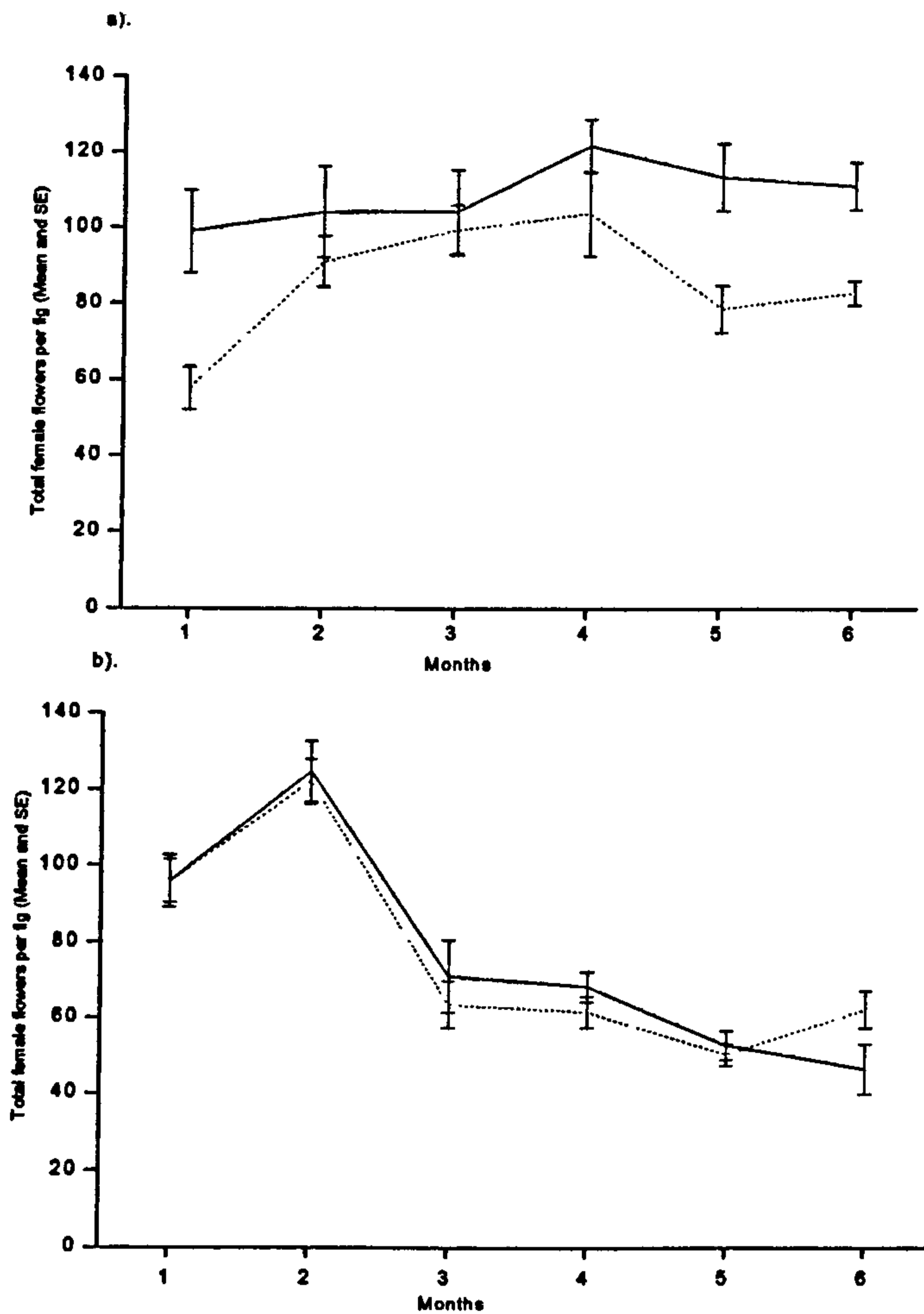


Fig. 2.3 The number of female flowers in figs grown for six months under high (solid line) and low (dashed line) nutrient conditions.
 a). Female plants, b). Male plants.

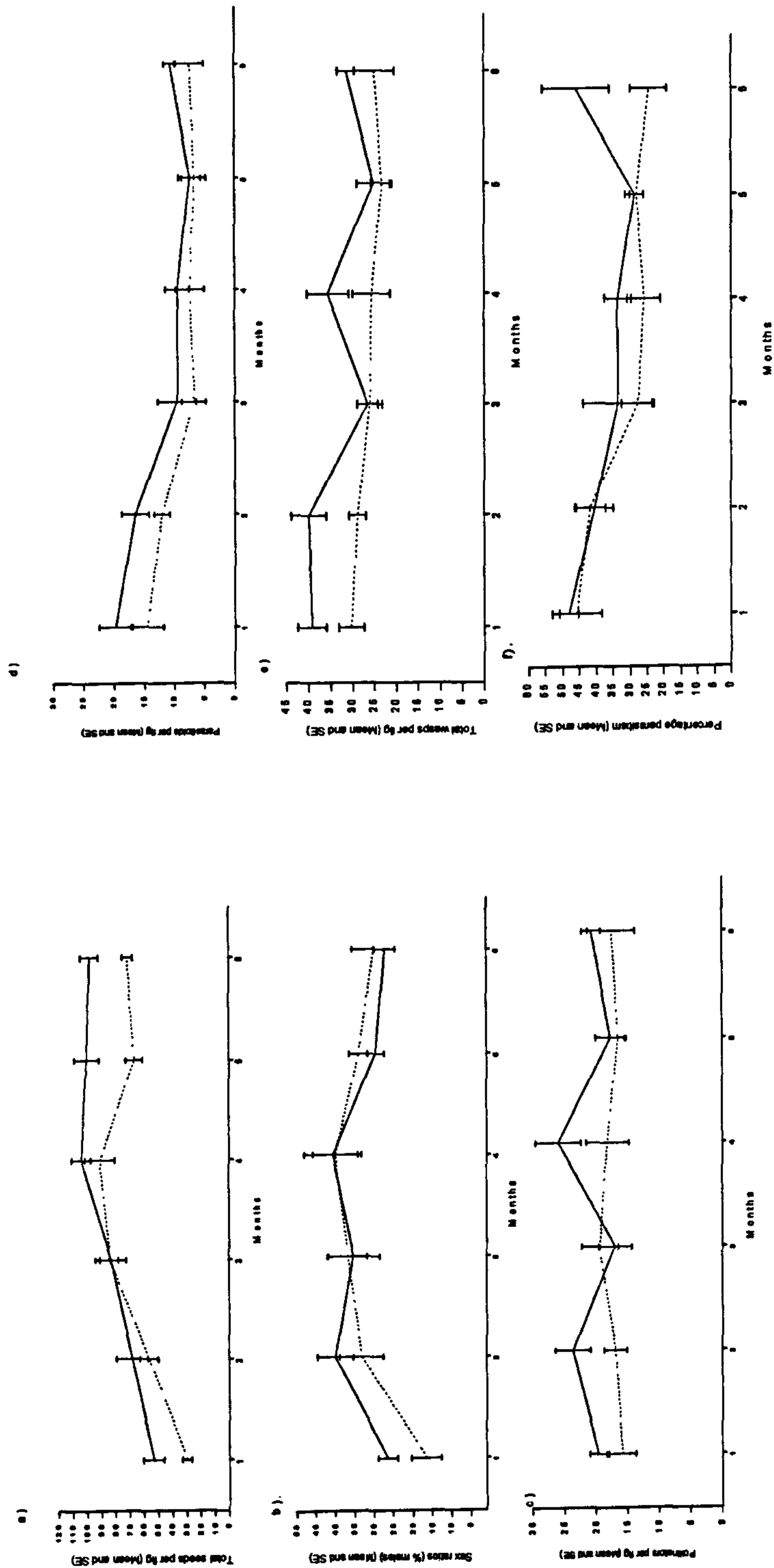


Fig. 2.4 Responses to varying nutrition over a six month period (high nutrition solid line, low nutrition dashed line).
 a). Number of seeds present in female figs, b). Sex ratio of pollinators in male figs, c). Number of pollinators in male figs.
 d). Number of parasitoids present in male figs, e). Total number of wasps (pollinators and parasitoids) in male figs, f). Percentage parasitism of pollinators in male figs.

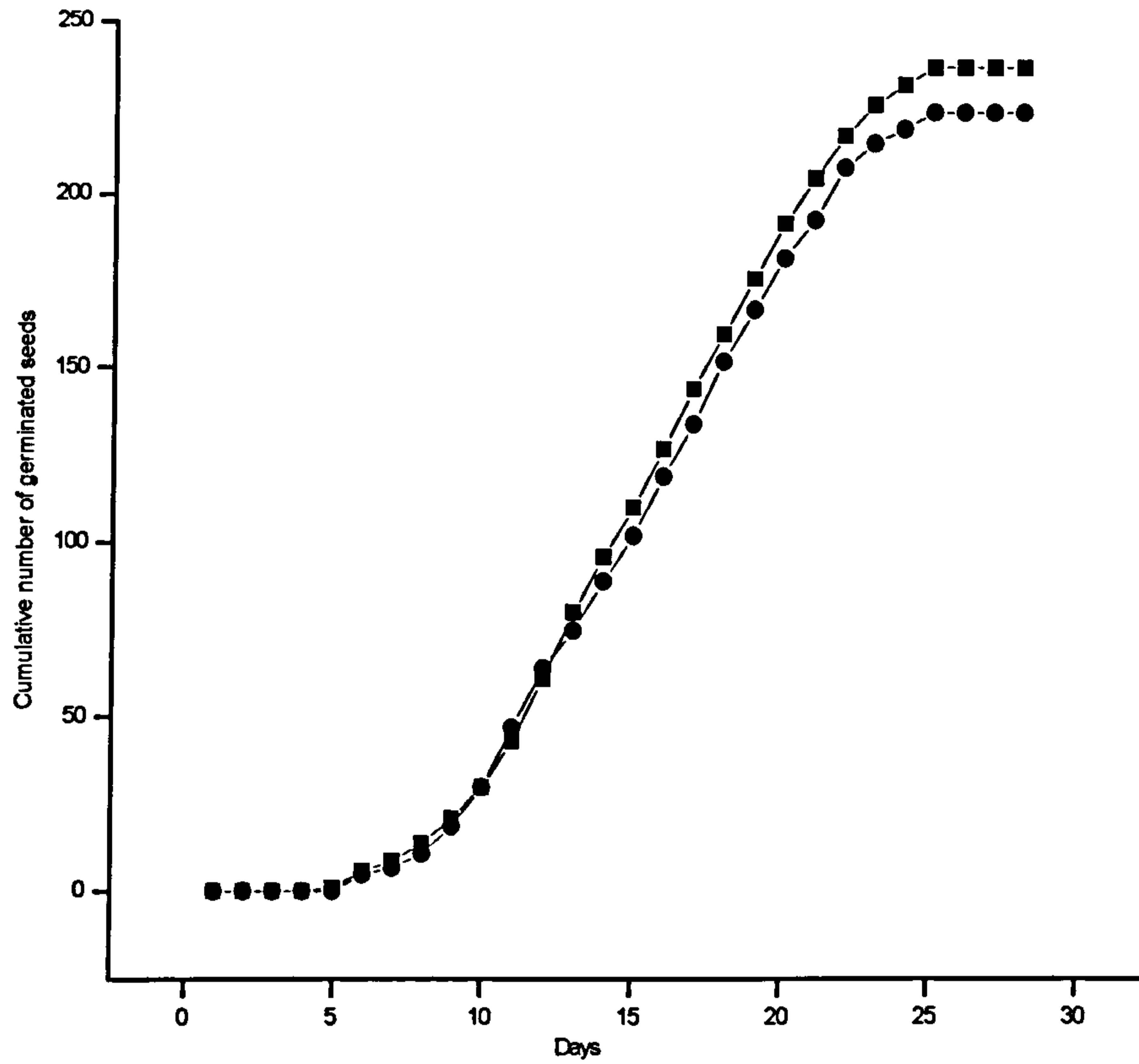


Fig. 2.5 Germination of seeds from figs grown under high (squares) and low (circles) nutrient conditions.

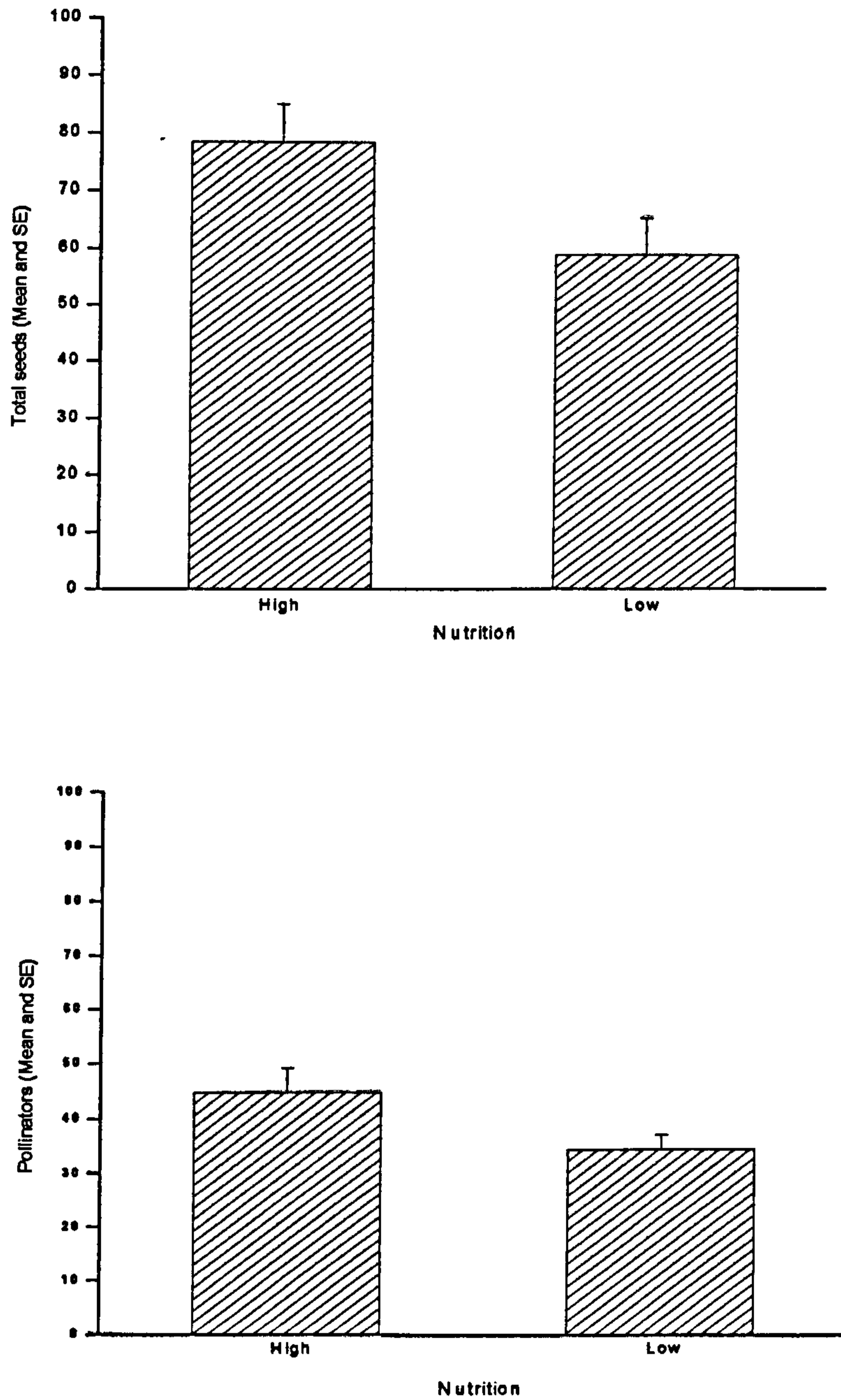


Fig. 2.6 The contents of figs that had been grown under contrasting nutrient conditions and entered by a single pollinator.
a). The number of seeds in figs of female plants, b). The number of pollinator progeny in figs of male plants (parasitoids were excluded).

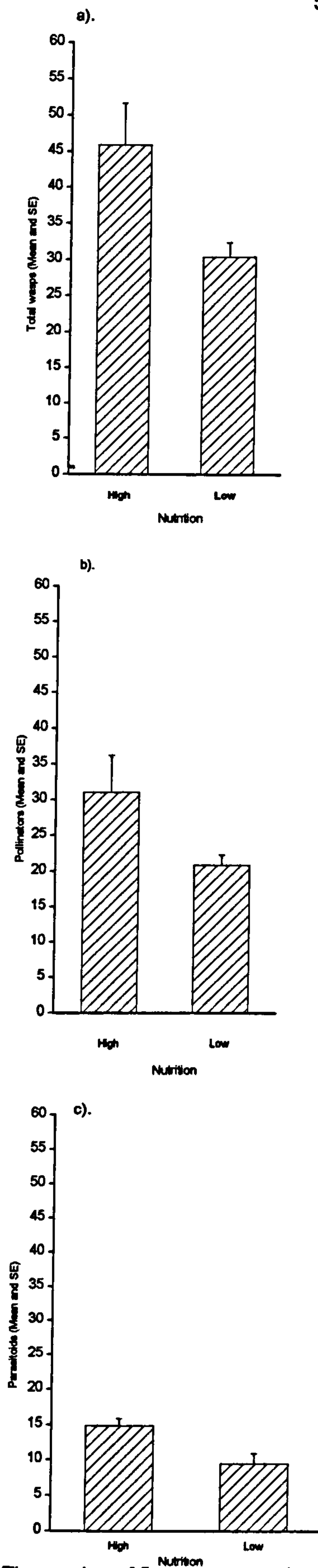


Fig. 2.7 The number of fig wasps emerging from male figs that had been grown under contrasting nutrient conditions for six months and entered by a single pollinator (parasitoids were allowed access).

a). Total wasps (Pollinators and parasitoids), b). Pollinators (in figs with parasitoids), c). Parasitoids.

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Table 2.1 Summary of growth parameters of female *Ficus montana* plants grown under high and low nutrient conditions (combined data from six monthly samples).

Variable	High nutrition			Low nutrition			F			Probability		Supplementary Nutrition Effects
	Mean	SE	SE	Mean	SE	SE	Month	Treatment (Month)	Month	Treatment (Month)	Month	
Young leaves	8.51	0.55	0.47	5.58	0.47	0.47	10.92	4.12	P<0.001	P<0.001	P<0.001	Increase
Mature leaves	49.73	2.77	1.70	34.63	1.70	1.70	12.39	6.05	P<0.001	P<0.001	P<0.001	Increase
Senescing leaves	2.65	0.37	0.46	3.76	0.46	0.46	1.93	0.87	0.09	0.52	0.52	None
Total leaves	60.89	2.66	1.45	43.97	1.45	1.45	8.92	7.87	P<0.001	P<0.001	P<0.001	Increase
Main stem length (cm)	134.73	5.93	5.26	121.81	5.26	5.26	41.42	1.60	P<0.001	0.15	0.15	None
Total stem length (cm)	251.41	16.15	9.93	174.68	9.93	9.93	29.97	7.72	P<0.001	P<0.001	P<0.001	Increase
Number of stems	7.53	0.40	0.42	7.20	0.42	0.42	1.62	0.38	0.16	0.89	0.89	None
A+B phase figs	38.80	2.81	0.90	9.20	0.90	0.90	5.85	27.61	P<0.001	P<0.001	P<0.001	Increase
C phase figs	20.55	2.73	0.52	5.78	0.52	0.52	9.00	11.40	P<0.001	P<0.001	P<0.001	Increase
E phase figs	11.31	1.26	0.53	5.26	0.53	0.53	7.73	8.91	P<0.001	P<0.001	P<0.001	Increase
Total figs	70.66	5.43	1.30	20.24	1.30	1.30	16.63	37.08	P<0.001	P<0.001	P<0.001	Increase

Table 2.2 Summary of growth parameters of male *Ficus montana* plants grown under high and low nutrient conditions (combined data from six monthly samples).

Variable	High nutrition		Low nutrition		F		Probability.		Supplementary Nutrition Effects
	Mean	SE	Mean	SE	Month	Treatment (Month)	Month	Treatment (Month)	
Young leaves	8.66	0.57	6.40	0.72	14.76	2.55	P<0.001	P<0.001	Increase
Mature leaves	51.5	2.80	35.80	2.10	16.00	6.60	P<0.001	P<0.001	Increase
Senescing leaves	2.48	0.38	2.31	0.17	0.97	0.97	0.44	0.45	None
Total leaves	62.64	2.67	44.51	2.12	6.90	7.35	P<0.001	P<0.001	Increase
Main stem length (cm)	147.31	6.89	127.30	5.82	31.61	2.19	P<0.001	P<0.001	Increase
Total stem length (cm)	253.58	16.11	182.83	10.14	23.12	6.01	P<0.001	P<0.001	Increase
Number of stems	8.35	0.44	6.95	0.42	1.02	2.53	0.40	P<0.001	Increase
A+B phase figs	31.30	3.13	8.11	1.33	6.09	11.78	P<0.001	P<0.001	Increase
C phase figs	40.55	6.00	10.43	1.72	11.66	9.72	P<0.001	P<0.001	Increase
D phase figs	1.01	0.25	0.28	0.95	4.40	3.55	P<0.001	P<0.001	Increase
E phase	3.85	0.62	1.35	0.25	14.07	8.94	P<0.001	P<0.001	Increase
Total figs	76.71	8.57	20.17	2.60	12.07	14.92	P<0.001	P<0.001	Increase

Table 2.3 The contents of female figs grown under high and low nutrient conditions (combined data from six monthly samples).

Variable	High nutrition		Low nutrition		F	Probability	Supplementary Nutrition Effects
	Mean	SE	Mean	SE			
Total female flowers	109.06	3.91	85.68	3.37	4.92	P<0.001	Increase
Seeds	84.56	4.15	66.63	3.64	3.82	P<0.001	Increase
Un-pollinated flowers	24.50	2.09	19.05	1.63	3.03	P<0.001	Increase

Table 2.4 The contents of male figs grown under high and low nutrient conditions (combined data from six monthly samples).

Variable	High nutrition			Low nutrition			F		Probability		Supplementary Nutrition Effect
	Mean	SE	Mean	SE	Month	Treatment (Month)	Month	Treatment (Month)	Month	Treatment (Month)	
Total flowers	92.64	4.51	87.18	4.10	47.63	0.61	P<0.001	0.72	0.72	None	
Female flowers	82.21	4.36	76.52	3.82	6.19	0.59	P<0.001	0.73	0.73	None	
Male flowers	10.43	.56	10.66	0.52	42.88	0.84	P<0.001	0.54	0.54	None	
Un-pollinated flowers	20.86	1.72	17.91	1.56	35.49	0.84	P<0.001	0.53	0.53	None	
Bladders	27.56	2.06	32.11	2.23	23.87	1.74	P<0.001	0.11	0.11	None	
Total pollinators	20.98	1.07	17.38	1.07	1.01	1.68	0.41	0.13	0.13	None	
Male pollinators	6.98	0.61	5.30	0.37	3.68	2.33	P<0.001	P<0.001	P<0.001	Increase	
Female pollinators	14.00	0.85	12.08	0.92	0.83	0.89	0.53	0.50	0.50	None	
Sex ratio (% male pollinators)*	33.18	2.03	31.07	2.19	3.65	0.88	P<0.001	0.51	0.51	None	
Total parasitoids	12.81	1.06	9.11	0.96	6.27	1.12	P<0.001	P<0.001	P<0.001	Increase	
Male parasitoids	4.35	0.45	3.43	0.55	3.24	0.42	P<0.001	0.86	0.86	None	
Female parasitoids	8.46	0.73	5.68	0.56	6.86	2.06	P<0.001	P<0.001	P<0.001	Increase	
Sex ratio (% male parasitoids)*	26.90	1.69	22.64	2.06	1.31	1.13	0.26	0.35	0.35	None	
Total wasps	33.79	1.60	26.50	1.36	3.05	2.35	P<0.001	P<0.001	P<0.001	Increase	

• Values were arcsine square root transformed for analysis

Table 2.5 The contents of female figs grown under high and low nutrient conditions and pollinated by a single fig wasp.

Variable	High nutrition		Low nutrition		F	Probability	Supplementary Nutrition Effect
	Mean	SE	Mean	SE			
Total female flowers	91.06	6.67	72.22	6.09	4.30	P<0.001	Increase
Seeds	78.21 (85.88 %)	6.65	58.84 (81.46%)	6.29	4.44	P<0.001	Increase
Un-pollinated flowers	12.85 (14.12 %)	0.91	13.38 (18.54 %)	1.38	0.10	0.75	None

Table 2.6 The contents of male figs grown under high and low nutrient conditions and pollinated by a single fig wasp (parasitoids were excluded).

Variable	High nutrition		Low nutrition		F	Probability	Supplementary Nutrition Effect
	Mean	SE	Mean	SE			
Total flowers	92.32	6.15	88.46	4.39	0.26	0.609	None
Female flowers	79.91	6.00	73.76	4.18	0.71	0.407	None
Male flowers	12.41	0.75	14.69	0.97	3.35	0.080	None
Un-pollinated flowers	9.66 (12.09%)	1.16	11.92 (14.00%)	0.97	2.23	0.149	None
Bladders	25.50 (31.90%)	3.70	27.46 (37.22%)	3.04	0.16	0.688	None
Total pollinators	44.75 (55.99%)	4.27	34.38 (46.61%)	2.62	4.42	P<0.001	Increase
Sex ratio (% males)*	25.56	1.85	28.20	2.83	0.44	0.512	None

* Values were arcsine square root transformed for analysis

Table 2.7 The contents of male figs grown under high and low nutrient conditions and pollinated by a single fig wasp (parasitoids not excluded).

Variable	High nutrition		Low nutrition		F	Probability	Supplementary Nutrition Effect
	Mean	SE	Mean	SE			
Total flowers	93.09	8.33	100.91	5.50	0.66	0.426	None
Female flowers	80.09	8.38	86.50	5.49	0.44	0.515	None
Male flowers	13.00	1.19	14.41	1.09	0.75	0.397	None
Un-pollinated flowers	9.55 (11.92 %)	1.60	14.25 (12.13 %)	1.56	4.21	0.054	None
Bladders	24.66 (30.79 %)	3.91	41.83 (37.76 %)	4.60	7.38	P<0.001	Decrease
Total pollinators	31.00 (38.69 %)	5.21	20.83 (24.08 %)	1.38	4.56	P<0.001	Increase
Sex ratio (% male pollinators)*	29.29	4.35	34.81	2.78	1.34	0.261	None
Total parasitoids	14.88 (18.58 %)	1.01	9.58 (11.07 %)	1.47	7.56	P<0.001	Increase
Sex ratio (% male parasitoids)*	26.10	3.60	18.9	3.21	2.40	0.138	None
Total wasps	45.88	5.76	30.41	1.99	8.00	P<0.001	Increase

* Values were arcsine square root transformed for analysis

3. When bigger is only better if you are female: inflorescence size and reproductive success in a dioecious fig tree

3.1. Abstract

I studied the relationship between inflorescence size and reproductive success in male and female figs of the dioecious *Ficus montana* grown under controlled conditions in the presence of its pollinator *Liporrhopalum tentacularis* Grandi and its main parasitoid *Sycoscapter* sp. indesc. Unlike monoecious figs which produce wasps, seeds and pollen in every fig, dioecious figs have female trees which produce seeds while male trees produce wasps and pollen only. Female figs that contained more flowers produced more seeds, but male figs that produced more flowers did not produce more female pollinators. Although the female pollinators laid more eggs in figs with more flowers the eventual number of female pollinators produced was reduced by *Sycoscapter* and other causes. There was no significant relationship between wasp sex ratio and the number of female flower in male figs.

3.2. Introduction

Fig trees (*Ficus* spp., Moraceae) are a highly species-rich group of over 700 plant species, mainly found in the tropics. They are characterised by their unique inflorescences (figs), each of which is a hollow sphere, lined on its inner surface by tens, hundreds or thousands of tiny flowers, depending on the species. Figs may be borne among the leaves, on specialised leafy branches, on the trunks or even underground and mature figs vary in size from just a few millimeters across to over 20 cm. They are mainly animal-dispersed, and are fed upon by more species of frugivorous vertebrates than any other plant group (Shanahan *et al.*, 2001).

Inflorescence size (the number of individual flowers present) is clearly correlated with fig diameter, which along with other 'design' features such as colour and location on the plant, determines which species of vertebrates feed on the figs, and subsequently disperse the seeds they contain (Shanahan *et al.*, 2001). Dispersal agents are therefore likely to act as a major selection pressure on the design of figs, including the number of flowers they contain, but interactions with other mutualists may also be of significance.

Along with their 'keystone' role as food for vertebrates (Shanahan *et al.*, 2001), fig trees are also well known because of their intimate and species-specific association with fig wasps, their only pollinators. Attraction of pollinators in general is often linked to floral apparency, at a variety of scales, with more individuals attracted to larger flowers (Goulson, 1999), to inflorescences that contain more flowers (Kudo and Harder, 2005) and to plants with more flowers in total, the 'floral display size' (Ohashi and Yahara, 2001). Attraction of fig wasps to figs is largely based on species-specific odours, released at the time when figs are ready to be pollinated, rather than visual cues (Ware *et al.*, 1993). Although the same principles are likely to apply, evidence that fig wasps are preferentially attracted to larger figs (or larger fig crops), is equivocal (Patel and Hossaert-McKey, 2000). Fig wasps may nonetheless generate selection pressures on inflorescence size in other ways.

In monoecious fig trees (where each fig produces a mixture of seeds and pollen-carrying fig wasps), the balance between seed and pollinator production is initially determined by the proportion of pollinated flowers that are destroyed by wasp larvae, which in turn depends on the number of accessible female flowers relative to the combined egg loads of the wasps that enter a fig (Nefdt and Compton, 1996). This is

then modified by the actions of the numerous species of non-pollinating fig wasps, some of which destroy pollinator larvae while others feed on the seeds.

In dioecious fig trees (which make up over half of all the species), each tree specialises in either wasp or seed production. Male and female plants tend to produce figs that have similar external diameters; at least at the time that pollination occurs, probably due to joint inter-sexual mimicry (Grafen and Godfray, 1991). The number of flowers in female figs is nonetheless often considerably higher than in male figs (Corlett *et al.*, 1990; Patel and Hossaert-McKey, 2000). The male figs of *F. asperifolia* from West Africa, for example, have around 1000 flowers, while female figs contain around 1700 flowers (Verkerke, 1989). The presence of male flowers (present only in male figs) accounts for much of this difference in those species where pollination is passive (and there are many male flowers), but it is also evident in actively pollinated species, where male flowers are much less frequent. Furthermore, in a small number of species, the typical pattern is reversed, with more female flowers in male rather than the female figs, which produce a small number of exceptionally large seeds (Corner, 1952). The observed differences in inflorescence size imply that the de-coupling of sexual functions present in dioecious fig species has allowed male and female figs to respond to sex-specific optima for this character. Female figs, for example, might benefit from higher numbers of pollinators than male figs (and therefore benefit from greater numbers of flowers), because of the increased genetic variability in the resultant seeds. Certainly in most species this optimum appears to be higher in female than male figs.

A further possible benefit of dioecy in fig trees is that interactions with non-pollinating fig wasps have also been partly de-coupled (Compton *et al.*, 1996). Pollinator larvae in male figs suffer from parasitoids and inquilines in the same way as seen in monoecious

figs, but seed predatory fig wasps (associated with female figs) have been recorded from very few *Ficus* species, and consequently levels of seed predation are typically much lower than in monoecious species.

Here we compare the relationship between inflorescence size and reproductive success in male and female figs of the dioecious *Ficus montana* grown under controlled conditions in the presence of the tree's pollinator and its main parasitoid. Previous studies (Chapter 2) have found that both sexes respond to enhanced soil nutrients by producing more figs, but only female plants respond by producing figs that contain more flowers. In order to see whether this difference might be adaptive, we determined whether fitness gains associated with larger inflorescence sizes were present in female figs, but not male figs, and examined the causes of any sexual differences.

3.3. Natural History

Populations of functionally dioecious fig trees comprise male plants that only produce pollen-carrying wasps and female plants that produce only seeds. Anatomically, fig trees are gynodioecious, with female figs possessing only female flowers (which if pollinated produce seeds), whereas male figs contain both male and female flowers, although the latter produce no seeds, just wasps, if a wasp has laid eggs in them.

Ficus montana Blume is a dioecious fig tree found in lowland South East Asia (Corner, 1952). It has a low, shrubby growth form and forms clumps through vegetative spread. The mature figs of both sexes are around 8-12mm in diameter. They are borne in the leaf axils and on older wood. Fruiting within plants is asynchronous, with all stages of fig development often present at the same time.

The pollinator of *F. montana* is the diurnal fig wasp *Liporrhopalum tentacularis* Grandi. Adult female *L. tentacularis* that enter male figs (foundresses) lose their wings and most of their antennae while passing through the ostiole, but routinely re-emerge after laying some eggs, then walk to and enter other figs, before laying again. One larva can develop in each galled ovule. Foundresses that enter female figs show similar behaviour, but fail to oviposit successfully. The sex ratios amongst *L. tentacularis* progeny vary according to how many eggs a foundress lays, which is influenced by how many other females share the figs with them (Moore *et al.*, 2005; Moore *et al.*, 2002). *L. tentacularis* appears to show no preference for entering larger figs (Moore *et al.*, 2003b).

Sycoscapter sp. is an undescribed solitary non-pollinating fig wasp that oviposits into male figs from the outside, through the fig wall. It acts as a parasitoid because it destroys the larvae of *L. tentacularis*, but may also feed on the remaining plant material inside the ovules (Chapter 7). In Indonesia, *Sycoscapter* sp. is widespread, occurring at almost all the sites where *F. montana* has been sampled (Pers. Obs, M. Zavodna and J. Moore, unpublished). No parasitic fig wasps that utilise female figs of *F. montana* have been reported, and significant seed predation by other insects has not been recorded.

3.4. Materials and methods

3.4.1. Glasshouse conditions

The plant and insect populations were maintained in a glasshouse at the Experimental Gardens of the University of Leeds. The plants originated from seeds collected at CIFOR HQ, Bogor, Indonesia and from the Krakatau Islands, Indonesia in 1995

(Moore *et al.*, 2002). The insects were collected from the same locations the following year.

The experimental plants (twenty male and twenty female) were grown from cuttings (100 mm woody shoots with heels) taken in November 2003. The cuttings were dipped in hormone rooting powder (50:50 mix of 0.1 % IBA and 0.4% NAA + 2.1 % Captan fungicide), inserted into expanded jiffy No 7 peat pellets and then placed in propagating trays over benches heated with soil warming cables.

After establishment they were transplanted to 15 cm pots and then finally into 20 cm pots in March 2004. Nutrient treatment was started at the end of April 2004. Each pot was provided with 45 cm³ of liquid fertilizer (Peter's Excel water-soluble fertilizer in crystalline form) every week. The plants received 1.4 g/litre (N= 0.01134 g, P= 0.0063 g and K= 0.01134 g). To allow for nutrient distribution within the soil, for different rates of absorption due to different moisture levels of soils, and for variation in root numbers, saucers were placed under the pots so that no feed would be lost as runoff. The pots were placed into equally spaced rows. The pots were rotated fortnightly to minimize any long-term variation in light levels.

3.4.2. Plant reproductive characters

Ten mature figs from female plants and 10 mature figs from male plants were collected each month for six months (April-October). The mean maximum temperature for this period was 28.8 °C and mean minimum temperature was 19.8 °C. There was no control over day length. Normally one fig was collected from each randomly-selected plant, but up to three figs were collected from the same plant when they were in short supply. Female figs were dissected under a binocular microscope to give counts of total seeds

and un-pollinated flowers. Male figs were placed in plastic containers covered tightly with a fine mesh. They were left for 24 hrs to let the wasps emerge naturally for counting, sexing and identification. The figs were then split open with fine forceps and any wasps remaining within the lumen were also counted, along with adult insects still present in their galled ovules. Along with ovules with wasp exit holes, the remaining female flowers were scored as either 'bladders' (developed galls that had not produced wasps) or as un-pollinated. The numbers of functionally male flowers were also counted.

3.5. Results

Within individual male figs the number of female flowers ranged from 35 to 157 with a mean of 82.21 in male figs ($SE \pm 4.36$). Total flowers (male + female flowers) ranged from 41 to 176 ($n = 60$) with a mean number of 92.64 ($SE \pm 4.51$). The total number of female flowers in female figs ranged from 20 to 176, with the mean number of 109.06 ($SE \pm 3.91$). There was no significant relationship between the numbers of male and female flowers present in male figs (Pearson correlation, $r = 0.21$, $P > 0.05$).

Reproductive success in female figs is linked to the number of seeds they contain, whereas in male figs an equivalent measure is the number of pollen-carrying female fig wasps. The number of female flowers in female figs had a significant effect on the number of seeds they produced, with figs that contained more flowers also producing more seeds (Linear regression model 1: $F_{1,59} = 175.5$, $P < 0.05$, $r^2 = 0.75$) (Fig 3.1). In contrast, the number of female pollinator progeny emerging from a male fig was not related to the numbers of female flowers present, meaning that larger figs were less efficient than small figs in terms of the proportion of flowers that were 'useful' to the plant (Linear regression model 1: $F_{1,59} = 2.50$, $P > 0.05$, $r^2 = 0.04$) (Fig 3.2).

The lack of a positive relationship between female flower number and the number of female fig wasps produced could be the result of a smaller proportion of the flowers being utilised by the wasps in larger figs, or a reduced success rate amongst the flowers that they had utilised. There was little evidence for the former, as the number of galled flowers (those containing wasps or empty galls 'bladders') in a fig was strongly correlated with female flower number (Linear regression model 1: $F_{1, 59} = 105.53$, $P < 0.05$, $r^2 = 0.623$) (Fig 3.3). Rather, the absence of a positive relationship between female flower number and female pollinator production resulted from changes in what the galled ovules contained.

Larger clutches of *L. tentacularis*, the pollinator of *F. montana*, have more female-biased sex ratios (Moore *et al.*, 2002). Consequently, given that larger figs offer more oviposition sites, they might have been expected to produce a higher proportion of female progeny, so changes in the progeny sex ratio were not expected to have contributed to the absence of a relationship. However, a significant positive relationship with female flower number was observed when total pollinator numbers (both males and females) were considered (Linear regression model 1: $F_{1, 59} = 7.67$, $P < 0.05$, $r^2 = 0.12$) (Fig 3.4). This was despite a non-significant relationship between progeny sex ratio and inflorescence size, (Linear regression model 1 with arc sine square root transformation: $F_{1, 59} = 0.22$, $P > 0.05$, $r^2 = 0.004$).

Each adult of the parasitoid *Sycoscapter* sp. that emerges from a male fig represents the loss of about one pollinator larva. When this species was included with the pollinators in the analysis, the relationship between female flower number was much more clear-cut, (Linear regression model 1: $F_{1, 59} = 23.56$, $P < 0.0001$, $r^2 = 0.29$) (Fig

3.5). Aggregation of parasitoids in larger figs could generate this effect, but although more parasitoids were reared from figs with more female flowers (Linear regression model 1: $F_{1, 59} = 17.5$, $P < 0.05$, $r^2 = 0.23$), parasitism rates were not significantly higher (Logistic Linear regression model 1 with arcsine square root transformation: $F_{1, 59} = 2.83$, $P = 0.098$, $r^2 = 0.05$). Bladders are galled ovules which failed to produce adult pollinators (or their parasitoid). Bladders were considerably more numerous in figs with larger numbers of flowers (Linear regression model 1: $F_{1, 59} = 49.52$, $P < 0.0001$, $r^2 = 0.68$) (Fig 3.6).

3.6. Discussion

We have shown that under our experimental tritrophic (plant-pollinator-parasitoid) conditions, female figs of *F. montana* that contain more flowers produce more seeds, but male figs with more flowers fail to produce more female pollinators to transport their pollen. There is not likely to be a compensatory benefit with wasps emerging from larger figs carrying more pollen, because figs with more female flowers do not necessarily contain more male flowers (N. Suleman Pers. Comm). Female reproductive success in this plant therefore clearly scales with inflorescence size, but male reproductive success (for which female pollinator production is a surrogate) does not. This finding is interesting in the light of our previous results from experimental manipulations of soil nutrients, where female plants, but not male plants, responded to enhanced soil nutrition by producing figs that contained more flowers. Our results are in contrast to those of Patel and Hossaert-McKey (2000), who also examined the relationship between female flower number and seed and wasp production in a dioecious fig tree, *F. hispida*. They detected a significant positive relationship between flower number and pollinator numbers (both sexes combined) in male figs, but found no such relationship with seed production in female figs. However, in their

experiments any parasitoids were excluded and consequently their effects on pollinator numbers were unknown.

In female *F. montana* figs, the weight of individual seeds in a fig is negatively correlated with the number of seeds it contains (S. Raja *et al.*, in prep). Germination rates were not found to be related to seed weight, but the slope of the relationship between flower number per fig and realised female reproductive success may nonetheless not be as dramatic as recorded here, due to competitive disadvantage associated with smaller seed size under natural conditions.

In male *F. montana* figs, it appears that although *L. tentacularis* females lay more eggs in figs with more flowers (or at least gall more flowers there), this is not translated into greater numbers of the pollen-carrying adult females that eventually emerge. This is because the numbers of female pollinators are reduced by *Sycoscapter* sp. parasitoids and other causes of pollinator larval mortalities that result in the production of increased numbers of bladders in larger figs. These factors alter the relationship between the reproductive success of male plants and size of their figs to the extent that larger figs are not more successful than smaller ones, and in terms of efficiency of the plant's resource use, are less successful.

The optimal progeny sex ratio for foundress fig wasps varies according to how many other foundresses lay eggs in a fig with her, and their relative contributions to combined clutch sizes (Moore *et al.*, 2002). In *L. tentacularis*, and probably other species of fig wasps, the increase in the proportion of male progeny as more foundresses enter a fig is linked to smaller clutches being produced by each foundress (Zavodna *et al.*, 2005) and a largely male eggs first strategy (S. Raja, in prep.). As

larger figs allow individual females to lay larger clutches (S. Raja, in prep.), this should favour the production of a higher proportion of the female progeny that are of benefit to the plant. However, no significant relationship between sex ratio and female flower numbers was detected, and consequently larger figs failed to gain from this putative advantage. The cause of this is uncertain, but it will reflect the combined oviposition patterns of all the pollinators that enter each fig.

If larger female figs with more flowers produce more seeds, but smaller male figs are more efficient, what prevents a divergence of inflorescence size? One explanation is that inter-sexual mimicry at the time that pollinator females enter figs is necessary to avoid pollinator avoidance of female figs (Grafen and Godfray, 1991). Furthermore, fig wasps only successfully reproduce if they enter male figs, and consequently any selection acting on their behaviour once they enter a fig (such as how long they stay before attempting to leave) is generated only in male figs, even though it has consequences for pollination in female figs. These constraints may prevent fixation of 'optimal' inflorescence size in both sexes.

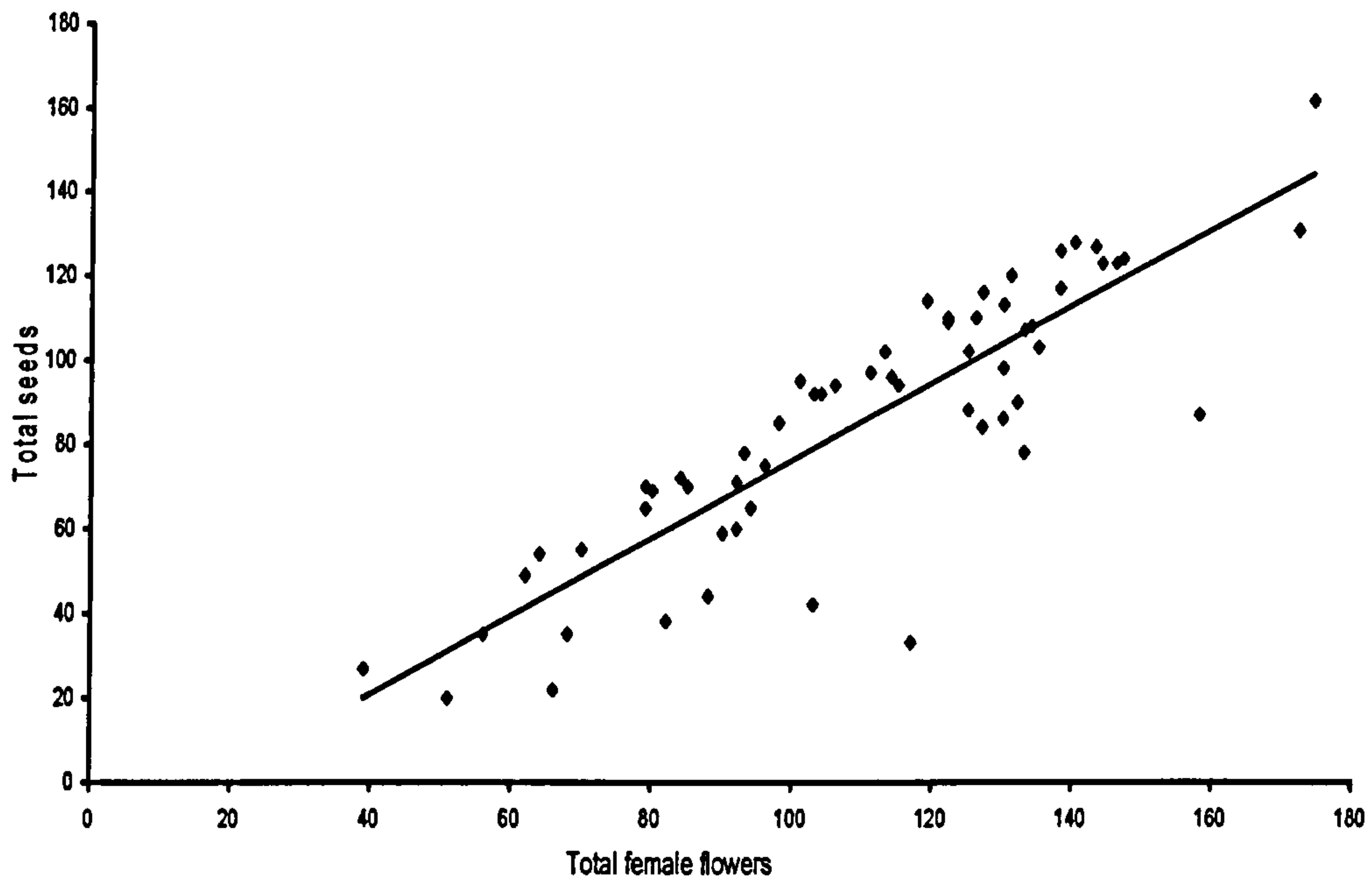


Fig. 3.1 The relationship between total female flowers and seed number in female figs of *Ficus montana*.

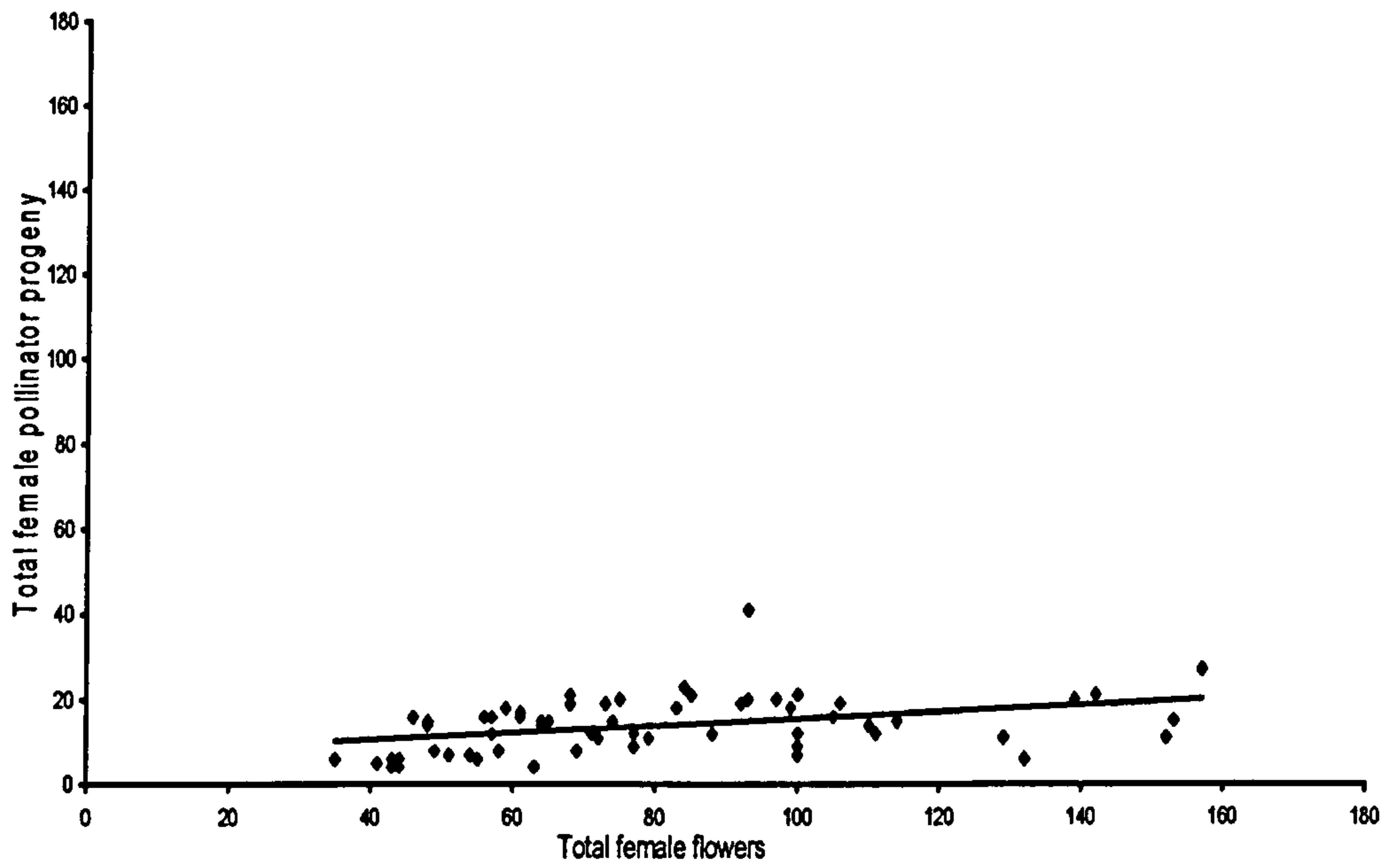


Fig. 3.2 The relationship between total female flowers and female pollinators in male figs of *Ficus montana*.

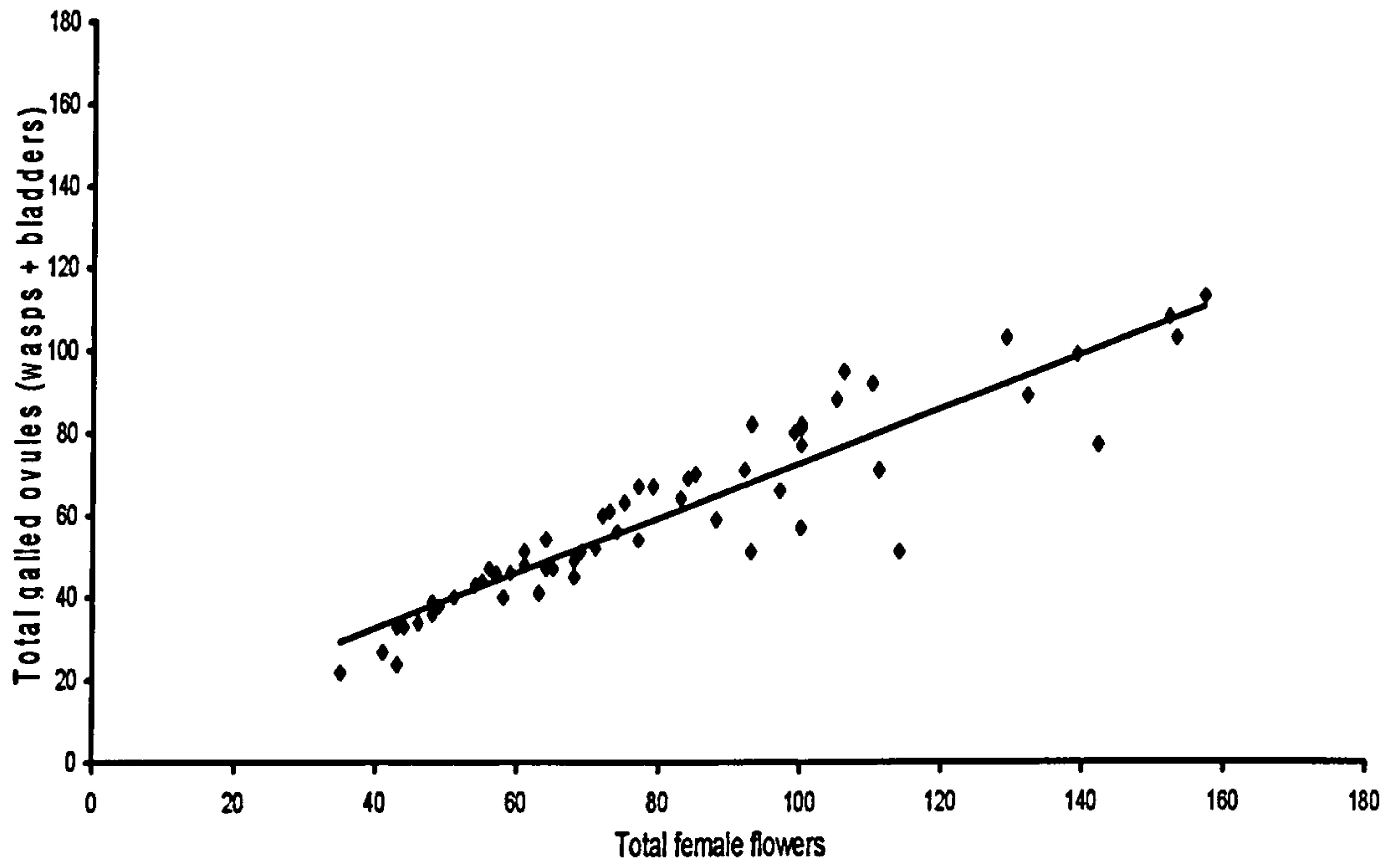


Fig. 3.3 The relationship between the number of female flowers in male figs and the number of galled ovules (wasps+ bladders) they contain.

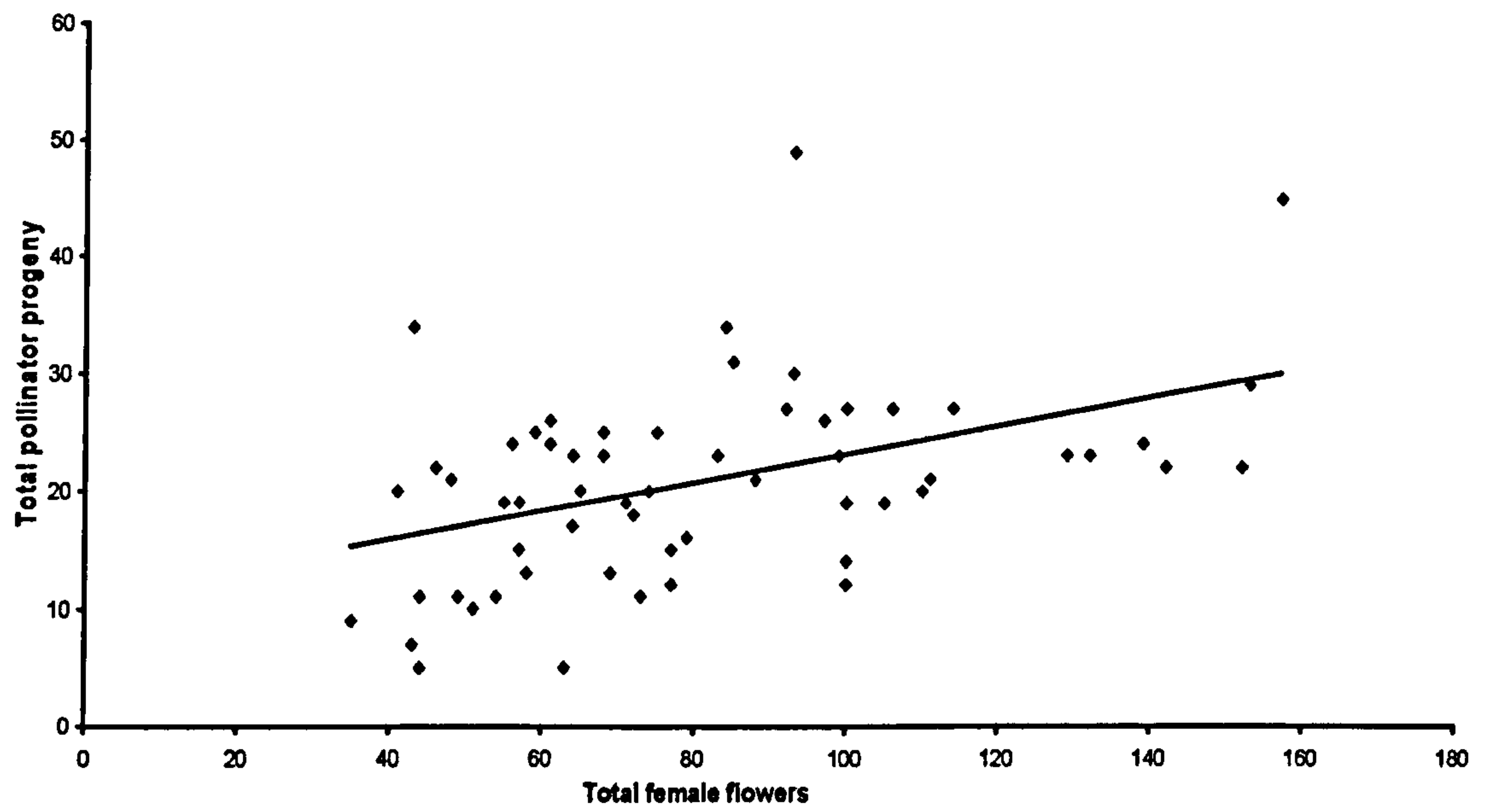


Fig. 3.4 The relationship between total female flowers and total pollinators in male figs of *Ficus montana*.

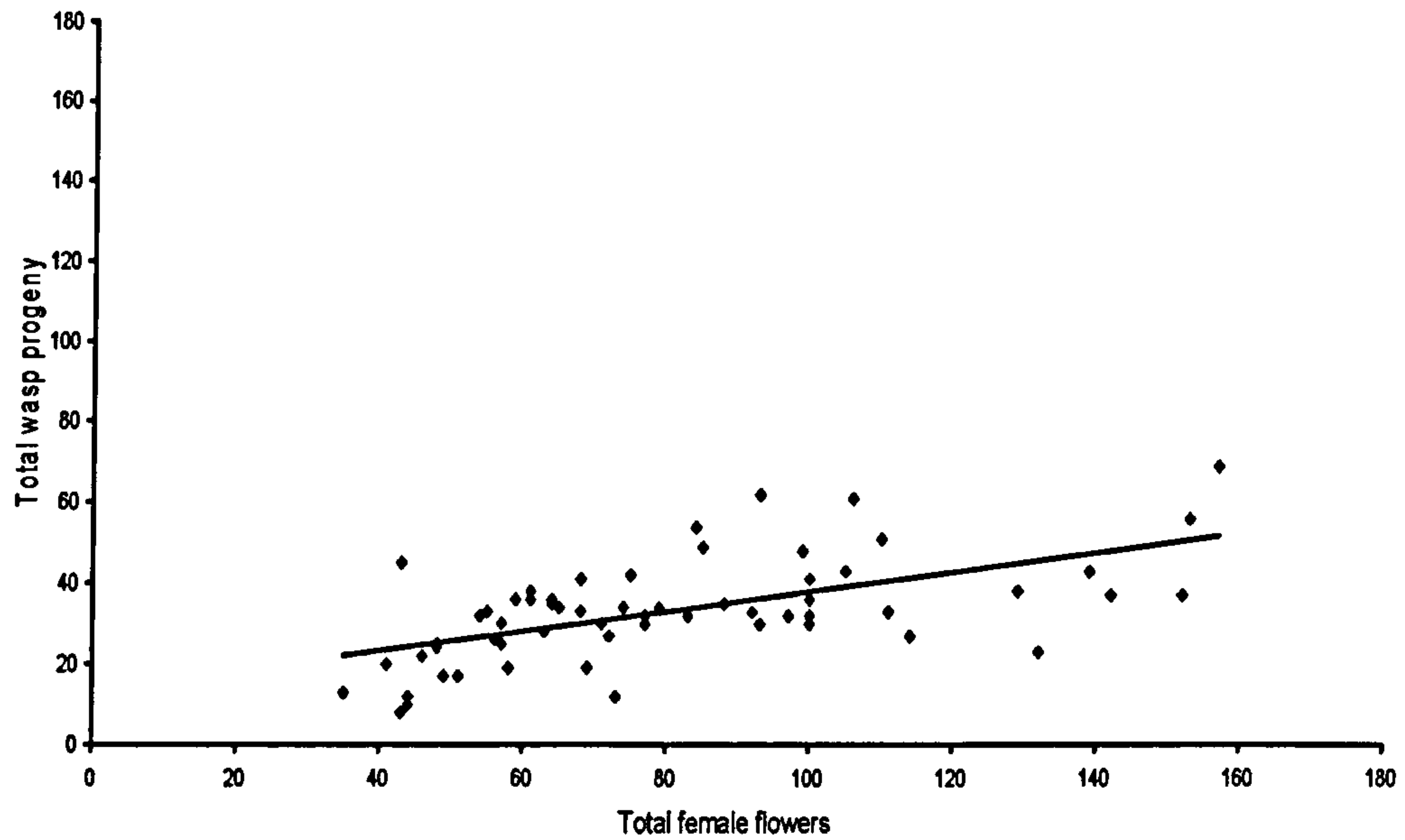


Fig. 3.5 The relationship between the number of female flowers present in male figs and the total number of fig wasp progeny (pollinators and parasitoids) that emerge from them.

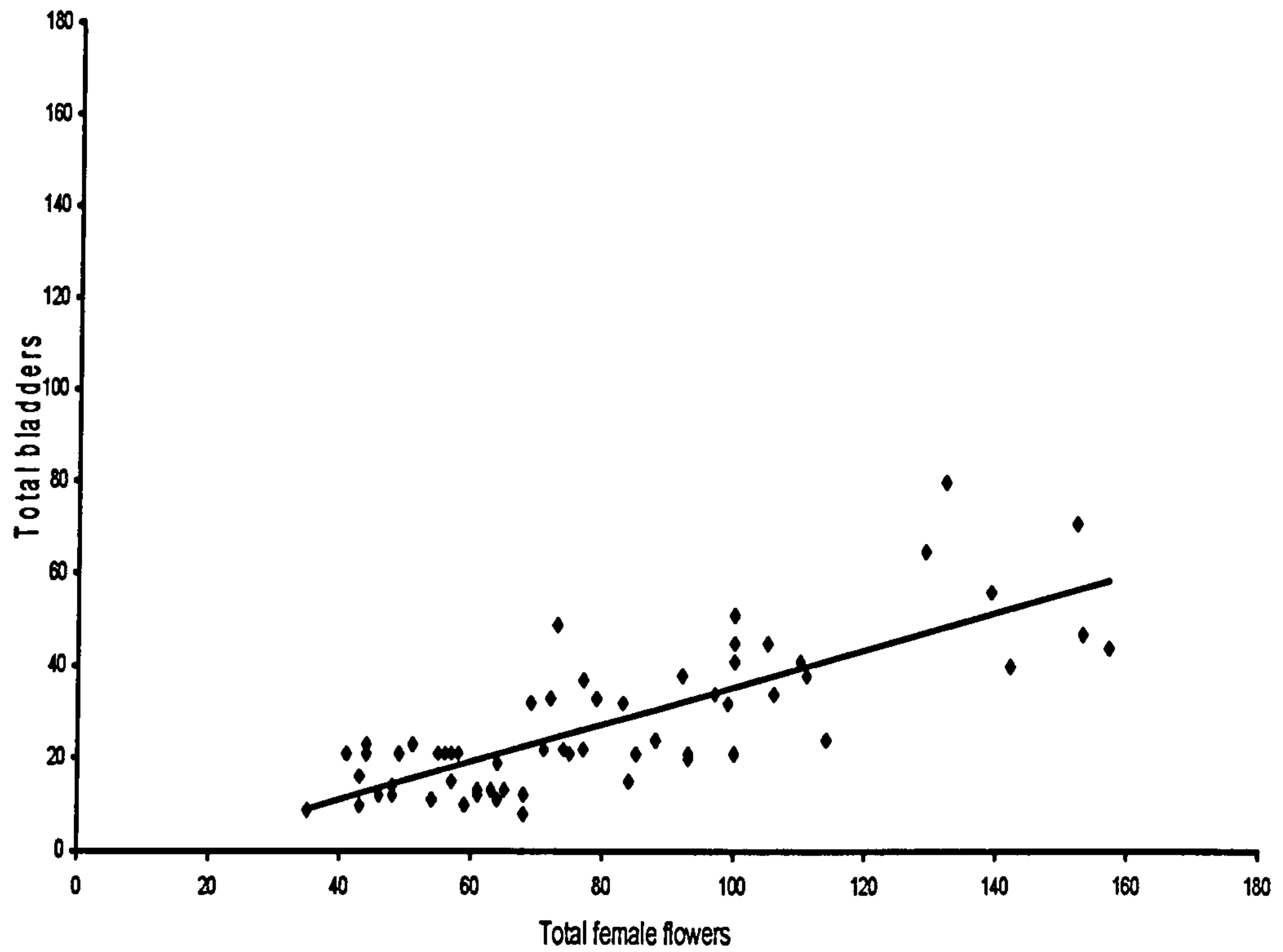


Fig. 3.6 The relationship between the total number of female flowers in male figs and the number of bladders (wasp-free galls) they contain.

4. Why do fig wasps pollinate female figs?

4.1. Abstract

The evolutionary conflict between functionally gynodioecious figs and wasps arises from the separation of seed and wasp production between the two sexes of the plant. Pollination and seed production depend upon fig wasps that leave no offspring if they enter a female fig, which has led many authors to question why they fail to distinguish between male and female figs and continue to enter the latter. A related question arises for those fig wasps that routinely re-emerge from figs they enter: why do they stop inside long enough to pollinate, rather than rapidly re-emerging? I investigated the timing and frequency of re-emergence of *Liporrhopalum tentacularis* wasps from male and female figs of *Ficus montana* and how different lengths of time spent inside the figs affects their progeny number (in male figs) and seed production (in female figs). I also used insecticide to kill the foundresses inside the figs at different times after they had entered the figs. Pollinator behaviour in female figs appears to closely reflect their behaviour in male figs. Once a pollinator has entered a female fig it is effectively dead, in so far as it can never reproduce, and natural selection can no longer operate to modify its behaviour. Consequently, the behaviour seen in female figs is likely to be reflecting what is best for the wasps once they have entered a male fig. Foundresses first start to re-emerge from both sexes after about one hour and keep on emerging through the next day. Rates of oviposition and pollination are rapid when the wasps first enter, but decline rapidly in both male and female figs. When fig wasps were killed within five minutes of entry into male figs, the figs always aborted, and so rapid emergence from female figs would not be expected because such a strong sanction is applied.

4.2. Introduction

Plants and their insect pollinators present one of the classic examples of mutualism. Quantification of the factors affecting the reproductive success of the participants in this partnership is central to the study of mutualisms in general (Bronstein, 1994; Herre *et al.*, 1999). Some insect-pollinated plant species have many pollinator species, whereas others have few or just one pollinator and in such cases the structure of their flowers is clearly adapted to facilitate the obligate relationship. Pollinating fig wasps (Hymenoptera, Agaonidae) are associated with fig trees (*Ficus*, Moraceae), which are characterised by their unique inflorescence, the fig (Weiblen, 2000). The fig is often called a fruit, but as it acts as a site of pollination and pollen production, it acts both as an inflorescence and compound fruit (Verkerke, 1989). There are two forms of breeding systems present in *Ficus*, monoecy and dioecy. The figs of monoecious species contain female and male flowers. They produce seeds, wasps and pollen within a single fig. The female wasps enter the figs through the ostiole carrying pollen from the natal figs, oviposit inside some of the female flowers and pollinate others (Weibes, 1979). Their larvae develop eventually into adults, mating occurs inside the fig and females exit carrying pollen. Gynodioecious (functionally dioecious) fig tree species have two different types of figs, occurring on separate trees. Female figs contain only female flowers and male figs contain mostly female flowers and some male flowers. The male fig trees produce wasps and pollen while female figs only produce seeds. In these dioecious fig trees the female wasps that enter figs on male plants can reproduce, just as in monoecious figs, but no seeds are produced, whereas foundresses entering female figs cannot lay eggs, but only act as pollinators.

How pollination is achieved depends upon the species of wasps, with two main categories (Galil and Eisikowitch, 1968; Ramirez, 1969). In some species pollen is transported passively, trapped on the body of foundresses and deposited incidentally in the figs. In other species, foundresses actively gather pollen from their natal figs into special structures on their bodies called pollen pockets and actively deposit the pollen directly on the stigmas of female fig flowers while ovipositing (Cook *et al.*, 2004). Such active pollination has evolved rarely (Cook *et al.*, 2004), and may have evolved in only three groups, involving Yucca moths (Pellmyr, 1996), Senita moths (Fleming and Holland, 1998) and figs wasps (Frank, 1985).

In the case of long-lived species such as fig trees, determining life time reproductive success is impractical. Whole tree productivity is a function of the number and content of figs produced during a tree's life (Herre, 1987) and the quality and quantity of seed dispersed (Shanahan *et al.*, 2001). Female pollinator wasps act as pollen dispersers and form the major part of a fig tree's investment in male reproductive function. Just as with seed, their numbers represent a more practical measure of fig reproductive success than life time recordings (Bronstein, 1988; Herre, 1989). To date, the majority of work on factors affecting fig tree reproductive success has centred on monoecious species (Herre, 1989; Corlett *et al.*, 1990; Cook and Power, 1996; Nefdt and Compton, 1996). In comparison, little is known about the dioecious species which make up about half of all fig tree species (Berg, 1989).

Pollen is dispersed by female pollinators, so the number of female wasps produced roughly equates to plant reproductive success through male function, while the number of

seeds produced reflects female reproductive function (Frank, 1985). Generally, it was assumed that after oviposition and pollination foundresses die in the figs (Hill, 1967, Ramirez, 1974, Hamilton, 1979), but Gibernau *et al.* (1996) showed that foundresses in several species of pollinators can re-emerge and subsequently oviposit in other figs. Moore *et al.* (2003a) showed that such re-emergence may be more common in dioecious than monoecious fig trees. While working on *Liporrhopalum tentacularis*, a pollinator of the dioecious *Ficus montana*, he also found that foundresses that re-emerged and entered a second female fig produced more seeds, indicating that individual *L. tentacularis* foundresses carry considerably more pollen than they disperse in any one single fig.

The mating system in dioecious fig species results in conflict between fig trees and their pollinators. For fig production to take place fig wasps must pollinate female figs. Even though some foundresses can re-emerge from female figs, they have lost their wings and antennae while entering the fig and so they are unable to reach a male tree, even though they may walk to, and enter several figs (Gibernau *et al.*, 1996). Thus, foundresses entering female figs always fail to reproduce and natural selection should favour the evolution of mechanisms to avoid them. In the long term this would be fatal for the mutualism as it would lead to the extinction of both fig tree and pollinator. Two main questions arise for those species that can re-emerge from the persistence of this clearly maladaptive behaviour of foundresses: why do they enter female figs and when they discover that it is a female fig where it is impossible to lay eggs, why do they not quickly re-emerge, after providing minimal or no pollination? The first question has been considered by many authors and the consensus is that the mutualism persists because foundresses are unable to discriminate between the fig sexes due to combination of

vicarious selection leading to inter-sexual mimicry and selection to rush for ovipositing sites, where the foundresses enter the first fig they encounter regardless of sex (Grafen and Godfray, 1991; Patel *et al.*, 1995). Exceptionally, *Blastophaga psenes*, the pollinator of *Ficus carica*, showed a preference for male figs over female figs (Anstett *et al.*, 1998). However, the production of male and female figs in temperate *F. carica* is temporally separated, so in nature very few male figs are available to foundresses at the time of year when they emerge. Host fig choice in fig wasps is based largely on species specific volatile blends released from the figs (Ware *et al.*, 1993). The discrimination shown by *B. psenes* may be linked to the small differences in volatiles produced by female and male figs of their species

Much less attention has focussed on why foundresses that are capable of re-emergence choose not to do so once they discover they are in female figs. One explanation for such behaviour is that foundresses that are in this situation have no further opportunity to reproduce and therefore natural selection can no longer operate on them. Consequently, the behaviour they exhibit in female figs reflects what would be appropriate if they had been lucky, and entered a male fig. If this is the case, then behaviour in female figs should closely mimic what occurs in male figs. To examine this question I compared the frequency and timing of re-emergence from male and female figs, and, as a measure for how active they are inside the figs, I recorded the rates at which they pollinate or oviposit at different time intervals after they enter.

4.3. Materials and Methods

I used a glasshouse population of the small shrubby fig tree *Ficus montana* (Subgenus: *Sycidium*, Section *Sycidium*) and the fig wasp *Liporrhopalum tentacularis* (Agaonidae). *F. montana* is a functionally dioecious species which despite its name is found in lowland forest edges, disturbed areas and similar habitats in South East Asia (Comer, 1952). Male figs are yellow to green in colour throughout their development whereas female figs are deep red when mature. Mature figs of both sexes are 8-12 mm in diameter. Fruiting within plants is typically asynchronous, with all stages of fig development often present on the same plant throughout the year.

Adult female *L. tentacularis* are diurnal. On entry into figs they lose their wings and most of their antennae while passing through the ostiole, but they are nonetheless capable of re-emerging. The sex ratios amongst their progeny vary according to how many eggs they lay, which is influenced by how many other foundresses share the figs with them (Moore *et al.*, 2002). *Sycoscapter* sp. is an undescribed solitary non-pollinating fig wasp that oviposits into male figs from outside. It destroys the larvae of *L. tentacularis* (S. Raja in prep.) The plant and pollinator populations were maintained in glasshouses at the Experimental Gardens of the University of Leeds, U.K. The plants originated from seed collected at CIFOR HQ, Bogor, Indonesia and from the Krakatau islands, Indonesia in 1995 (Moore *et al.*, 2002). The insects were collected from the same locations the following year.

Prior to experimental manipulations, mature male figs were left in plastic containers with mesh lids for 24 hours, so that the wasps could emerge naturally. Foundresses that

emerged from these figs were introduced into receptive male and female figs (sensu Galil and Eisikowitch, 1968) with a fine paintbrush. The figs were then surrounded by bags (made of fine mesh) to avoid further entries by other foundresses and also to catch foundresses emerging from the figs.

One hundred foundresses were introduced in total to 100 figs (50 male: 50 female) and after every hour the bags were observed to determine the timing of emergence of the foundresses from the figs. Vaseline was applied around the figs to avoid the re-entry of foundresses into the figs. The female figs were left on the plants for almost seven weeks (covered with fine mesh cloth to prevent entry by other foundresses) until maturity and dissected later to determine the seeds produced. For the male figs the same procedure was carried out again with the figs covered with fine mesh cloth to prevent entry by other foundresses and oviposition by *Sycoscapter* sp. The numbers of progeny produced by foundresses that emerged after varying lengths of time were compared.

Preliminary experiments with the natural insecticide pyrethrum (pre diluted, Fisons Ltd, U.K.) showed that contact with one microlitre was sufficient to kill foundresses in less than one minute. They also confirmed that pyrethrum is effective inside the figs and that it does not affect the eggs and larvae produced by the foundresses (Chapter 6).

Foundresses were killed at varying times after they entered the male and female figs (2, 2.5, 5, 15, 30, and 60, 120 and 240 minutes). This was achieved by gently introducing a syringe through the ostioles then injecting the pyrethrum. The figs were bagged afterwards to prevent the entry of other females and oviposition by *Sycoscapter* sp. When mature,

male figs were picked and placed singly into nylon mesh bags. The progeny present were sexed and counted. Mature female figs were opened and the seeds counted

4.4. Results

Thirty three (66 %) of the foundresses emerged from the male figs and thirty seven (74 %) emerged from the female figs. Consequently, there was no clear cut difference in the frequency of emergence of wasps from male and female figs (Chi square, $X^2 = 0.005$, $df = 1$, $P > 0.05$). The foundresses started emerging after one hour in both fig sexes and keep on emerging until the next day. The largest number of foundresses emerged 3-4 hrs after entry in both sexes of figs (Fig 4.1, Fig 4.2).

In male figs, there was no relationship between time spent (natural) in a fig and the total number of wasp progeny produced (Linear regression model 1: $F_{1, 53} = 0.21$, $P > 0.05$, $r^2 = 0.00$). The number of potential oviposition sites might influence progeny numbers but an analysis of covariance using female flower numbers as a covariate again showed no significant relationship between the numbers of progeny of foundresses and the time they spent inside the figs (ANCOVA, $F_{(3, 49)} = 1.51$, $P = 0.22$, Fig 4.3). Figs where foundresses did not re-emerge were not included in this analysis.

No relationship was found between the time (artificial) spent in female figs and the number of seeds they produced (Linear regression model 1: $F_{1, 29} = 2.14$, $P > 0.05$, $r^2 = 0.06$), nor the percentage of flowers forming seeds (Linear regression model 1: $F_{1, 29} = 2.02$, $P > 0.05$, $r^2 = 0.06$). There was also no significant difference in seed numbers when

female flower numbers were included as a covariate (ANCOVA, $F_{(6, 23)} = 1.07$, $P = 0.40$, Fig 4.4).

Figs that were injected 2 and 2.5 minutes after foundress entry were all aborted. In male figs, when foundresses were killed after varying lengths of time the brood size achieved varied from 6.44 ± 0.44 (Mean \pm SE) in the first five minutes to 27.80 ± 1.90 (Mean \pm SE) after 240 minutes (Fig 4.5). The speed of oviposition decreased rapidly over time (Fig 4.6), but as expected, brood sizes were significantly different for wasps allowed to oviposit for different lengths of time (Kruskal Wallis, $X^2 = 61.8$, $df = 5$, $P < 0.001$) and there was a positive relationship between available time and the total number of wasps produced (Linear regression model 1: $F_{1, 68} = 360.60$, $P < 0.001$, $r^2 = 0.84$).

There was a positive relationship between available time in female figs and the total number of seeds produced (Linear regression model 1: $F_{1, 57} = 26.84$, $P < 0.001$, $r^2 = 0.32$) and the percentage of flowers that were pollinated (Linear regression model 1: $F_{1, 57} = 50.71$, $P < 0.001$, $r^2 = 0.47$). The rate of pollination (as recorded by the numbers of resulting seeds) decreased with time, with 3.51 ± 0.54 (Mean \pm SE) seeds produced per minute in the first five minutes and 0.26 ± 0.36 seeds per minute produced over the course of 240 minutes (Fig 4.7). The total number of seeds produced varied considerably with different pollination times, ranging from 17.50 ± 2.70 to 64.5 ± 8.80 . Significantly different numbers of seeds were also produced with varying pollination times when female flower numbers were a covariate (ANCOVA, $F_{(5, 52)} = 36.42$, $P < 0.001$, Fig 4.8).

Two way analysis (after log transformation) of pollination and oviposition speeds showed a significant difference, with flowers being more rapidly pollinated than oviposited into (Two Way ANOVA, $F_{(1, 129)} = 139.98$, $P < 0.001$) as well as a significant interaction with time as pollination rates tailed off quickly as the fig flowers were saturated with pollen (Two Way ANOVA, $F_{(5, 129)} = 6.35$, $P < 0.001$).

4.5. Discussion

We did not find any difference in the timing and frequency of foundresses emerging from male and female figs. The numbers of progeny produced in male figs and the number of seeds in female figs was also found to be independent of the time spent by foundresses before they re-emerged. However, when we interrupted their oviposition or pollination artificially at different times by killing the foundresses, we found significant differences in the numbers of both progeny and seeds.

As a result of the mating system in dioecious fig species, a potentially costly source of conflict exists between fig tree and pollinator wasp. For fig production to take place, female wasps must pollinate female figs. However, even though foundresses entering female figs fail to reproduce, and should be selected to avoid them, given the obligate nature of female fig pollination, the evolution of such discriminatory abilities could ultimately be fatal for the mutualism, leading to the extinction of both fig and pollinator.

Contrary to earlier opinion, recent work has indicated that foundresses are often able to re-emerge from the first figs they enter, whether the figs are male or female and can then enter second or third figs (Gibemau *et al.*, 1996; Moore *et al.*, 2003a and S. Raja in prep.).

Male trees may be selected to allow foundresses to re-emerge because it results in more efficient use of pollinator resources, as on female trees pollinator numbers may often be limited. Certainly, re-emerging *L. tentacularis* foundresses are capable of pollinating two or more female figs (Moore *et al.*, 2003a). Foundresses that entered two figs produced considerably more seeds than females that did not remerge from the first fig they entered. Thus it is also apparent that female *F. montana* trees benefit from more efficient pollen dispersal if they allow *L. tentacularis* to re-emerge. In contrast, foundresses re-emerging from male figs produced only slightly more progeny than those who did not re-emerge (Moore *et al.*, 2003a) although there may be additional benefits for both plant and insect through spreading the risk.

Re-emerging foundresses cannot fly off in search of other trees as they lose their wings and most of their antennae, so those that enter female figs always fail to reproduce and so they are not subject to selection once they enter a fig. We suggest that they behave in the same manner in both tree sexes because foundress behaviour is selected in male figs. In order to gain the advantage of multiple fig entry on female trees, male trees have to allow foundresses to re-emerge from their own figs and the wasps have to benefit from such behaviour. Such selection where one party (the female fig) exerts pressure on another (the male fig) through its interaction with a third organism (the female pollinator), has been termed vicarious by Grafen and Godfray (1991). This phenomenon also explains the lack of differences in foundress re-emergence rates from male and female figs. Selection of this type may also account for why male trees of other dioecious fig species allow foundresses to re-emerge from figs. A further advantage of allowing foundresses to enter more than one fig is that it is likely to increase the genetic diversity of the seeds that are produced,

and the opportunity for pollen competition, as more foundresses are likely to have entered each fig. There advantages for dioecious figs may also be the reason why the incidence of foundress re-emergence is apparently lower among monoecious fig species than dioecious ones. An increase in foundress density in monoecious figs often results in an increase in male pollinators relative to females due to “sex ratio adjustment” and a decrease in seed production (Anstett *et al.*, 1998). Hence for monoecious figs the advantages of multiple fig entry in terms of increasing fitness in male reproductive function will have to be higher than in dioecious male figs for them to be selected to allow foundresses to re-emerge.

Selection also operates on foundresses on male figs to determine the optimal time for the wasps to exit. We found that the time that foundresses spent in female figs was similar to that spent in male figs. A foundress presumably leaves a male fig when on average potential gains from leaving outweigh those from staying. An element of chance will influence how many foundresses will be able to enter and lay eggs in second or third figs. Given that pollinator sex ratios become more female biased in larger clutches it is slightly better for the plant if the wasps stay longer. So from the plant point of view it would be the best to have pollinators inside for longer periods. For the plants, gains from an increasing number of seeds in one fig are balanced by gains in having additional figs pollinated, mediated by the inevitable risk that many foundresses will die along the way. We predict that the optimum from the plant’s point of view should be influenced by the chance of entering the subsequent figs, so the better the chance of survival, the earlier the wasp should be encouraged to leave. Such ‘encouragement’ can only be generated via fitness gains accruing from pollinator behaviour on male plants, but mortality factors are likely to be similar on the two sexes. From the wasp’s point of view it should spend the

same amount of time in a female fig as it would spend in a male fig to optimise its reproductive success. In agreement with this, we found that the time spent in male and female figs were very similar.

The rates of pollination and oviposition under both natural and controlled conditions were found to be same, with both falling over time. Pollination rate was quite high as compared to the rate of oviposition, which shows that female pollinators unload pollen faster than they can lay eggs. As the proportion of ovules with eggs laid in them increases, so the time spent searching without laying eggs increases and thus the rate of oviposition decreases with time (Yu *et al.*, 2004). Also there is evidence that foundresses may first oviposit in shorter styled flowers (Compton *et al.*, 1994) and only later into longer styled flowers, which have longer handling times, so oviposition rate drops over time.

Females of *F. montana* are active pollinators (N. Suleman in prep.) and pollen is also abundant as the ratio of male flowers to female progeny in figs of *F. montana* can be as high as 1:1. Most female pollinators might be expected to carry abundant pollen. An individual fig tree's production of seeds or wasps in any given fruiting episode may be pollinator limited, or it may be the case that figs do not have enough resources to develop seeds or wasps. Therefore the relationship between wasp, fig and the reproductive properties of the fig and of the wasp are quite complex.

The vicariate selection seen in the mutual mimicry of male and female figs also extends to selection on the behaviour of wasps once they enter female figs. If our hypothesis is correct, for fig tree species in which there is no realistic chance of emerging females ever

successfully ovipositing in second figs on male plants, then re-emergence behaviour should not be evident in either plant sex. Figs that are produced in isolation, well away from other figs, would be an example. Also fig tree species where ant predation is constantly high on male plants.

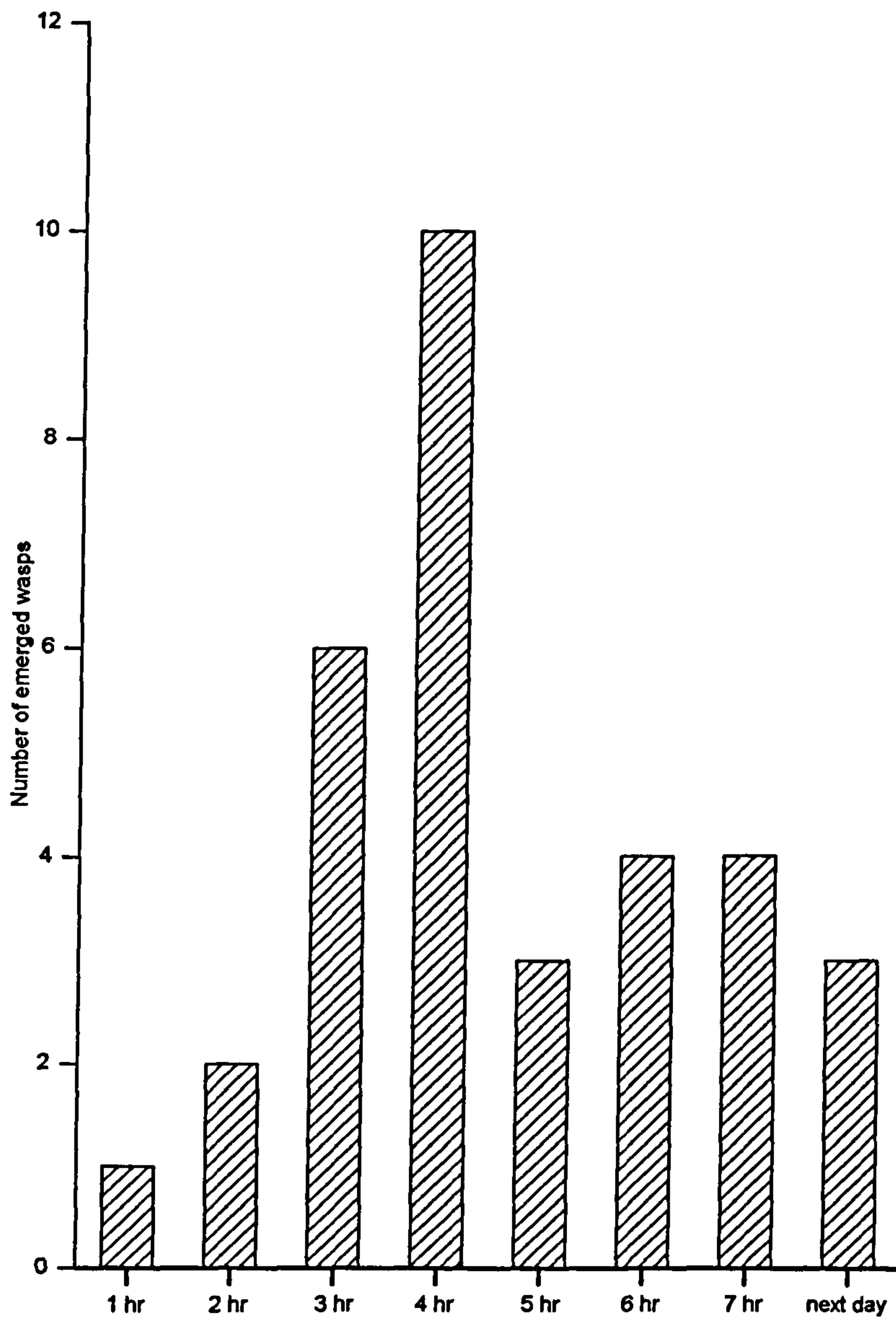


Fig. 4.1 The timing of emergence of naturally emerging foundresses from male figs.

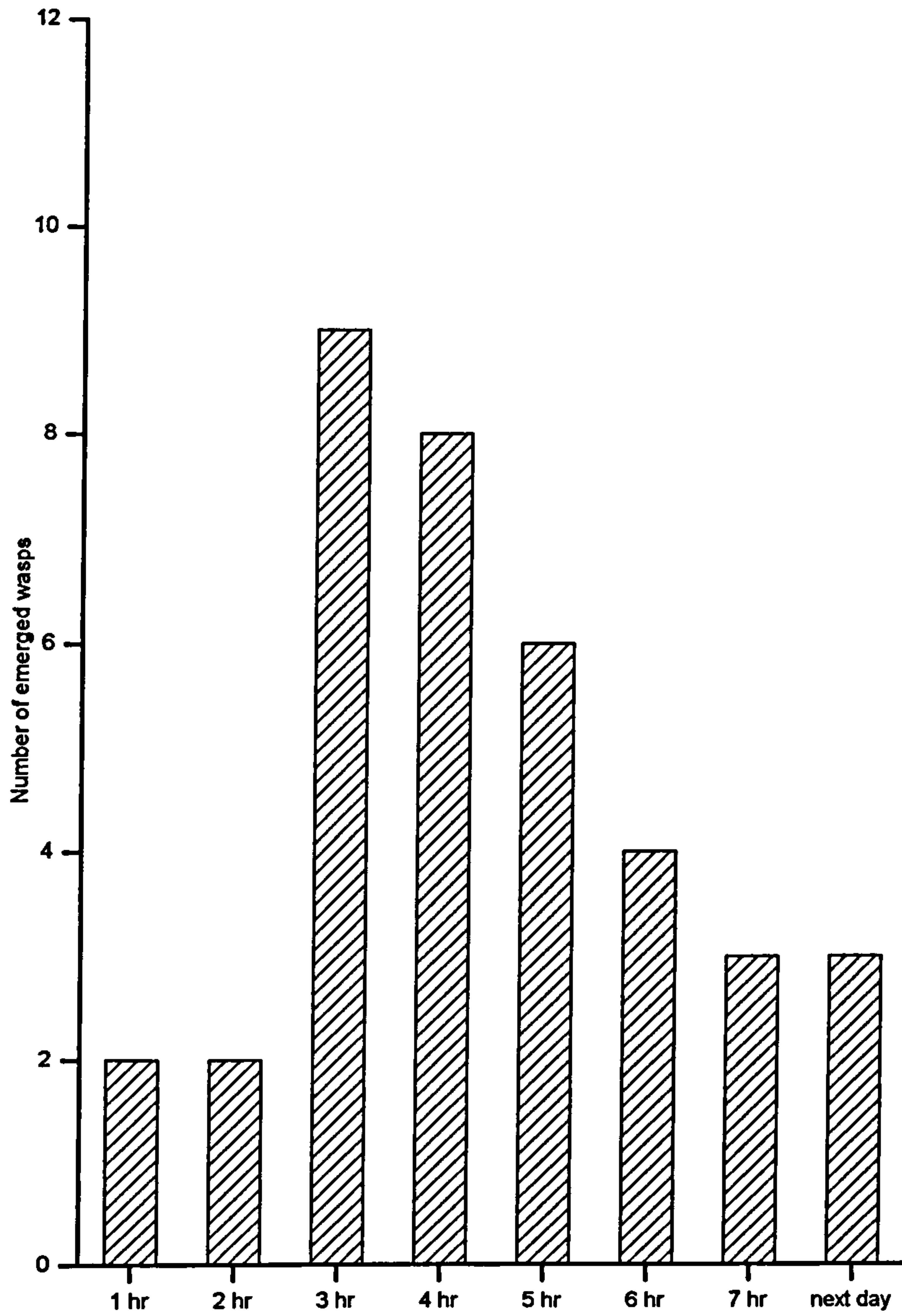


Fig 4.2. The timing of emergence of naturally emerging foundresses from female figs.

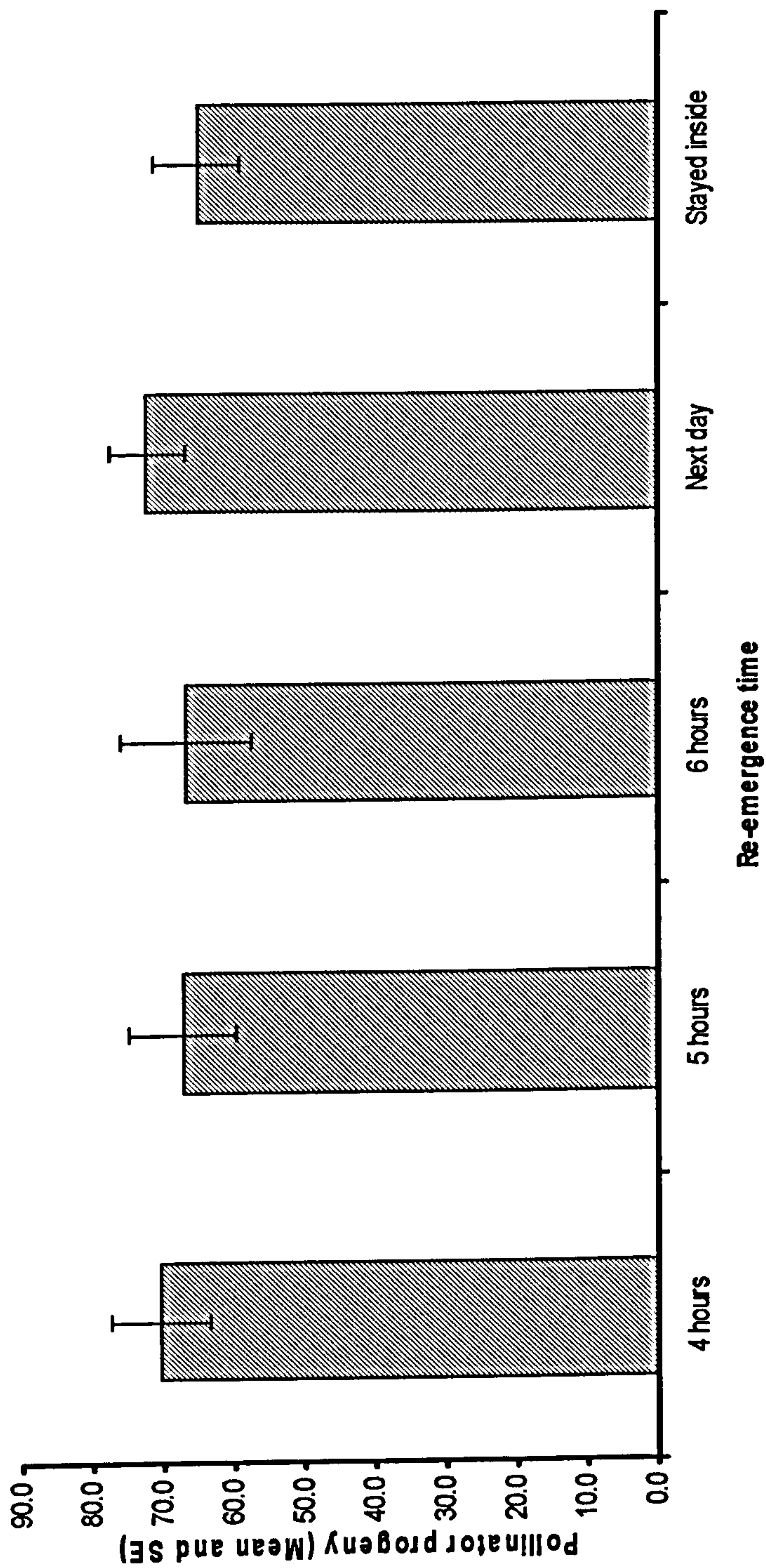


Fig. 4.3 Progeny produced by naturally emerging foundresses that re-emerged from the figs after different time intervals

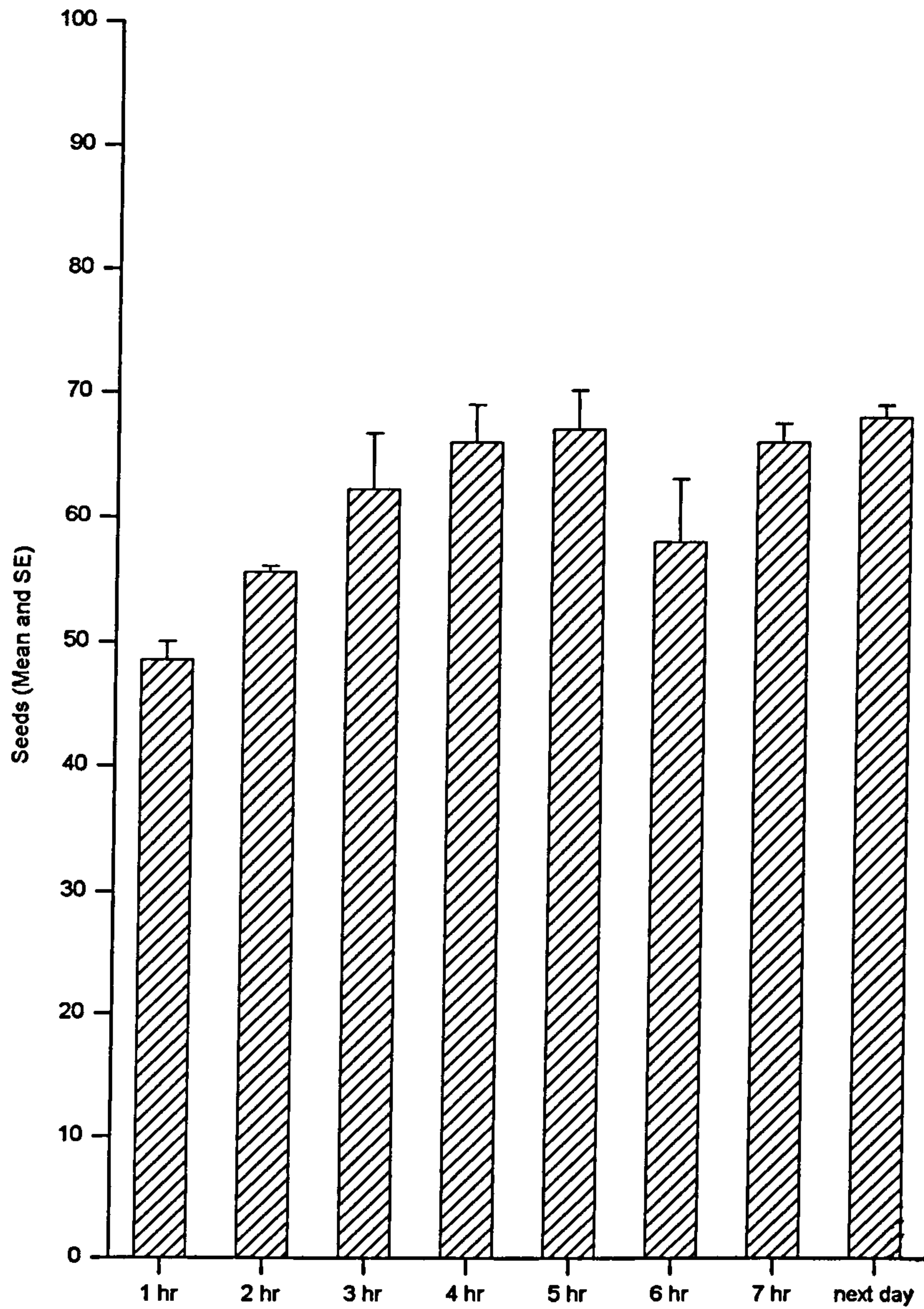


Fig. 4.4 The number of seeds produced from female figs when naturally emerging foundresses were present in figs for varying times.

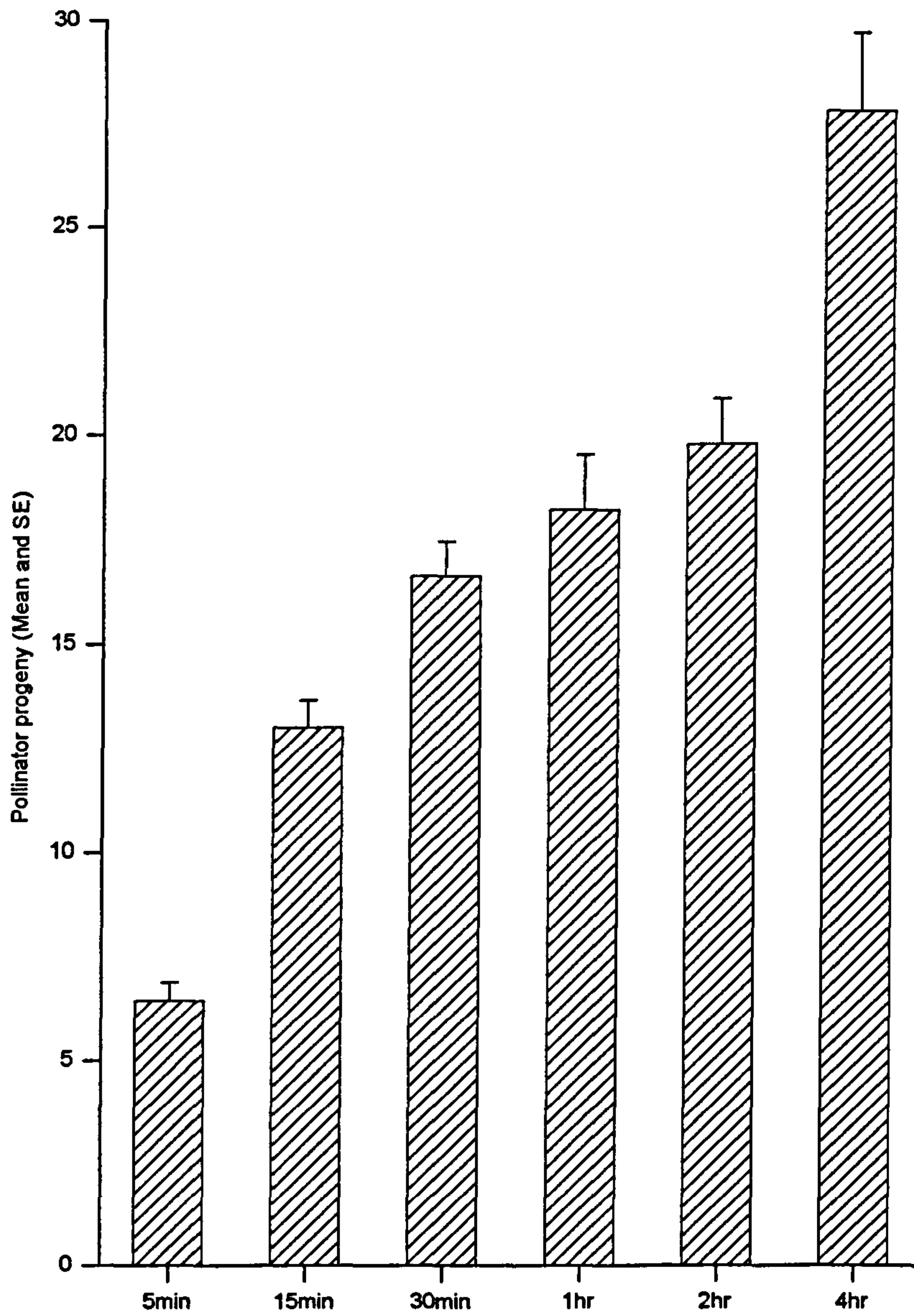


Fig. 4.5 The number of pollinator progeny produced in male figs when foundresses were experimentally manipulated to be in figs for varying times.

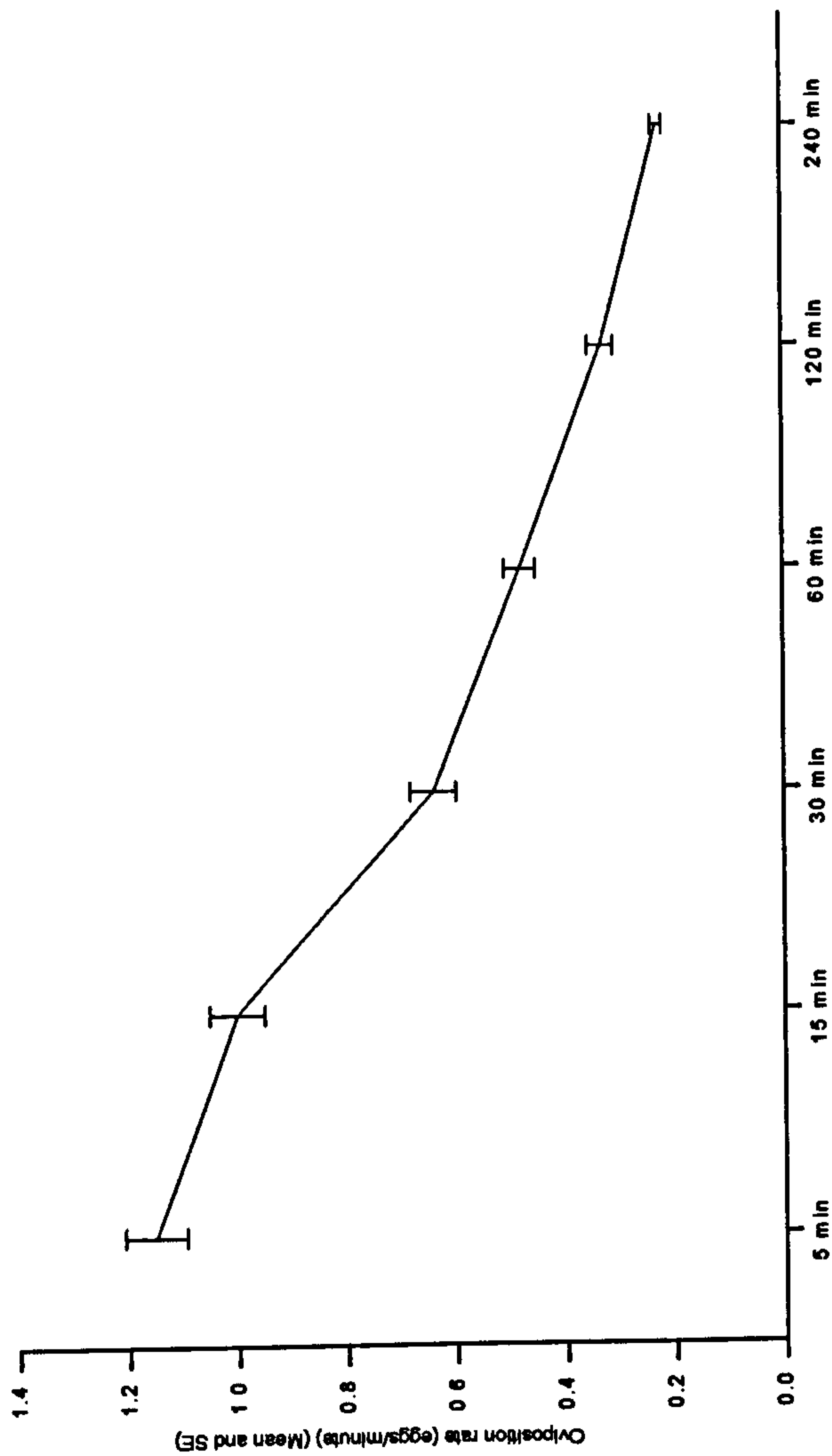


Fig. 4.6 Changes in the estimated rates of oviposition by winged pollinators during experimentally controlled time spent inside a fig. Rates at each time interval are calculated over the entire period since they entered.

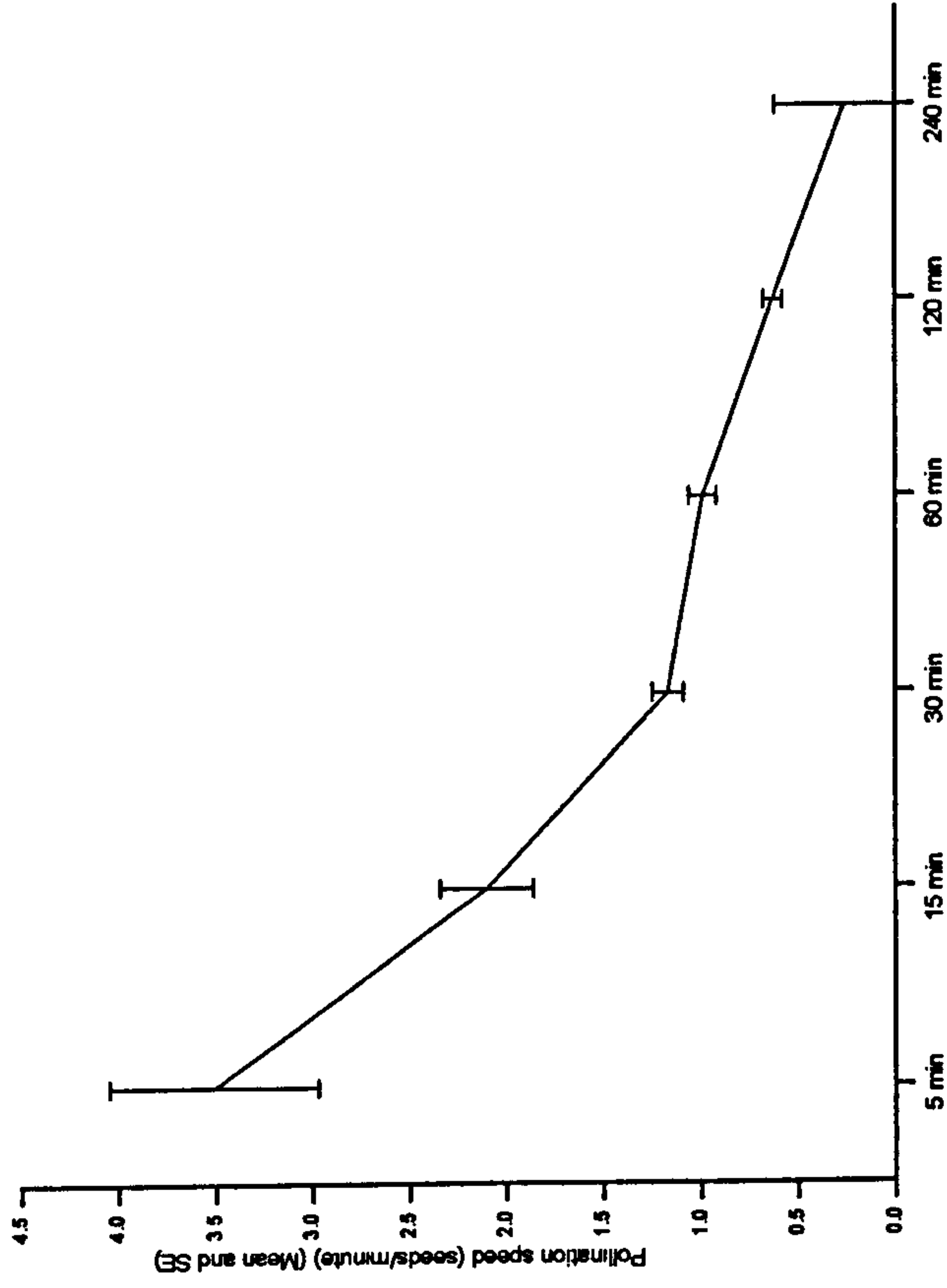


Fig. 4.7 The number of seeds produced per minute for experimentally manipulated pollination times in female figs. Rates at each time interval are calculated over the entire period since they entered.

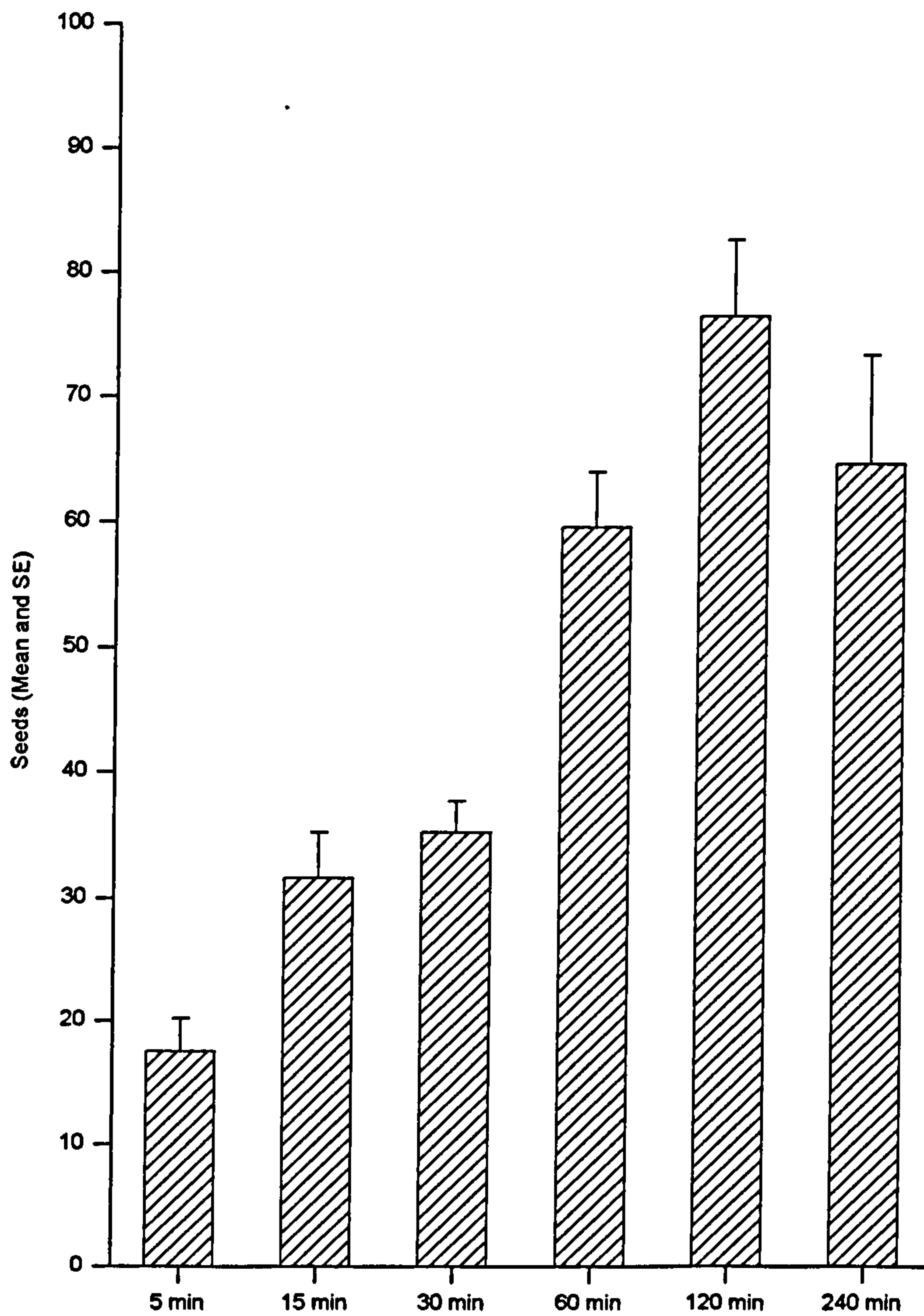


Fig. 4.8 The total number of seeds produced by female figs when foundresses were allowed to pollinate for different periods of time.

5. Decision making in fig wasps: Factors influencing foundress re-emergence

5.1. Abstract

Each *Ficus* species is generally pollinated by a single species of fig wasp which must enter a fig through its ostiole, in order to gain access to the flowers where they lay their eggs. Pollinators are typically trapped in the figs and die inside them, but some pollinators can re-emerge and subsequently enter and oviposit in further figs. I investigated factors affecting the likelihood of re-emergence of the Asian fig wasp *Liporrhopalum tentacularis* from male *Ficus montana*, its gynodioecious fig tree host. Likelihood of re-emergence is not influenced by wasp age, flower number (fig size), or timing of entry into the figs, but re-emergence is more frequent from older figs that have been waiting longer to be pollinated.

5.2. Introduction

The quality of host plants for insects varies considerably and both egg laying and time allocation decisions by insects therefore become very important for successful selection of host plants (Green, 1984). Foraging insects have to track changes in host plant quality as their time can potentially be used more effectively on other hosts. The decision made by an insect whether or not to leave its host and select another host plant is likely to be one of the key factors determining its success. Which mechanism an animal uses strongly depends on the available information on patch and habitat quality (Iwasa *et al.*, 1981). Factors determining departure from host patches have been studied in a number of insect parasitoids and large variability in the behaviour between species as well as individuals is

commonly observed. A number of theoretical models attempt to predict the host time allocations that maximise an individual's fitness (Iwasa *et al.*, 1981; Mangel, 1987; Sirot and Bernstein, 1997). According to these models motivation to oviposit is determined both by physiological (egg load and life span) and ecological variables (host quality and host availability). One of the first models to describe optimal patch use is the marginal value theorem (MVT, Charnov, 1976), which predicts that a single animal exploiting a patch of resources should leave it and search for another host when its rate of gain drops below the expected mean rate in the whole habitat. Mangel's (1987) model predicts that when egg load is high parasitoids should attack a large number of hosts because the expected number of future encounters is not sufficient for all eggs to be laid. Thus females when faced with hosts which are difficult to handle often reject them. Each host rejection induces a significant increase in the tendency of wasps to leave the patch.

Two main factors, structure and quality of the host, influence oviposition strategy, and the choice either to exploit or to leave a host (Alphen *et al.*, 2003). In a good environment (containing only unparasitized hosts) each oviposition of *Anaphes victus* (Hymenoptera, Mymaridae) an egg parasitoid of *Listronotus orgeonensis* (Coleoptera, Curculionidae) in the patch increased the motivation to stay in the patch (Van Barren *et al.*, 2005), but when the female rejected a host, its tendency to leave the patch increased. Age of parasitoid as well as egg load can also influence parasitoid patch exploitation strategies (Charnov, 1976; Mangel, 1989), but working on *Pachycrepoideus vindemmiae* (Hymenoptera, Pteromalidae), a solitary parasitoid species, Goubault *et al.* (2005) found no linear relationship between increasing age of females and their patch-leaving tendency.

Hirose *et al.* (2003) also found that in parasitoids host age influences host quality as it is difficult for some females to oviposit in hosts that are in advanced stage of development. Hence on the basis of theory and empirical observations, we can say that if the environment is of good quality animals should spend a relatively longer amount of time there and there will be lower tendency to leave the host. The general context of this work is that it provides an understanding of decision-making process in insects from a functional point of view.

A classical example of mutualism involves the highly coevolved relationship between *Ficus* plants (Moraceae) and their phytophagous obligate pollinating wasps (Hymenoptera, Agaonidae) (Galil, 1977; Weiblen *et al.*, 2000). Pollinators and their host fig plants often exhibit one-to-one host specificity though the number of exceptions is rising (Ramirez, 1969; Weibes, 1979; Compton *et al.*, 1996). Fig wasps are the exclusive pollinators of fig trees and are generally host tree specific (Weibes, 1979). They locate their particular species of host tree through plant specific volatile cues (Ware *et al.*, 1993).

Around half the species of fig trees are monoecious and half are functionally dioecious (though anatomically gynodioecious). Monoecious species have one type of tree where each fig contains both male and female flowers (Verkerke, 1989). The male flowers produce pollen and the female flowers develop into seeds or become galled by the fig wasps. When the adult female wasps emerge they actively or passively collect pollen which is then transferred once they successfully reach another fig that is at an earlier stage of development. Dioecious species have distinct male and female trees (Verkerke, 1989). Female trees have figs with no male flowers that prevent any pollinators that enter from

laying eggs, so they only produce seeds. In contrast, male figs allow wasp oviposition, but are typically unable to produce any seeds, only (pollen-carrying) fig wasps.

The length of the receptive period during which figs are attractive to pollinators can be less than five days (Galil and Eisikowitch, 1968; Bronstein and Hossaert-McKey, 1996), but a much longer period of receptivity was observed in *F. exasperata*, lasting from five to seven weeks (Patel, 1996). Similarly, in *F. carica* the receptivity of female figs can last up to three weeks (Khadari *et al.*, 1995). Un-visited figs can remain receptive for several weeks, and continue to increase in diameter (Weiblen *et al.*, 2000). Noting that the ostiolar bracts become less tightly packed as fig diameter increases during the receptive period, the authors predicted that later entered, larger figs should be easier for foundresses to re-emerge from (Khadari *et al.*, 1995). After the entry of a pollinator, receptivity is lost quickly (Ramirez, 1974; Janzen, 1979), though in *F. carica* and *F. aurea*, individual figs apparently extend their receptive period for a few days if they are visited by only a single pollinator wasp (Khadari *et al.*, 1995). It has been determined that un-pollinated figs of *Ficus montana* can remain receptive for seven days or more (N. Suleman in prep).

Until recently it was assumed that after pollination and oviposition pollinators die in the first figs they enter (Hill, 1967; Ramirez, 1974; Frank, 1985; Bronstein, 1988). However, Gibernau *et al.*, (1996) showed that foundresses of several species re-emerge from the figs after pollination and oviposition. They also found out that the frequency of re-emergence from figs varied among species and suggested that some figs could be unable to prevent re-emergence due to the time of receptivity at which they are entered by pollinators. In *Ficus microcarpa*, only 63.9 % of pollinated figs contained pollinators, again indicating

that they routinely re-emerge from the figs (Chen *et al.*, 2001). Patel *et al.* (1995) determined that ostiolar bracts repeatedly become less tightly packed when a fig increases in diameter, and this might be the reason that later entered figs should be easier for foundresses to re-emerge from. It is also suggested that re emergence is much more common from dioecious than monoecious species (Moore *et al.*, 2003a)

Moore *et al.* (2003a), investigated why the pollinator fig wasp *L. tentacularis* re-emerges from the figs of *F. montana*. It was shown that foundresses contained more eggs than male figs contained oviposition sites (measured as the number of female flowers). They found that frequencies of re-emergence were unaffected by either foundress density or the number of oviposition sites (female flowers) in male figs. Similarly, fig diameter at the time of foundress entry also did not affect foundress re-emergence.

In this paper we further investigate the re-emergence behaviour of *L. tentacularis* from male figs of *F. montana*. We examine a variety of factors that might influence this decision including female flower number, fig and wasp age. We also examined whether immediate (two foundresses were simultaneously introduced into a single fig) and sequential (after introducing first foundress the second foundress was introduced an hour later) entry of pollinators into figs influenced the likelihood of re-emergence.

5.3. Materials and Methods

5.3.1. Natural History

The development stages of the fig fruits are generally subdivided according to the terminology devised by Galil and Eiskowitch (1968). The stages before the wasp entry are

called the A or pre-floral phase and this phase may take some three weeks. In the receptive or B phase the wasps enter the fig through the ostiole. This phase may last a few (3-6) days and when no wasps enter the fruit, it may extend longer. The subsequent phase is the C or inter-floral phase, in which the wasps develop in the male fruits, and seeds develop in the female figs. The C phase can last from two to six or up to ten weeks, depending on the fig species. At the D or emission phase the next generation of wasps emerges, the anthers open in male figs and the female wasps leave the figs, loaded with pollen. This phase may take 3-4 days (Ramirez, 1974). After the wasps have left, the figs ripen further and become attractive to animal dispersers (E phase).

Newly emerged foundresses of *L. tentacularis* contain an average of around 104 eggs while male and female figs of *F. montana* contain an average of about 87 and 95 female flowers respectively, although flower numbers are highly variable (Moore *et al.*, 2003b). It has also been found that most of the time wasps do not survive overnight, but if they do survive, their reproductive success was the same as that of recently-emerged wasps (N. Suleman in prep). Foundresses of *L. tentacularis* are willing to re-enter the figs they emerged from as often no suitable figs are nearby (Personal observation). Zavonda (2004) found that the progeny numbers of foundresses of *L. tentacularis* that share a fig are often unequal in size. The sex ratios of *L. tentacularis* broods become less female biased with increasing foundress numbers (Moore *et al.*, 2002) and Moore *et al.* (2005) using micro satellite markers have shown that this is related to the size of the clutch laid in a particular fig.

A population of the South–East Asian pollinator fig wasp *L. tentacularis* is maintained on its dioecious host plant *F. montana* in glasshouses at the Leeds University Experimental Gardens. The fig trees originate from seeds collected at the Centre for International Forestry Research (CIFOR) plantation, West Java, Indonesia and from Rakata, also in Indonesia. The wasps also originated from CIFOR and have been in culture since 1996 (Moore, 2001).

We conducted experiments examining the effects of the following factors on re-emergence of foundresses from male *F. montana* figs.

To examine the effect of fig age on the frequency of foundress re-emergence, figs in the late pre-floral (Galil and Eiskowitch, 1968) phase on trees in the glasshouse population were identified and fine mesh bags were placed over them. One to two weeks later, when the figs had entered the female receptive “B” phase of development, single foundresses were placed individually on the ostioles with a moist paint brush and allowed to enter the figs. The figs were bagged afterwards to prevent further entries of foundresses and the area around the ostioles of the figs was covered with vaseline so that any emerging foundresses could not re-enter the figs. Twenty four hours later the number of foundresses (dead or alive) found inside each bag was recorded. The procedure was carried out using figs on the first day when they were receptive (day 0, n = 30), a day later (day 1, n = 25) and the day three (day 3, n = 25). The onset of receptivity was determined by holding the figs by fingers and when they were very soft it was said to be 0 day receptive, while as the days proceed they become harder.

To determine the effect of wasp age on the frequency of re-emergence, figs were again bagged at the pre-floral stage to prevent pollinator entry. When they were receptive (day 0), the bags were removed from half of the figs and single foundresses that were freshly emerged that morning from other figs were allowed to enter with the help of fine paint brush ($n = 26$). The figs were bagged afterwards to prevent further entries of foundresses and ostioles were treated as above. The process was repeated in the late afternoon with older wasps ($n = 26$) which had emerged from natal figs that morning. Next day, the number of wasps that had emerged into the bags was recorded.

To examine the effect of female flower numbers on re-emergence, figs in the late pre-floral stage were identified and bagged as before. Single foundresses were introduced into each fig and were treated as before. The next day any emerged foundresses were counted and the figs were dissected to count the number of female flowers present ($n = 52$ figs).

To determine the effect on re-emergence of foundress entry time into figs, receptive figs were prepared as before. In some of the figs ($n = 34$ figs) foundresses were placed individually on the ostiole and after one foundress had entered a second was allowed to enter the same fig immediately. With other figs there was a delay of one hour before the second wasp was allowed to enter ($n = 30$ figs).

5.4. Results

Fig age had a significant effect on the likelihood of re-emergence by the female wasps introduced in the morning, with the proportion of foundresses re-emerging increasing with

the age of the figs (Day 0 = 46.6 % re-emergence, Day 1 = 68.0 %, and Day 3 = 80.0 %, Chi square, $X^2 = 6.84$, $df = 2$, $P < 0.05$) (Fig 5.1).

Out of 26 young wasps introduced in the morning into the figs, 53.8 % emerged while 46.1 % stayed inside the figs. Out of 26 older wasps introduced in the afternoon 61.5 % emerged and 38.4 % remained inside the figs. Wasp age therefore did not significantly affect the likelihood of re-emergence from the figs (Chi square, $X^2 = 0.315$, $df = 1$, $P > 0.05$) (Fig 5.2).

Flower numbers from the re-emerged figs (young wasps, 0 day receptive figs, $n = 26$ figs) ranged from 55-96 (80.00, Median), while the figs where pollinators stayed ranged from 47 -140 flowers (80.00, Median). The re-emergence of foundresses again not significantly affected by the flower numbers present inside the figs (Mann Whitney, $U = 73.0$, $P = 0.59$, median).

The above results were all based on figs where a single foundress entered. When 35 pairs of foundresses were introduced in close succession 58 % re-emerged from figs, while 41.9 % stayed inside the figs (both emerged = 18, both remained inside = 13 and one remained inside = 4). Comparison with the re-emergence of foundresses when only single foundress was allowed to enter the figs to the simultaneous entry of two foundresses did not find any significant effect (Chi square, $X^2 = 0.258$, $df = 1$, $P = 0.61$).

Out of 30 pairs of foundresses that entered the figs one hour apart, 55.6 % re-emerged and 44.4 % remained inside the figs (both emerged = 15, both remained inside = 12 and one

remained inside = 3). A comparison of single foundress and sequential entry emergence rates found no significant difference (Chi square, $X^2 = 0.01$, $df = 1$, $P = 0.90$).

The likelihood of a wasps emerging from figs where two wasps entered simultaneously (13 remained, 18 emerged) or sequentially (12 remained, 15 emerged) was also not significantly different (Chi square, $X^2 = 0.03$, $df = 1$, $P = 0.84$).

5.5. Discussion

L. tentacularis foundresses routinely emerge from male *F montana* figs. We found that re-emergence rates were independent of female flower numbers (equivalent to the number of potential oviposition sites) in figs entered by a single foundress wasps and we also did not find any change in the likelihood of re-emergence by foundresses that entered together with one another or an hour apart. Re-emergence was also unaffected by wasp age (morning or afternoon of the day of emergence). The only factor that was found to influence the likelihood of re-emergence was the age of the figs, with wasps more likely to have vacated older figs.

In some fig species, oviposition sites within figs are routinely limiting (Nefdt, 1989; Herre, 1989) and for species that do not routinely vacate the figs this leads to dead foundresses inside the fig cavity with eggs still in their ovaries. Gibernau *et al.* (1996) suggested that foundresses might re-emerge from figs because of such oviposition site limitation, with emergence allowing them to increase the number of their offspring by ovipositing in a second fig. Contrary to this, in *L. tentacularis* we found previously those foundresses that re-emerged and laid more than one clutch did not produce significantly

more offspring than foundresses who failed to re-emerge (N. Suleman *et al.*, in prep). Moore *et al.* (2003a) found that newly emerged foundresses of *L. tentacularis* usually contain significantly more eggs than the number of female flowers in male figs of *F. montana*. Because of this, it is clearly advantageous for this species to re-emerge and to oviposit in another male fig. Oviposition site competition will be more intense in figs with more than one foundress, and in figs with smaller numbers of female flowers. However, we did not find an increase in re-emergence rates with greater oviposition site limitation. All the figs probably contained fewer oviposition sites than even one foundresses contained eggs, so lack of effect may reflect a ubiquitous shortage.

We have recently found (N. Suleman in prep) that re-emerging foundresses often failed to lay the second clutch and therefore have a risk of producing fewer offspring than those that do not re-emerge. There is considerable variation in brood size, and progeny numbers of the two groups are not necessarily different. Thus, although *L. tentacularis* foundresses often suffer oviposition site limitation, there is no clear advantage of re-emergence in terms of increasing the number of offspring.

Moore *et al.* (2003) found that re-emergence in *L. tentacularis* increased with foundress density and according to him this indicates that they re-emerge because of oviposition site limitation, but they could not assess it directly. Gibernau *et al.* (1996) proposed that foundresses of *Ficus aurea* are commonly in competition and thus their re-emerging behaviour allows pollinators to oviposit in less crowded figs. It was also hypothesized by Greef (1997) that foundresses re-emerge from figs because they can maximize their fitness by laying a second male biased clutch in a fig in which another foundress had laid a

female biased clutch, if they have knowledge of other foundresses having oviposited in those figs. Thus if foundresses laying two clutches in un-entered figs they should follow the same oviposition strategy in each fig (Greef, 1997). However, if a wingless foundress enters a fig which has already been oviposited in, then this may actually be beneficial, because she can lay mainly sons that mate with the female progeny of the first wasp that have entered. There is lot of variation in fig wasp system and the available data shows contradictory results. *L. tentacularis* showed no differences in the size and sex ratio of the first and subsequent clutches laid by re-emerging foundresses in previously un entered figs (Moore *et al.*, 2003a; N. Suleman in prep), but in another case the clutch sizes laid by wingless foundresses were found to be significantly smaller and more male biased than their first clutches (also N. Suleman, in prep).

Like *L. tentacularis*, some foundresses of *B. psenes*, which also re-emerges (Gibernau *et al.*, 1996) also go on to lay a second clutch of similar size and sex ratio to the first. However the re-emerging foundresses produce around twice as many offspring overall compared to non re-emerging foundresses. Gibernau *et al.* (1996) subsequently noted that oviposition site limitation is not the cause of re-emergence in this species as up to six foundresses can oviposit in a fig before mean clutch size decreases. Because *B. psenes* oviposition sites are rarely limiting, clutch sizes are not affected, and this is a major determinant of the brood sex ratio (Kjellberg *et al.*, 2005). Reflecting this, and in contrast *L. tentacularis*, *B. psenes* foundresses do not adjust their sex ratios in response to the presence of other foundresses in the fig.

Adult female pollinator wasps are carriers of various fungi, bacteria and nematodes which feed on the wasps or the figs (Herre, 1993). Gibernau *et al.* (1996) suggested that by re-emerging and dying outside foundresses might reduce the risk of transmission of pathogens to their offspring. But according to our results it seems unlikely that *L. tentacularis* foundresses re-emerge from figs to minimise the risk of disease and pathogen transmission, because the foundresses often enter the second figs and die in there, so they can infect these figs and their subsequent clutches as well (N. Suleman in prep).

What other factors influence the re-emergence of foundresses from figs? There is believed to be a link with plant breeding system, with re-emergence more common from dioecious than monoecious figs (Gibernau *et al.*, 1996). The ostiole is clearly important and ostiole sizes and shapes differ across species (Verkerke, 1989). Fig trees may have a limited control over their ostiole tightness and as figs dramatically increase in diameter over the receptivity period, the ostiole may become increasingly loose. Individual figs can remain receptive for 2-3 weeks and increase in diameter during receptivity (Khadari *et al.*, 1995). If ostiole tightness is the factor determining re-emergence, then pollinators which enter the figs at an early stage of receptivity might be expected to have lower rates of emergence than those which visited late and Gibernau *et al.* (1996) postulated that figs that were entered later might be unable to prevent re-emergence, because the ostiolar bracts had become loose. The un-pollinated figs of *F. montana* grow from 4mm to 6.5 mm before finally aborting (Moore, 2001), but do not change noticeably in the first three days. This implies that the foundresses are more likely to leave older figs because they are of lower quality (There is no evidence of this however).

Amongst other groups of wasps, Brodeur *et al.* (1996) and Van Driesche (1988) showed that *Cotesia glomerata* (Hymenoptera, Braconidae) can discriminate between hosts of different ages, with first and second instars more acceptable than third instar hosts. Our results suggest that ovipositing females were presumably responding to a decline in host quality. Similarly the parasitoid *Gryon obesum* Masner (Hymenoptera, Scelionidae), was offered *Euschistus conspersus* Unler (Hemiptera, Pentatomidae) of different ages it was found that there was lower acceptance and oviposition success per patch, reduced body size and survival time, and lowered progeny when hosts of 5 days old were compared with 0 day old (Hirose *et al.*, 2003). These results are consistent with our results showing female foundresses left the figs more readily as they become older. Poor host conditions are a stimulus to leave the host not only in parasitoids, but also bugs (Saks *et al.*, 1988), honey bees (Skalicki *et al.*, 1988), bumble bees (Keaser *et al.*, 1996) and birds (Brzek and Konarzewski, 2001).

The causes and relative costs and benefits of re-emergence behaviour may differ between pollinator wasp species. It is clear that further work on a variety of fig species and pollinator wasps is required before any general conclusions should be drawn for the causes and consequences of re-emergence of fig wasps.

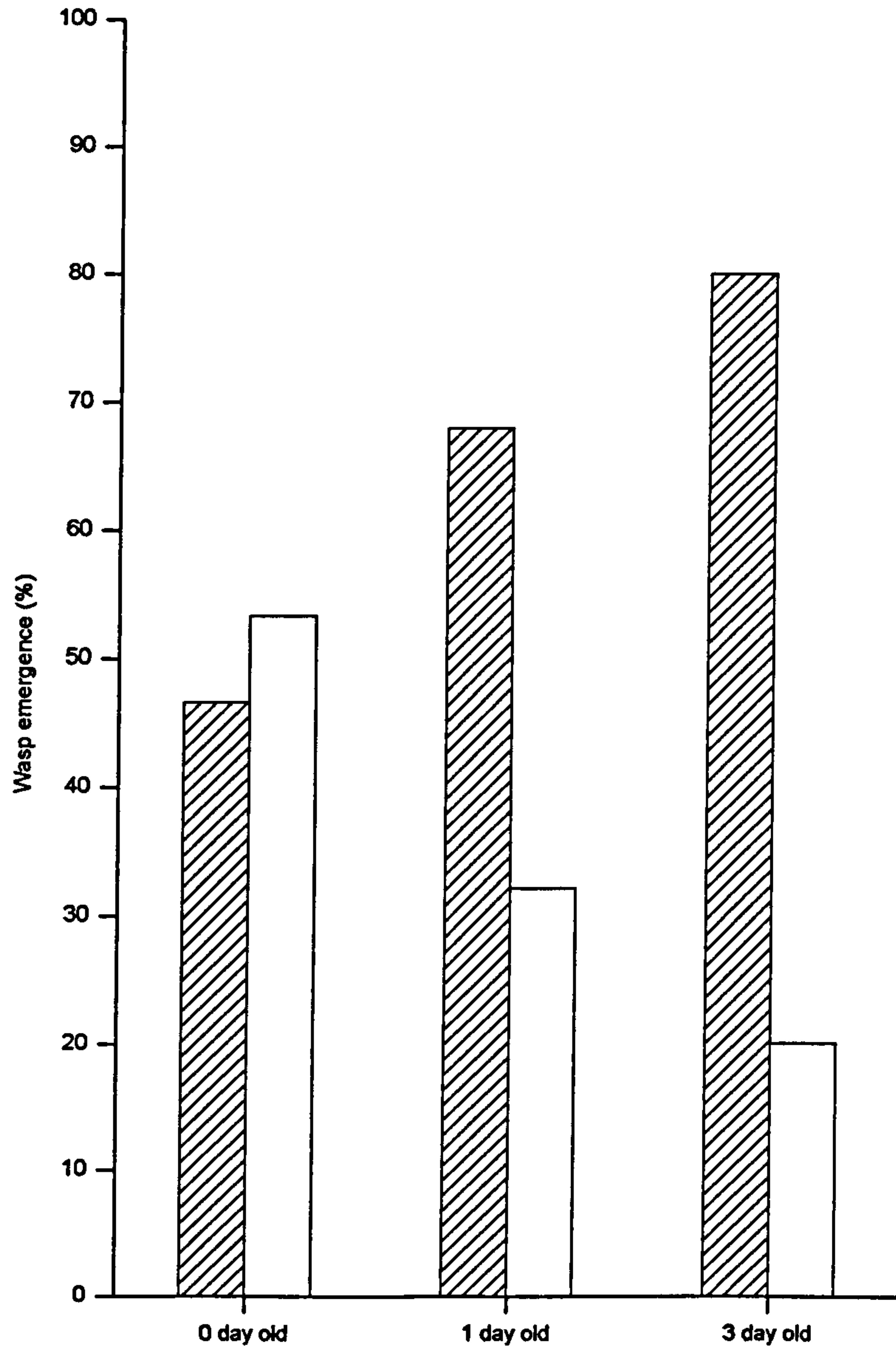


Fig. 5.1 Pollinators that emerged from figs (shaded bars) and those that remained inside (open bars) in relation to the age of the figs (how long they had waited to be pollinated). Wasps were allowed to enter the figs in the morning.

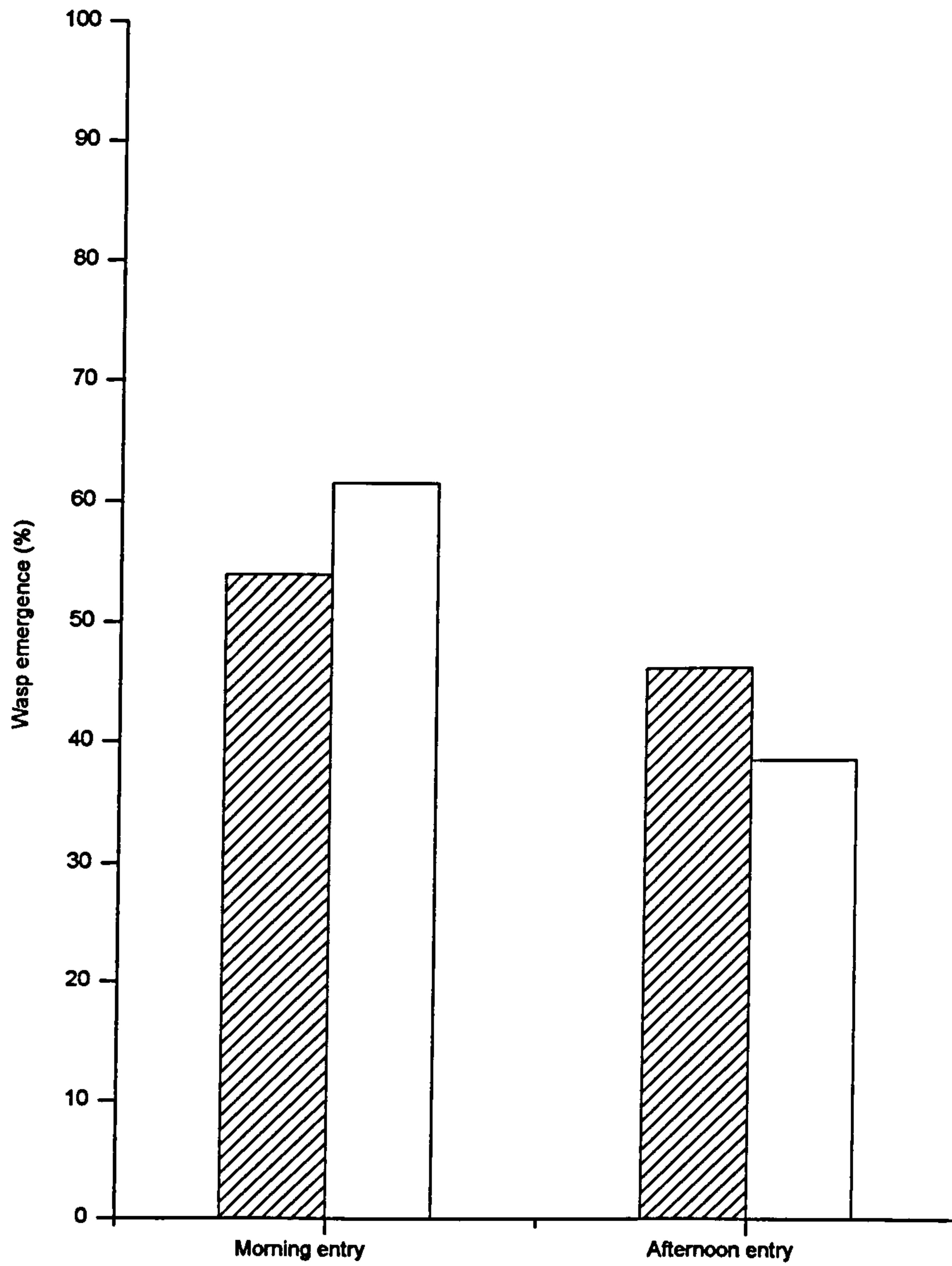


Fig. 5.2 Pollinators that emerged from figs (shaded bars) and those that remained inside (open bars) in relation to the age of the foundresses. Young wasps emerged in the morning were allowed to enter the figs in different times.

6. Mostly male eggs first: the mechanism for sex ratio adjustment in a fig wasp that can enter several figs

6.1. Abstract

Fig wasps, the pollinators of fig trees, have been widely used in studies of sex ratio evolution, especially local mate competition (LMC) because they produce less female biased sex ratios as the number of foundress females sharing a patch increases. Previous studies of *Liporrhopalum tentacularis* the pollinator of *Ficus montana* have shown that progeny sex ratios were linked to clutch size rather than foundress number per se. *L. tentacularis* foundresses can re-emerge from figs and subsequently lay in additional figs, but lose their wings on entry into their first fig. I determined the sequence of egg laying of winged and wingless *L. tentacularis* foundress by terminating oviposition sequences after different lengths of time. The foundresses laid most of their male eggs early in a sequence and consequently the sex ratio of the progeny became more female biased the longer they were allowed to oviposit. The responses of winged and wingless foundresses to varying foundress numbers were different. Wingless foundresses did not display sex ratio adjustment to foundress number, because they produced smaller clutches than winged foundresses that were not reduced in size in shared figs.

6.2. Introduction

Fisher (1930) was the first to explain why most species have 50:50 sex ratios. He showed that in populations with panmictic matings there should be equal investment in the production of male and female offspring because in any population with an excess of females, individuals producing males would be a selective advantage, and vice

versa. However, sex ratios in many hymenopterans are not 50:50. Being generally haplodiploid, this may be related to their form of sex determination as their fertilized eggs develop into females and unfertilized eggs develop into males (Crozier, 1975). Thus a female wasp may be able to control the sex ratio of her offspring by controlling the fertilization of her eggs (Cole, 1981; Suzuki *et al.*, 1984).

When mating occurs between the offspring of a single female, sibling males compete for mates. Hamilton (1967) showed that such local mate competition (LMC) leads to female biased optimal sex ratios which reduce competition between males (Taylor, 1981). Thus in structured populations with sib matings, Fisher's principle no longer holds (Hamilton, 1967) as under these conditions brothers compete to inseminate their sisters and the optimal strategy for foundresses is to invest more in female progeny, thereby producing more daughters and just enough sons to mate with them. If more than one female contributes offspring to an isolated brood, the intensity of LMC decreases and less female biased progeny sex ratios are expected (Hamilton, 1967; Werren, 1980). Thus sex ratios are expected to vary according to the number of females contributing to a joint brood within a patch.

One mechanism by which sex ratio adjustment can be achieved is sequential oviposition of male followed by female eggs. This ensures that enough males are present to inseminate all the females, while not requiring detailed predictions of how many eggs will be laid in total. The egg parasitoid, *Trichogramma evanescens* (Hymenoptera) follows this male eggs first strategy (Waage and Lane, 1984), and Waage (1982) also found that females of *Gryon atriscaps* (Scelionidae) usually deposit male eggs in the first host egg parasitized in a run. Such a pattern is also found in *Trissolcus mitsukurii* (Hokyo *et al.*, 1966) and some other solitary parasitoids attacking

small host egg masses (Strand, 1988). Nefdt and Compton (1996) found that the female fig wasps, *Elizabathiella baijnathi* and *C. capensis* preferentially oviposit into shorter styled flowers and as these become utilized, progressively longer styled flowers are inhabited. Most male progeny of both wasps were found in shorter styled flowers, whereas females were found in both short as well as longer styled flowers. This indicates that these fig wasps may also lay many or all of their male eggs first, followed by female eggs. This pattern is not universal, however as the parasitoid *Pediobius foveolatus* (Hymenoptera) for example lays male eggs late in an oviposition bout (Mary and Barrows, 1989). Werren (1980) showed that *Nasonia vitripennis* (Pteromalidae) adjust the sex ratio of their broods according to whether they are the first or second wasp to parasitize a host. The average sex ratio of the first wasp is strongly female biased, but when a second wasp attacks a host it lays fewer eggs and adjusts the proportion of sons to the local level of LMC. Oviposition sequences can be extremely precise. *Gryon japonica* (Scelionidae), a solitary egg parasitoid, lays a male egg in the second host egg of an oviposition sequence and female eggs in the others. The sequence is reset after a three hour interval, allowing *G. japonicum* to produce precise sex ratios in response to the size of host egg batches (Noda and Hirose, 1989).

Fig wasps, the pollinators of figs (*Ficus*; Moraceae), are often used to study the effects of LMC and inbreeding on sex ratio strategies. *Ficus* is a plant genus characterized by its unique inflorescence, the syconium or fig (Weiblen, 2002) which is lined on its inner surface by hundreds of tiny flowers. Fig wasp foundresses enter figs and produce one offspring per female flower (Jousselin *et al.*, 2001). Pollination and galling of the ovules occurs at the same time. Foundresses usually lose their wings and part of their antennae on entry through the ostiole, and are often unable to re-emerge again. Consequently they often pollinate only one fig, where all their eggs are laid. Mating

takes place within the figs, and as only one or a small number of foundresses will have laid their eggs there, this leads to frequent mating between siblings and inbreeding.

Unvisited figs can remain receptive for several weeks (Patel *et al.*, 1995). Until recently it was assumed that after pollination and oviposition the pollinators die in their figs (Frank, 1985, Bronstein, 1988), but Gibernau *et al.* (1996) showed that foundresses of several species re-emerge from the figs after pollination and oviposition. In *Ficus microcarpa*, for example, around one third of pollinated figs contain no pollinators (Chen *et al.*, 2001) presumably because pollinators have re-emerged. Such re-emergence appears to be particularly common amongst figs of dioecious rather than monoecious species (Moore *et al.*, 2003a).

There is increasing evidence that sex ratio adjustment, in some fig wasps at least, is achieved mainly by laying male eggs first followed by mainly or only female eggs (Kinoshita *et al.*, 2002). Kjellberg *et al.* (2005) also found that in some fig pollinating species male numbers increased with female numbers, showing that females do not begin by first laying a constant number of male eggs and then subsequently laying female eggs. Kjellberg *et al.* (2005) suggested that in fig wasps sex ratios may be negatively correlated with clutch size. This was also confirmed by Moore *et al.* (2005), who used maternity analysis involving micro satellite markers to show that it cannot be assumed that *Liporrhopalum tentacularis* foundresses contribute equally to multi-foundress broods and that their sex ratios are determined by their clutch sizes, but not foundress numbers. They also predicted that sex ratio adjustment in response to foundress number would only be evident in situations where foundresses were competing for oviposition sites (Zavonda, 2004, Moore *et al.*, 2002). Here we directly recorded the oviposition sequence of *L. tentacularis* by terminating oviposition

sequences after varying lengths of time. We also tested whether sex ratio adjustment is generated by a combination of laying male eggs first and oviposition site limitation by comparing the responses to foundress numbers of females entering their first fig ('winged' foundresses with larger egg loads) and entering their second figs ('wingless' foundresses with fewer eggs to lay). As oviposition sites will rarely be limiting for wingless foundresses we predicted that they would not display sex ratio adjustment.

6.3. Materials and Methods

F. montana Burm is a functionally dioecious species of fig tree, which despite its name is found in lowland forest edges, disturbed areas and similar habitats in South East Asia (Corner, 1952). It has a shrubby growth form, rarely reaching two metres in height under natural conditions, and through vegetative spread it can form loose clumps 10 m or more in diameter. Fruiting within plants is typically asynchronous, with all stages of fig development often present on the same plant.

The pollinator of *F. montana* is the diurnal fig wasp *L. tentacularis* Grandi. Adult females of *L. tentacularis* that enter figs (foundresses) lose their wings and most of their antennae while passing through the ostiole, but are nonetheless capable of re-emerging and finding new figs to enter. Consequently they can pollinate and lay their eggs in several figs on one plant. One larva develops in each galled ovule in male plants. Foundresses that enter figs in female trees fail to oviposit, but continue to pollinate. Pollinator fig wasps are pro-ovigenic, carrying their entire lifetime complement of mature eggs when they emerge as adults from their galls (Copland and King, 1973). Most females survive for less than 24 hours as adults, but if they do survive to a 2nd day, they can lay as many eggs as recently-emerged wasps (N. Suleman in prep.).

I utilized a glasshouse population of *L. tentacularis* and its host *F. montana* housed at the Experimental Gardens, University of Leeds (UK). *F. montana* and its pollinator originated from the Centre for International Forestry Research (CIFOR) plantation, West Java, Indonesia and from Rakata (Krakatau), Indonesia and have been maintained continuously since 1995 (Moore *et al.*, 2002).

6.3.1. Sex ratio adjustment with varying time available for oviposition

In order to determine which eggs are laid during specific periods of an oviposition sequence, we killed ovipositing females at different intervals of time after they had entered the figs. A preliminary experiment with the insecticide pyrethrum (pre diluted, Fisons Ltd, U.K.) using twenty females showed that contact with one micro-litre was sufficient to consistently kill the females in less than one minute. This was repeated under more natural conditions by introducing single females into twenty figs with a fine brush and then injecting the pyrethrum using a very fine syringe through the ostiole of the figs. When the figs were opened 10 minutes later, all the females were found to be dead. In the third preliminary trial we examined whether the pyrethrum also affected the eggs laid by the foundresses or the fig itself. We introduced single foundress into ten different figs and after that injected one micro-liter of pyrethrum inside each fig. The figs were then placed in fine cotton bags, so that no more females could enter. After 6-8 weeks, seven of the ten figs had matured normally, and contained numbers of wasp progeny similar to control figs where no insecticide was present.

After the preliminary experiments we used the poisoning technique to record the progeny sex ratios and oviposition rates of winged and wingless foundresses at varying

times after their entry into figs. Winged foundresses were collected after being allowed to emerge naturally from their natal figs and then introduced singly into the figs with the help of a fine brush. For wingless females we introduced a single foundress into figs and bagged them afterwards. They were observed hourly and any foundresses that emerged were introduced into another fig in the same way.

Each winged and wingless female was allowed to oviposit for 5, 15, 30, 60, 120 or 240 minutes before we interrupted oviposition by gently introducing a syringe through the ostiole and injecting pyrethrum to kill it. The figs were bagged afterwards to prevent the entry of any other foundresses and attacks by parasitoids. After 6-8 weeks (Stage D *sensu* Galil and Eisikowitch, 1968) the figs began to soften, indicating that the wasp progeny were about to emerge. The figs were picked and placed singly into nylon mesh bags for the next generation of wasps to emerge. All the wasp progeny were then counted and sexed.

6.3.2. Sex ratio adjustment with varying numbers of foundresses

Mature male figs were left in plastic containers for 24 hours with mesh lids, so that the wasps could emerge naturally. Foundresses that emerged from these figs were introduced into receptive male figs with a fine paint brush, as before. The foundresses were introduced at densities of one, two or three per fig into receptive figs, one after the other. The figs were then placed in fine mesh bags to avoid further entries by other pollinators and to stop egg laying by parasitoids. The experiment was repeated with wingless foundresses that had already entered another fig. Single foundresses were introduced into receptive figs, and any that re-emerged were collected with a paint brush and then released into another receptive fig. Wingless foundress densities of one, two or three, were introduced as before. A few weeks later the figs were mature and

were placed for 24 hours in plastic containers for the wasps to emerge. All the wasps were then counted and sexed.

6.4. Results

6.4.1. Sex ratio adjustment with varying times available for oviposition

Initial rates of oviposition were high, more than one egg per minute, during the first five minutes after foundresses entered the figs, but the rates of oviposition by both the winged and wingless lone foundresses decreased rapidly with time (Fig 6.1). The brood sizes they achieved were similar after five minutes (7.12 progeny \pm 1.34 (Mean \pm SE) for winged females and 6.44 progeny \pm 0.376 for wingless females) but as oviposition rates decreased more sharply in wingless females, by 240 minutes they averaged less than half the progeny produced by winged foundresses (54.47 progeny \pm 2.47 for winged females compared with 27.81 progeny \pm 1.92 for wingless females).

Around half the male progeny were produced within the first five minutes, although small numbers of sons continued to be produced throughout the experiment (Table 6.1, Fig 6.2). The number of male progeny produced varied significantly for different time intervals both for winged (Kruskal Wallis, $X^2 = 33.64$, $df = 5$, $P < 0.004$) and wingless (Kruskal Wallis, $X^2 = 17.00$, $df = 5$, $P < 0.001$) foundresses. Production of female progeny by wingless foundresses slowed down more rapidly after 30 minutes, and winged foundresses produced significantly more daughters (Table 6.1, Fig 6.3). Reflecting this dichotomy, progeny sex ratios of winged foundresses were significantly more female biased beyond 30 minutes after entry into figs than those of wingless foundresses (Table 6.1, Fig 6.4). Bladders (galled ovules which failed to produce

offspring) were not significantly different in figs entered by the two groups of foundresses at any single time interval (Table 6.1).

The plateau times for winged and wingless foundresses inside figs for varying lengths of time are summarized in Table 6.2. Beyond the times indicated, no significant increases in numbers were present.

6.4.2. Sex ratio adjustments with varying number of foundresses

As expected from previous studies (Herre 1987, Moore *et al.*, 2002) the numbers of winged foundresses (entering their first figs) had a significant effect on their progeny sex ratio, with the proportion of males increasing with increasing numbers of foundresses contributing to combined figs (ANOVA, $F_{(2,59)} = 23.20$, $P < 0.001$, Fig 6.5). Increasing numbers of winged foundresses resulted in fewer progeny being produced, not more (Table 6.3, Fig 6.6) perhaps because of interference effects. This change was associated with fewer female progeny in figs with more than one foundress (Fig 6.7) whereas the numbers of male progeny rose slightly (Fig 6.8).

Lone wingless foundresses averaged about 30 progeny, less than half that produced by winged foundresses (Table 6.3). Wingless foundresses entering their second figs responded differently to foundress density (Table 6.3). The wingless foundresses did not adjust the sex ratios in relation to foundress number as there was no difference in their sex ratios with increasing foundress density (ANOVA, $F_{(2, 27)} = 0.04$, $P = 0.95$, Fig 6.5). Although the numbers of their female progeny did not differ significantly with foundress number, their male progeny and total progeny numbers were significantly higher in figs shared by multiple foundresses (Table 6.3, Figs 6.6, 6.7 and 6.8).

The relationship between foundress state (winged or wingless), foundress number and progeny production are summarized in Table 6.4. Significant interaction terms were present, showing that foundress states respond differently to foundress density. Winged foundresses had more total progeny and female progeny, with mean values $50.03 (\pm_{SE}) 2.55$ and 32.46 ± 3.55 for female pollinators, 57.63 ± 2.46 and 42.17 ± 4.23 for total progeny by winged and wingless foundresses respectively.

6.5. Discussion

In this chapter the sex ratio strategies of *L. tentacularis*, the pollinator of *F. montana*, were investigated experimentally. It was found that the sequence of egg laying is similar for both the winged and wingless foundresses, as they laid most of their male eggs early in each oviposition sequence and consequently the sex ratios of the progeny were significantly different when they were allowed different lengths of time to oviposit. We also found that the responses of winged and wingless foundresses to foundress density were different, with only winged foundresses displaying sex ratio adjustment. The underlying cause of this difference was that wingless foundresses laid smaller clutches than winged foundresses. These were independent of foundress number because oviposition sites were less limiting.

Until recently it was assumed that after pollination and oviposition fig wasp foundresses die in the first fig they enter (Frank, 1985; Bronstein, 1988). Therefore, tests of LMC theory have often used the number of dead females present in a fig after dissection to deduce the number of foundresses contributing to combined broods (Kinoshita *et al.*, 1997). Gibernau *et al.* (1996) subsequently showed that foundresses of several species re-emerge from their figs after pollination and oviposition, and a

survey by Moore *et al.* (2003a) showed that re-emergence of foundress fig wasps may be quite common, occurring in 68 % out of 50 surveyed species. A further problem for simple models of sex ratio adjustment in fig wasps is that foundresses contributions to brood can vary (Greef and Compton, 1996; Kathuria *et al.*, 1999). Molbo *et al.* (2003) also showed by using micro-satellite markers that in figs with multiple dead foundresses some females make no contributions whatsoever to total broods. Our recent work has shown that in *F. montana* the *L. tentacularis* foundresses routinely re-emerge from the figs and can successfully lay eggs in two or more male figs (Moore *et al.*, 2003a; S. Raja in prep.). The re-emerging (wingless) foundresses are only able to walk short distances in search of other receptive figs to enter, and these will often already have been entered by other foundresses. Foundresses of this species typically contribute to shared broods unequally, producing smaller and less female-biased clutches than they would have produced when ovipositing alone (Moore *et al.*, 2005). Thus in this species foundress counts lead to an underestimation of both the numbers of wasps that entered the figs and the numbers that oviposited in them (M. Závondá Pers. Comm).

All previous experimental studies of fig wasps and Local Mate Competition have focused on the sex ratio strategies of winged foundresses. Our results show that wingless foundresses sometimes do not display sex ratio adjustment to foundress number, because they produced smaller clutches than winged foundresses that were not reduced in size in shared figs.

According to Waage and Lane (1984) oviposition sequence is the most important factor influencing sex ratio. There is a growing body of evidence that sex ratio adjustment in fig wasps is achieved by laying predominantly male eggs initially,

followed by mainly or only female eggs, with the eventual sex ratio determined by the total number of eggs that are laid (Nefdt, 1989; Kinoshita *et al.*, 2002). Our results also showed that the rates of oviposition by both the winged and wingless lone foundresses decreased with time, but oviposition rates decreased more sharply in wingless females and at the end of the experimental time their progeny numbers averaged less than half that of winged foundresses.

Different parasitic wasps have different rules for producing male and female eggs during single oviposition bouts. Gregarious parasites often lay many eggs at once, which results in variable sex ratios (Iwasa *et al.*, 1984). Several wasp species oviposit a fixed ratio of sons and daughters with sons appearing earlier, but not necessarily first in an oviposition bout (Waage, 1982). Putters and van den Assem (1985) showed that *Nesolynx albiclavus* (Eulophidae) a gregarious parasitoid of tsetse flies produce a male egg in the sixth egg of a run and lay no additional males in the next 10-20 eggs. They were unable to explain this strategy of laying a male egg exclusively on the sixth host egg parasitized. There are even some species which oviposit a fixed number of sons, and increase or decrease the number of females in response to host quality, as in *Anisopteromalus calandrae* (Pteromalidae) which always lays a male egg at the end of a bout (Assem *et al.*, 1984). The females of *L. tentacularis* also use a fixed mechanism of sex ratio control, as their sequence of egg laying is independent of whether they are laying their first clutch or the subsequent ones. They always lay mainly male eggs at the start of oviposition and the sequence of egg laying is reset when they are allowed to enter the second fig. This 'sons early' strategy ensures that subsequent daughters will be inseminated, but also that the numbers of females sharing a male varies according to the total numbers of eggs laid.

We also found that clutch size and oviposition duration are positively correlated, which is similar to that in *Apanteles glomeratus* (Braconidae) (Ikawa and Suzuki, 1981), but in contrast to certain trichogrammatids (Feijen and Schulten, 1981). Although we did not find any difference in the number of males produced by winged and wingless foundresses at different time intervals and even at the end of the oviposition time, the total progeny size of the former was almost double that of the wingless one. The reason is quite apparent, as the wingless females had already laid their first clutch so they have lower loads of eggs available for their subsequent clutch.

Dijkstra (1986) found that *Colpoclypeus florus* (Eulophidae), a gregarious ectoparasitoid of larvae of leaf rollers, deposited male eggs in the last few eggs of a clutch. He concluded that *C. florus* had more knowledge of the total resource of its host. But we argue that a later placement of males in fig wasps would be disadvantageous because if a female failed to lay the male eggs at the end she would lose the entire brood, if she was not sharing with other foundresses, because the female progeny need the help of males to emerge.

Combined brood sex ratios (percentage males) were significantly higher in figs with more than one winged foundress. This is consistent with many other studies showing that in fig wasps the proportion of males increases with foundress density (Greef and Compton, 1996, 1997; Kinoshita *et al.*, 2002; Moore *et al.*, 2002). The results suggest that the first foundresses had not managed to complete their oviposition when the next foundress was forced to enter the fig, as the number of eggs they eventually produced was reduced compared to foundresses in single foundress figs. This reduction probably reflects interference between the foundresses. As the later eggs in an oviposition bout are largely female, this had the effect of making their clutch sex ratios less female

biased, and closer to the clutch sex ratio of the second foundress. In those cases where a first foundress has left a fig (Kathuria *et al.*, 1999), then fewer oviposition sites will be available to the second female and she will be forced to lay fewer eggs, and a clutch that is therefore less female biased.

When we increased the density of winged foundresses there was no effect on total brood size, showing that each female was laying fewer eggs. Less female biased progeny sex ratios were observed and an increased number of males relative to figs with one foundress. The cost of male mortality is potentially high in pollinating wasps, as in the absence of the males which chew an exit hole through the fig wall, females are unable to exit the figs (West *et al.*, 1997). Thus the cost of mortality of males in small broods will be higher because of the increased chances of too few males surviving to chew the exit hole. Although we found that male progeny numbers increased with female progeny numbers, the reason that a foundress lays mainly male eggs initially, may be as insurance.

The wingless foundresses appeared to behave in a different way than winged foundresses. Wingless lone foundresses produced small clutches with more males than one winged foundress and when more than one wingless foundress was introduced into a fig they did not adjust their sex ratios in the way suggested by LMC. It can be assumed from their behavior that wingless foundresses already had laid their first clutch, so they probably acted to maximize their reproductive potential by laying more male eggs to take advantage of the female progeny from the first foundress.

Kjellberg *et al.* (2005) have shown that sex ratios are negatively related to clutch size. This was also confirmed by Moore *et al.* (2005), who showed that *L. tentacularis*

foundresses contribute unequally to two foundress broods and adjust their sex ratios according to the size of clutch but not foundresses density.

This sex ratio study is the first which takes into consideration both winged and wingless foundresses, but still much remains to be understood about pollinator fig wasp sex ratio strategies. The next challenge lies in unveiling what changes they are able to respond to and what other adjustments they can make to their sex ratios.

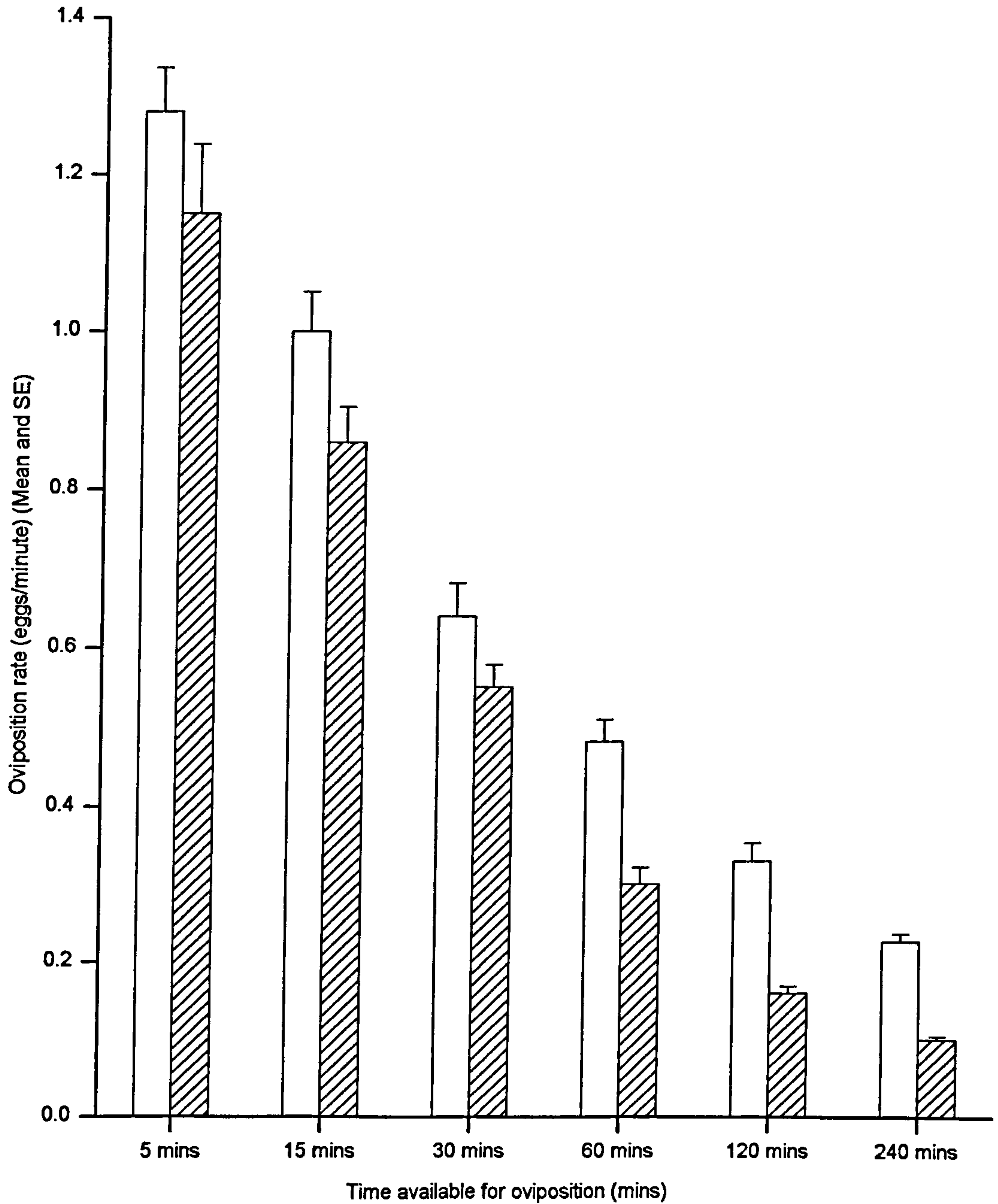


Fig. 6.1 Changes in the estimated rates of oviposition by winged (open bars) and wingless (hatched bars) foundresses during the time they spent inside figs. Rates at each time interval are calculated over the entire period since they entered.

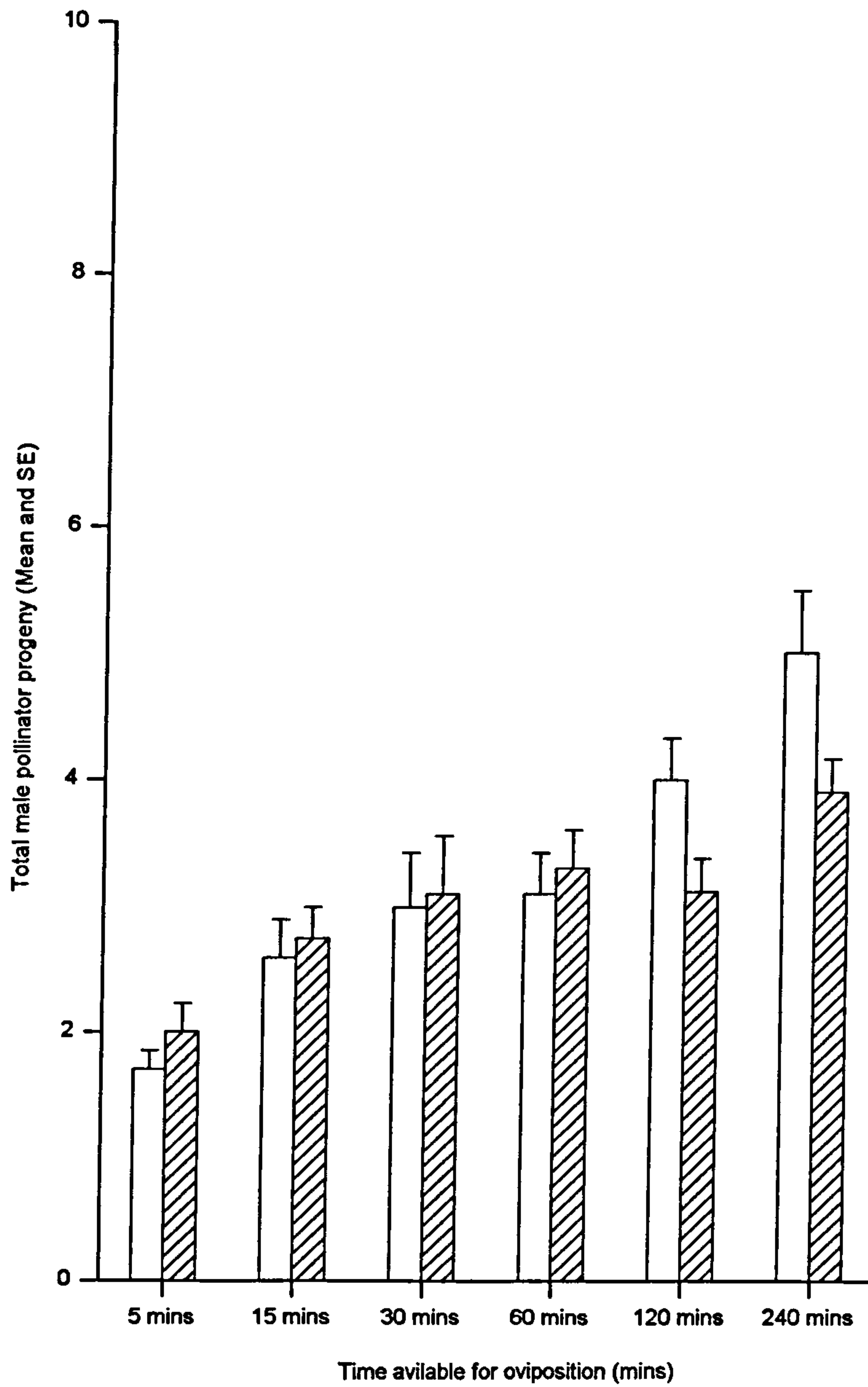


Fig. 6.2 The total numbers of male pollinator progeny of lone winged (open bars) and wingless (hatched bars) females when they were allowed to survive for different periods of time after entering a fig.

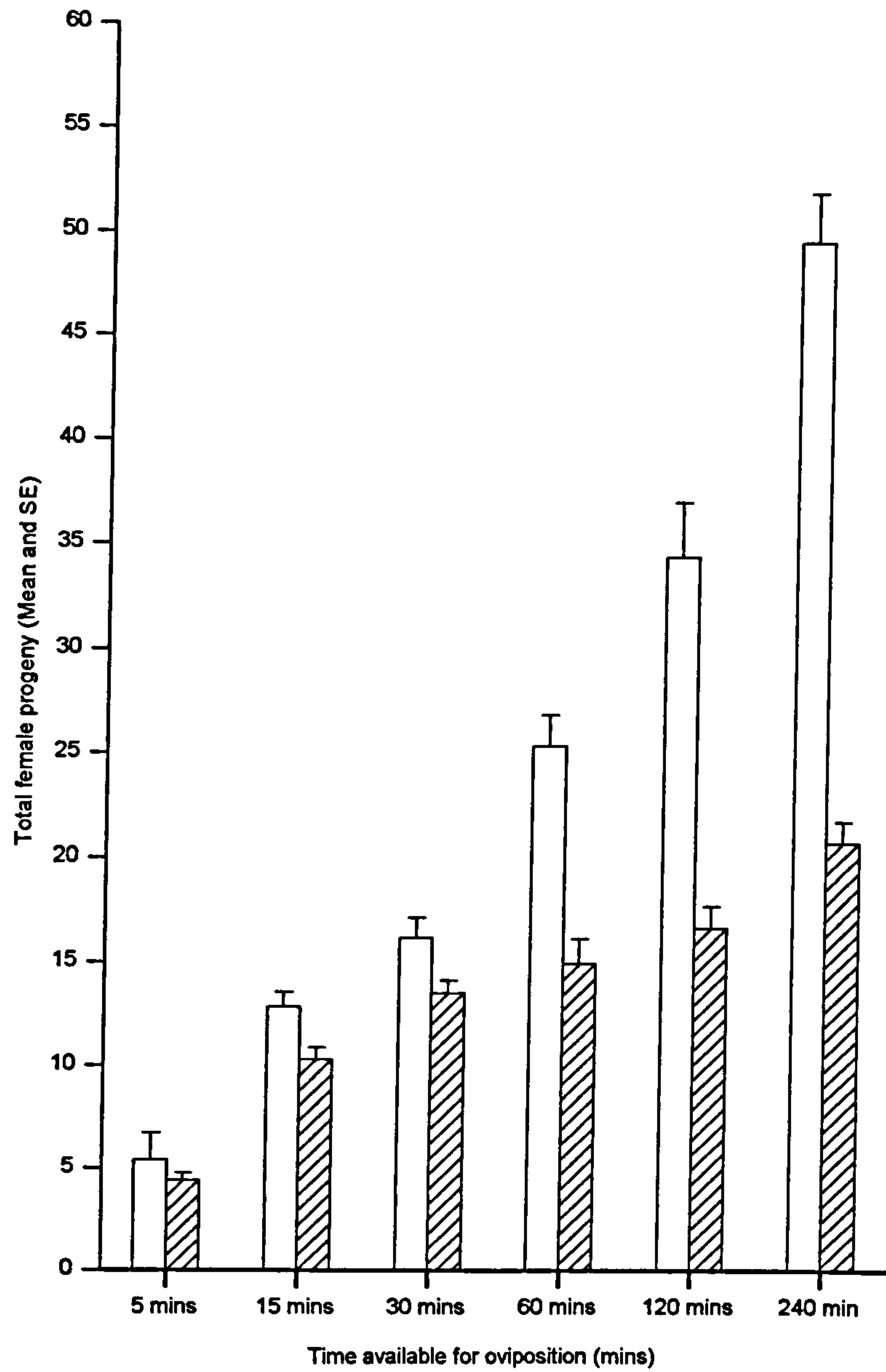


Fig. 6.3 The total numbers of female progeny of lone winged (open bars) and wingless (hatched bars) foundresses when they were allowed to survive for different periods of time after entering a fig.

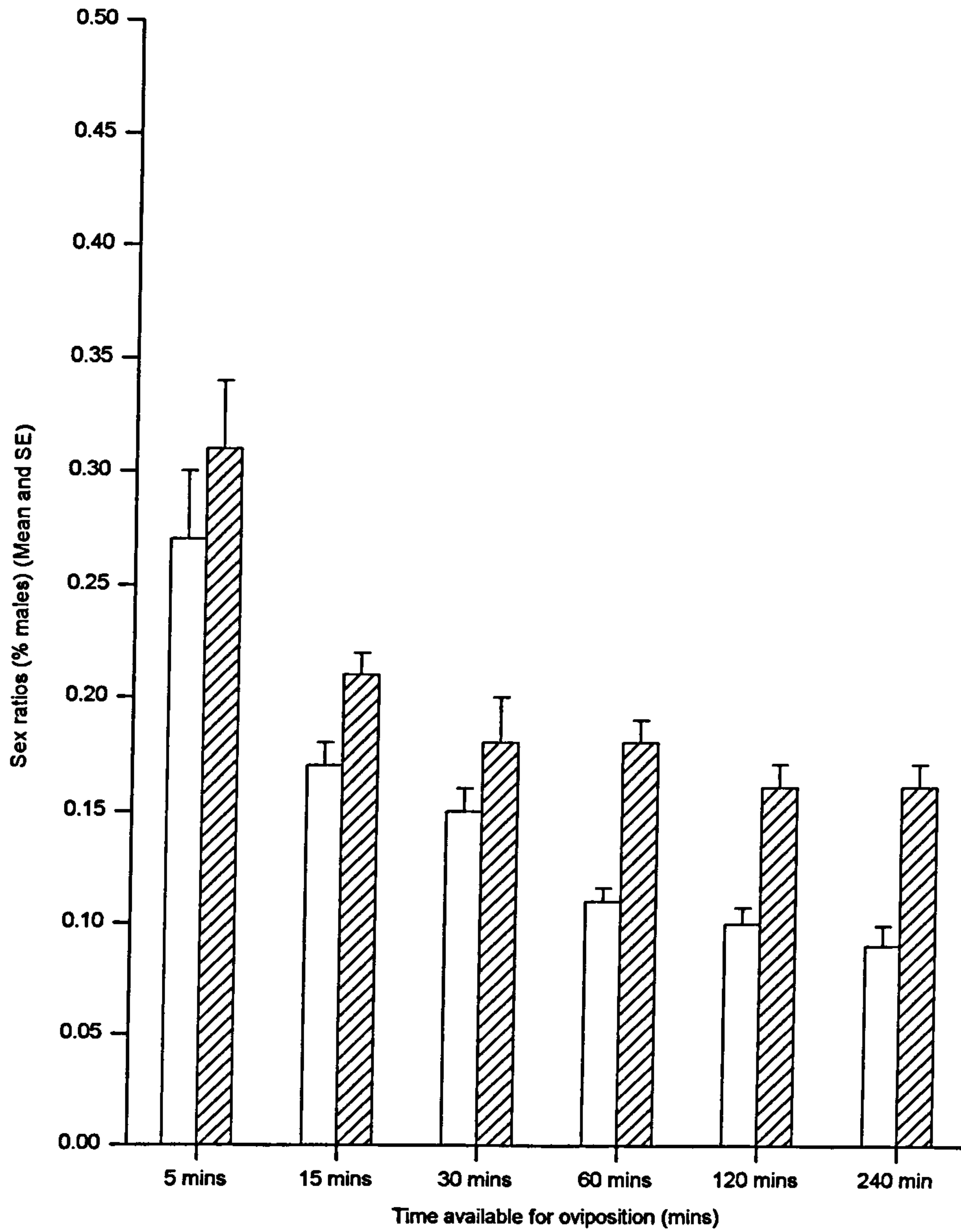


Fig. 6.4 The progeny sex ratios (% males) of lone winged (open bars) and wingless (hatched bars) foundresses in figs when they were allowed to survive for different periods of time after entering a fig.

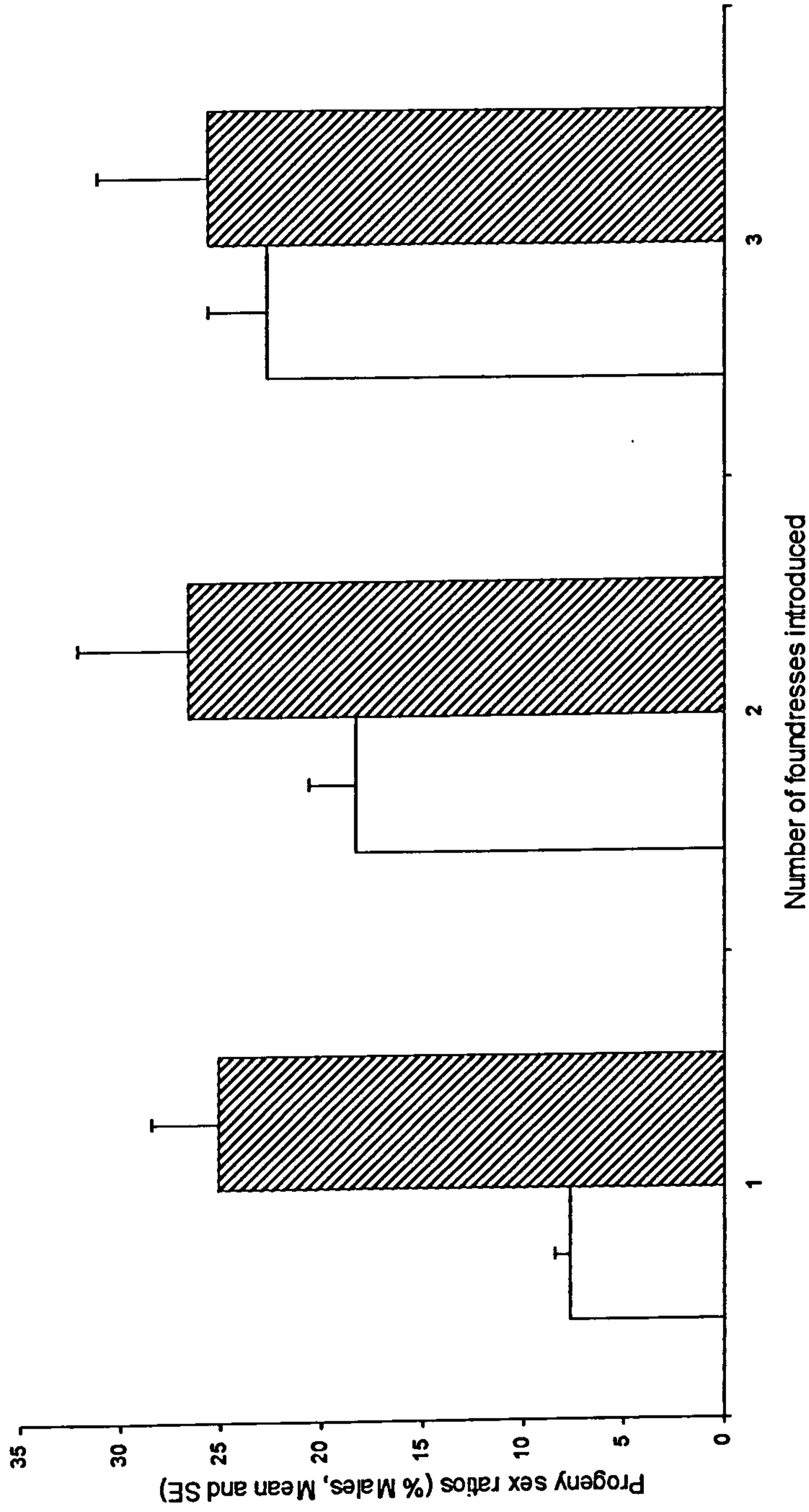


Fig. 6.5 The progeny sex ratios of winged foundresses entering their first figs (open bars) and wingless (hatched bars) foundresses entering their second figs, when alone or sharing figs with one or two other foundresses that were introduced in rapid succession. Sample sizes per treatment were 26, 21 and 13 for winged and 9, 9 and 10 for wingless foundresses.

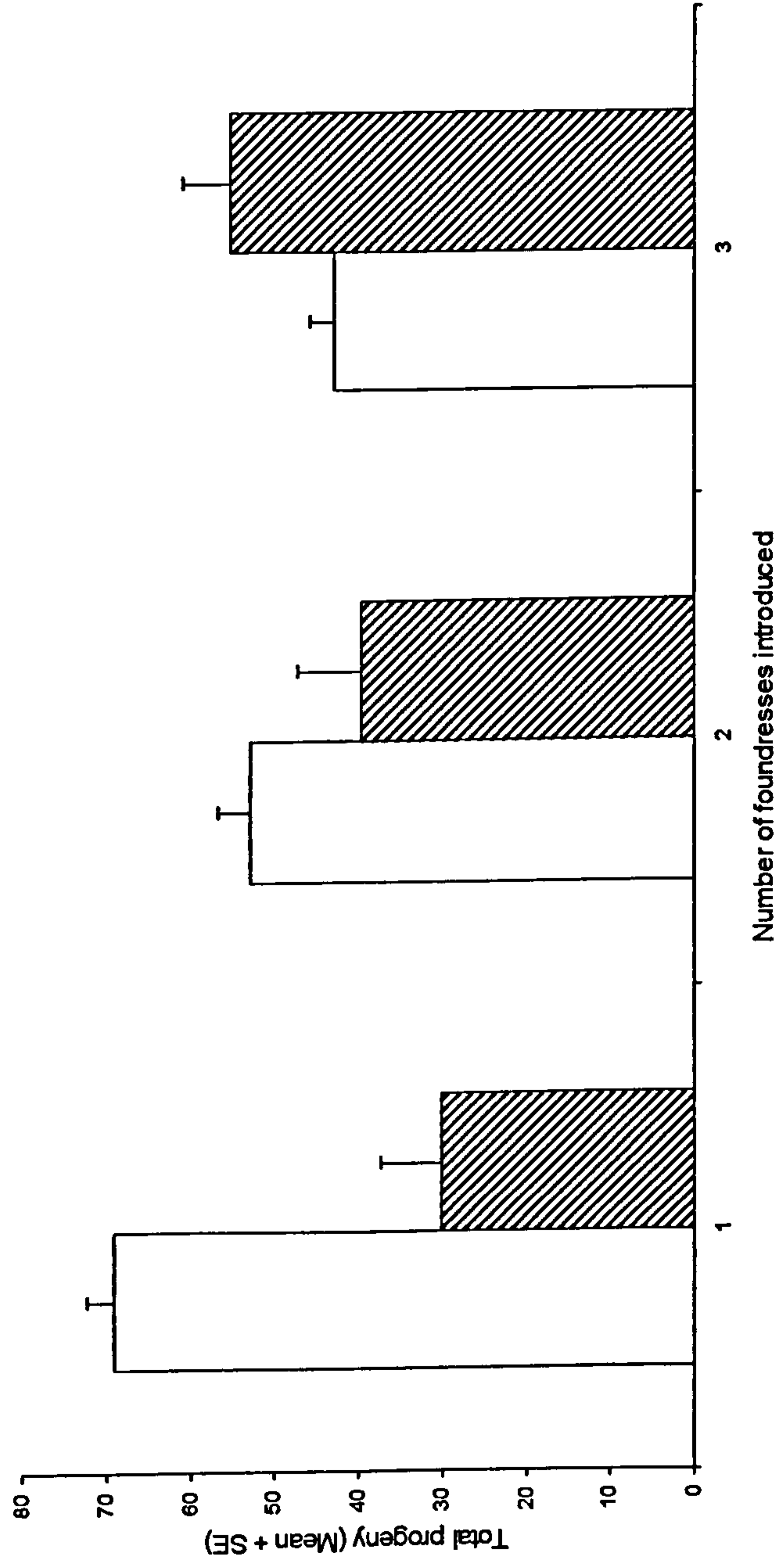


Fig. 6.6 The numbers of progeny (both sexes) produced by winged (open bars) and wingless (hatched bars) foundresses, when alone or sharing figs with one or two other foundresses that were introduced in rapid succession.

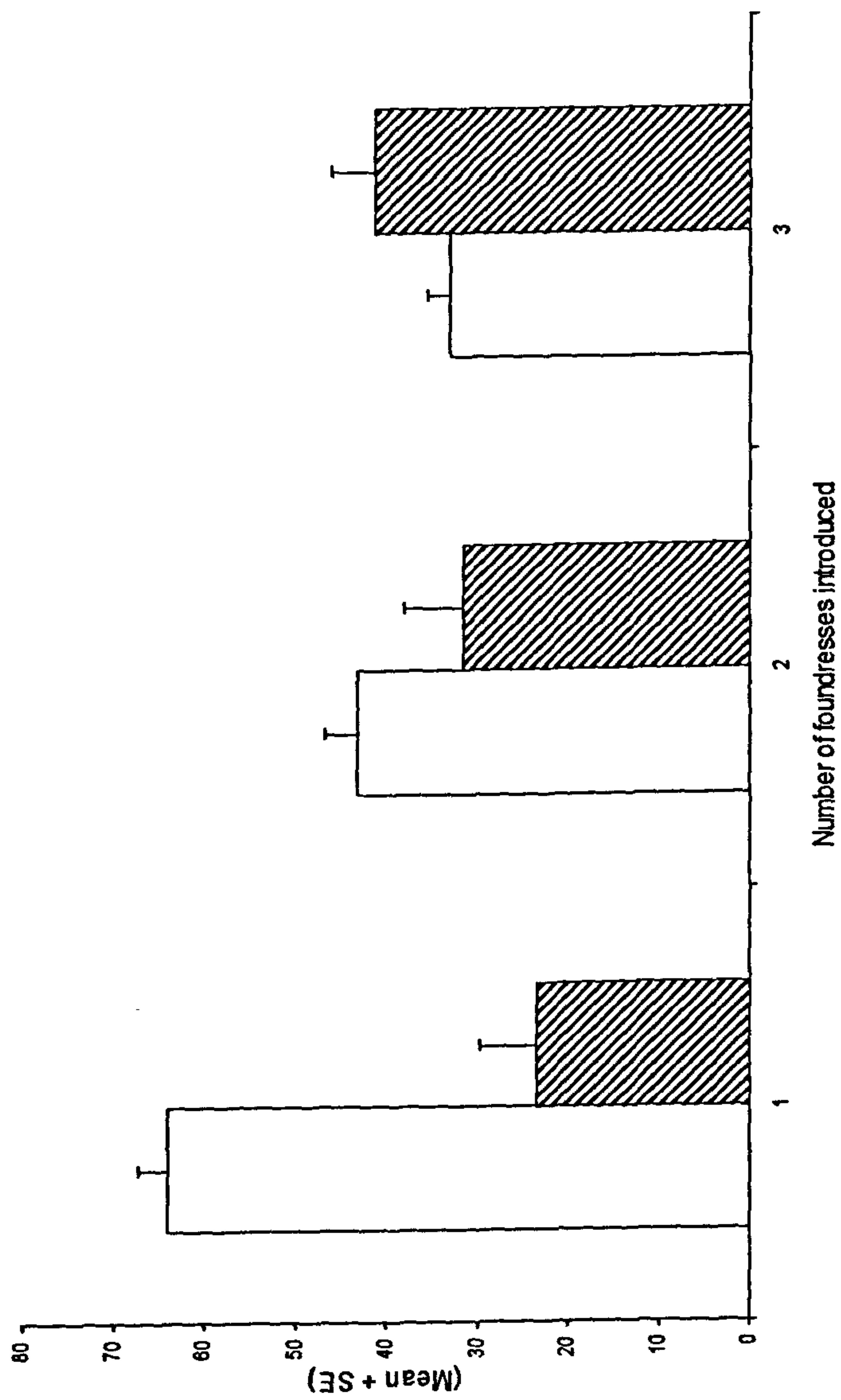


Fig. 6.7 The total number of female progeny of winged (open bars) and wingless (hatched bars) foundresses, when alone or sharing figs with one or two other foundresses that were introduced in rapid succession.

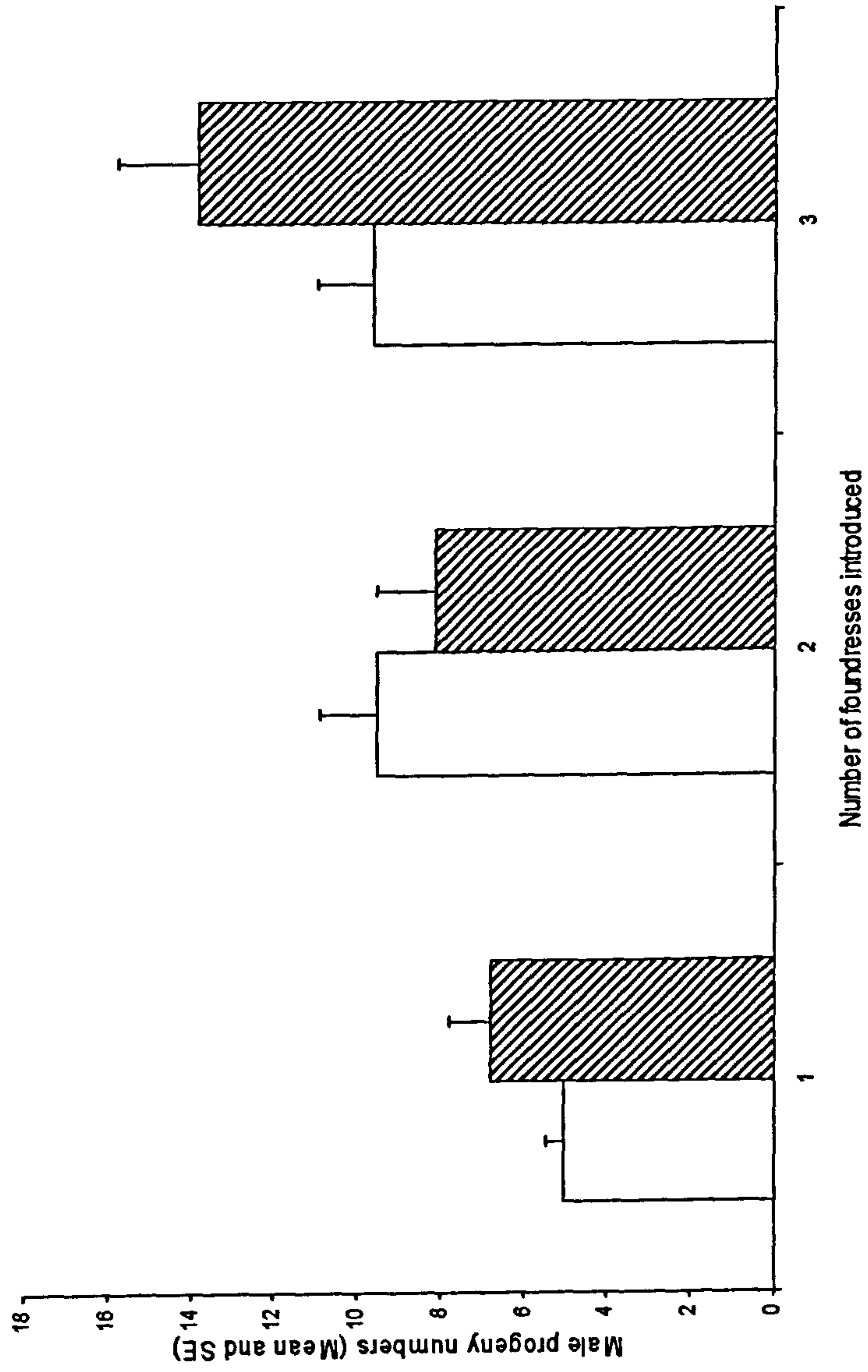


Fig. 6.8 The numbers of male pollinators produced by winged (open bars) and wingless (hatched bars) foundresses, when alone or sharing figs with one or two other foundresses that were introduced in rapid succession.

Table 6.1 Comparisons of progeny numbers, progeny sex ratios and bladders between figs entered by wingless and winged female pollinators that were allowed to survive for different periods of time after entry into figs (Kruskal Wallis tests with one degree of freedom).

Oviposition time (minutes)	Male progeny	Female progeny	Total progeny	Progeny sex ratios (% males)	Bladders
5	X ² 1.14 Sig NS	X ² 0.36 Sig NS	X ² 1.12 Sig NS	X ² 0.09 Sig NS	X ² 0.10 Sig NS
15	X ² 0.05 Sig NS	X ² 4.72 Sig NS	X ² 3.61 Sig NS	X ² 1.35 Sig NS	X ² 0.001 Sig NS
30	X ² 1.00 Sig NS	X ² 3.21 Sig NS	X ² 2.69 Sig NS	X ² 0.98 Sig NS	X ² 0.95 Sig NS
60	X ² 0.18 Sig NS	X ² 14.36 Sig ***	X ² 13.01 Sig ***	X ² 11.16 Sig **	X ² 0.38 Sig NS
120	X ² 5.64 Sig NS	X ² 13.57 Sig ***	X ² 13.57 Sig ***	X ² 6.85 Sig *	X ² 0.70 Sig NS
240	X ² 2.34 Sig NS	X ² 18.34 Sig ***	X ² 17.39 Sig ***	X ² 14.32 Sig ***	X ² 0.26 Sig NS

*indicates P<0.05
 ** indicates P<0.01
 ***indicates P<0.001
 NS indicates Not significant

Table 6.2 The plateau times for fig wasps progeny production inside figs where lone foundresses were killed after varying lengths of time (Kruskal Wallis). Beyond the times indicated, no significant increases in numbers were present.

Plateau time (mins)	Total progeny		Male progeny		Female progeny		Sex ratio (% males)		Bladders	
	W	WL	W	WL	W	WL	W	WL	W	WL
240+	240+	240+	120	60	240+	240+	120	15	60	30

W = winged foundresses
 WL = wingless foundresses

Table 6.3 The effects of foundress density on combined brood sizes in winged foundresses (entering their first fig) and wingless foundresses (entering their second figs).

	Foundress numbers	Winged foundresses Mean \pm SE (N)	Range	Wingless foundresses Mean \pm SE (N)	Range
Female progeny	1	63.92 \pm 3.37 (26)	33-94	23.44 \pm 6.32 (9)	8-71
	2	43.28 \pm 3.65 (21)	20-84	31.55 \pm 6.57 (9)	3-77
	3	33.15 \pm 2.55 (13)	15-50	41.40 \pm 4.75 (10)	17-64
ANOVA		$F_{(2,59)} = 20.57, p < 0.001$		$F_{(2,59)} = 2.39, p = 0.11$	
Male progeny	1	5.04 \pm 0.41 (26)	2-9	6.78 \pm 1.01 (9)	2-12
	2	9.52 \pm 1.37 (21)	3-25	8.11 \pm 1.41 (9)	4-15
	3	9.62 \pm 1.31 (13)	4-19	13.80 \pm 1.95 (10)	6-24
ANOVA		$F_{(2,59)} = 8.70, p = 0.001$		$F_{(2,59)} = 5.97, p = 0.008$	
Total progeny	1	69.04 \pm 3.44 (26)	35-101	30.22 \pm 7.11 (9)	14-83
	2	52.80 \pm 3.87 (21)	24-88	39.66 \pm 7.45 (9)	9-92
	3	42.76 \pm 2.88 (13)	25-60	55.20 \pm 5.66 (10)	27-81
ANOVA		$F_{(2,59)} = 12.62, p < 0.001$		$F_{(2,59)} = 3.60, p = 0.04$	

Table 6.4 The effect of wing presence and foundress density on progeny numbers and sex ratios (Two way analysis of variance).

Factors tested	Male progeny		Female progeny		Total progeny		Sex ratio (arcsine transformed)					
	F	df	P	F	df	P	F	P				
Winged/wingless	1.97	1	0.16	15.18	1	<0.001	10.40	1	0.002	18.86	1	<0.001
Foundress numbers	9.74	2	<0.001	1.31	2	0.27	0.26	2	0.76	5.92	2	0.004
Winged/wingless *												
Foundress density	2.23	2	0.11	14.19	2	<0.001	13.04	2	<0.001	5.48	2	0.006

7. Interactions between pollinator and non-pollinator fig wasps associated with a dioecious fig tree

7.1. Abstract

Ficus plants and their species-specific pollinator fig wasps represent an obligate plant-insect mutualism, but fig species also shelter a community of non-pollinating wasps that consist of gall makers and parasitoids. I studied interactions between *Liporrhopalum tentacularis*, the pollinator of a dioecious fig species *Ficus montana*, and its non-pollinator *Sycoscapter* sp. indesc. We found that *Sycoscapter* sp. oviposits 2-3 weeks after pollinator entry, when host larvae are present in the figs. No negative correlation was found between the numbers of the two wasp species emerging from the figs, which would suggest they were using different resources. However, in experiments where the numbers of pollinator foundresses entering a fig were controlled, *Sycoscapter* sp. significantly reduced the numbers of the pollinator. Consequently, it can be concluded that *Sycoscapter* sp. is a parasitoid, of *L. tentacularis*, although it may also feed on plant tissues as well.

7.2. Introduction

Insects are killed by a diverse collection of natural enemies that include vertebrate predators, parasitoids, fungi, bacteria, nematodes and viruses. The impact of these natural enemies is highly variable, can range from being severe enough to destroy populations in a particular area to the trivial (Eber and Brandl, 1994; Washburn and Cornell, 1981). The identification of the significance of these factors and the prediction of when and where they are going to occur are therefore central to an understanding of an insect population dynamics (Bradford *et al.*, 1997).

Parasitoids have long been popular subjects for ecological studies, firstly because of their importance as biological control agents and secondly, because they are ideal for developing relatively simple population models (Hassell, 2000). The characteristics that influence the susceptibility of herbivores to parasitoids has received considerable attention (Askew, 1961; Cornell and Hawkins, 1993). Endophytic feeding often protects herbivores from predation, but parasitoids in contrast are not deterred by endophagy (Bradford *et al.*, 1997).

The relationship between pollinating wasps (Agaonidae) and their host fig trees (*Ficus*) is a classic example of obligate mutualism (Galil, 1977; Janzen, 1979). Each fig tree depends upon its pollinating wasp to provide pollination services to its host and in turn fig trees provide breeding sites for pollinator progeny (Weibes, 1979; Rasplus, 1996).

Figs are also hosts to a number of other wasps that develop inside them, but they do not have any role in pollination. These wasps include gallers, parasitoids and inquilines that destroy other fig wasps but also feed on plant tissues (Compton and van Noort, 1992; West and Herre, 1994; Kerdelhue and Rasplus, 1996). The non pollinating wasp communities vary greatly between fig species (Compton and Hawkins, 1992) both in terms of wasp species number and community structure. These non-pollinating wasps appear to often be host specific, like the pollinators (Ulenberg, 1985). Each particular species of non pollinating wasp oviposits at a specific stage of fig maturation, but regardless of oviposition timing all fig wasp species usually emerge from galls inside the figs at the same time and typically use the exit hole that is chewed by the male pollinating wasps to emerge from figs (Kerdelhue *et al.*, 2000).

Kerdelhue *et al.* (2000) identified three ecological groups of non-pollinators on the basis of their timing of oviposition. Early and late gallers tend to oviposit at and after the time of pollinator entry, while parasitoids oviposit later. Gall makers often oviposit very early in fig development and induce large galls. Their development inside figs does not depend upon the pollinators. Most gallers usually lay their eggs from the outside of the figs, but there are some gall makers that enter through the ostiole and oviposit from the fig cavity. Gallers typically act as competitors of the pollinating species (Kerdelhue, 2000). The fig community also consists of parasitoids whose larvae develop directly at the expense of pollinators larvae and inquilines that feed upon the gall of other fig wasp larvae. Inquilines can be considered as functional parasites if they out compete the original inhabitant of the gall, causing them to starve to death. They have long ovipositors that are capable of piercing the figs from outside. They attack the flowers containing other fig wasps and either consume the host or starve it by feeding on the gall tissues. By definition, parasitoids of the pollinators cannot be found in the seed figs of dioecious species, as their development depends on pollinator larvae (Weiblen *et al.*, 2000) and female figs typically have very small, specialised fig wasp communities.

All of these non-pollinating wasps potentially have a negative impact on pollinator populations (Compton *et al.*, 1994; Herre and West, 1997; Kerdelhue *et al.*, 2000). Multiple species can develop side by side in a single fig and in New World figs, at least, non-pollinators usually out number the pollinator's offspring (Bronstein, 1991; Bronstein and Hossaert McKey, 1996).

Little is known about the natural history of non-pollinating wasps and their interactions with the mutualism (Hamilton, 1979; Bronstein, 1991; Compton and Hawkins, 1992).

Although there are some studies on the impact of non-pollinators on the fig pollinator mutualism (Galil *et al.*, 1970; Bronstein, 1991; West and Herre, 1994; Cook and Power, 1996), they have received little attention.

I studied the non pollinating *Sycoscapter* fig wasp (Agaonidae, Sycorytinae sensu Boucek) (an undescribed species) present in glass house population together with *L. tentacularis* a pollinator wasp of *F. montana*, a dioecious fig tree. Dioecious figs have fewer ovary layers compared with monoecious figs and it has been proposed that the incidence of parasitism has been limited by dioecy (Kerdelhue and Rasplus, 1996), possibly because an absence multiple ovary layers allows pollinators to exclude non-pollinators competitively. In Indonesia, *Sycoscapter* sp. is widespread, occurring at almost all the sites where *F. montana* have been sampled and is usually the only non-pollinator present there (Zavodna, 2004; S. Compton Pers Comm.). No fig wasps that utilise female figs of *F. montana* have been reported, and significant seed predation by other insects has not been recorded.

Sycoscapter species in general are assumed to be parasitoids of pollinator larvae (Compton, 1993; Cook and Power, 1996; Kerdelhue and Rasplus, 1996; Kerdelhue *et al.*, 2000), but conclusive evidence is lacking. In order to better understand the biology and impact of *Sycoscapter* sp. we observed its timing of oviposition and its relationship with the pollinating wasp. This involved monitoring the numbers of pollinators in figs relative to the numbers of *Sycoscapter* sp. and foundress introduction experiments, where pollinator progeny were reared in the presence and absence of *Sycoscapter* sp.

7.3. Materials and Methods

F. montana is a small pioneer gynodioecious (functionally dioecious) shrub or scrambler found along rivers or in distributed forest in S.E. Asia (Corner, 1952, Berg, 1989). Like many other dioecious figs, *F. montana* has asynchronous fruiting within plants, allowing pollinators and parasitoids to cycle among a small number of trees or even on an individual plant. Pollinating females of *L. tentacularis* seek out the fruits at the receptive stage, enter, oviposit, pollinate and then either die or sometimes leave the fruit in an attempt to oviposit in another fig. The non-pollinating wasp (*Sycoscapter* sp. *indesc.*) need fruits that have already been pollinated (*pers obs*). It oviposits from the outside of the figs. The larvae develop into adults inside the ovules of the fig. Male are apterous and mating occurs inside the female galls. Both species complete their development at the same time, hatch and mate. At this time the male fig flowers have mature pollen and the female fig wasps transport the pollen to the next crop of receptive figs.

The work described here was carried out under controlled conditions at the Experimental Gardens of Leeds University. This is the only such captive population of fig wasps and their host plants in the world. The wasps originated (as did the trees) from the Centre for International Forestry Research (CIFOR) plantation, West Java and from Rakata, Krakatau islands, Indonesia (Moore, 2001).

7.3.1. Timing of oviposition of *Sycoscapter* sp.

Compton (1993) used a sequential bagging technique to determine the timing of oviposition by non-pollinating species of *Ficus burtt-davyi* in South Africa. He found

that gall forming *Otitessella* species were active on the trees at the same time as the pollinators, while the presumed parasitoid species of *Philotrypesis* and *Sycoryctes* (*Sycoscapter* sensu Boucek) peaked several weeks later. He also determined that adults of these species appeared in figs from a narrow range of exposure periods, corresponding to the times when ovipositing females were present on the trees. Yan *et al.* (2005) similarly determined the timing of oviposition of three non-pollinators on *Ficus hispida*.

Using the same technique, sequential bagging was used to find the timing of oviposition by *Sycoscapter* sp. One female pollinator was introduced into each fig and all the figs were bagged afterwards to prevent entry by more pollinators. Three figs from the first replicate group were exposed to *Sycoscapter* sp. after one week and remained open for that whole week. At the end of the week those three figs were bagged again and three others were exposed. The same process was repeated till the end of the sixth week so that some figs were exposed to *Sycoscapter* sp. throughout the period after pollinator oviposition. The same experiment was replicated six times on different dates to control for variation in *Sycoscapter* sp. abundance over time.

7.3.2. The impact of *Sycoscapter* on pollinators in the general glasshouse population

Fig fruits at C phase were selected and labelled and left with tags on them. After about 3-4 weeks, when these fig fruits were mature and had reached D phase, they were collected and dissections were carried out to determine the number of *L. tentacularis* and *Sycoscapter* sp. and thereby infer the effect of *Sycoscapter* sp. on the pollinators.

7.3.3. Impact of *Sycoscapter* in figs with a controlled number of pollinators foundresses

Receptive B phase male fig fruits were selected and divided into two equal groups. In both groups one pollinator foundress was introduced to each fruit with the help of a camel hair brush and the fruits were then bagged with fine mesh to prevent further entry of foundresses. In the first group, when the figs reached a suitable age for *Sycoscapter* sp. egg laying (C phase), at least eight females were introduced into each bag and the same process was repeated for four days. The second group was left with bags, preventing any *Sycoscapter* oviposition. When the figs were mature (D phase), they were collected and dissections determined the number of pollinators, non-pollinators, un-pollinated flowers and bladders (empty galled flowers, which may have had fig wasps egg laid in them).

7.3.4. Larval biology

Mid and late C phase male figs in the general glasshouse population were selected, and the galls inside them were dissected to search for the larvae of *Sycoscapter* sp.

7.4. Results

7.4.1. Oviposition timing

Sycoscapter sp. only oviposited during a period of 2-3 weeks after the pollinators had laid their egg in the figs (Fig 7.1, wk 1 = 12, 2 = 13, 3 = 14, 4 = 10 and 5 = 10, and 6 = 10 figs). At this time host larvae are available to attack, but plant material is still present inside the galled ovules alongside the pollinator larvae. The timing of oviposition by *Sycoscapter* pointed to it being either a late galler or early parasitoid.

7.4.2. The impact of *Sycoscapter* sp. on pollinators in the general glasshouse population

Out of 114 figs observed, only seven had no parasitoids present. The number of pollinator progeny and *Sycoscapter* sp. progeny per fig averaged 18.85 ± 1.03 (\pm SE) and 12.31 ± 0.71 (\pm SE) respectively. The maximum numbers of progeny present were 55 (*L. tentacularis*) and 36 (*Sycoscapter* sp.). Pearson product correlation indicated that there was no association between pollinators and parasitoids (Pearson correlation, $r = 0.004$, $P = 0.97$) (Fig 7.2).

7.4.3 Figs with a single foundress

In contrast to the results in the general population, *Sycoscapter* sp. significantly reduced the numbers of pollinator progeny inside figs that had been entered by a single foundress (ANOVA, $F_{(1, 18)} = 9.97$, $P < 0.05$) (Fig 8.3, Table 8.1). An analysis of covariance with total female flower number as the covariate again showed that there was a highly significant difference between pollinator progeny numbers in figs with and without *Sycoscapter* sp. (ANCOVA, $F_{(1, 16)} = 18.82$, $P < 0.001$). This fall in pollinator numbers was not due to some form of killing of pollinator larvae during *Sycoscapter* sp. oviposition, as we did not find a significant influence of *Sycoscapter* sp. on the numbers of bladders (ANOVA, $F_{(1, 18)} = 0.12$, $P = 0.72$, Table 7.1). This conclusion is reinforced by comparing the combined number of *Sycoscapter* sp. and pollinator progeny with the other figs where only pollinators were present (Fig 7.3). The total number of wasps in the figs were very similar.

In all of the 150 dissected ovules only a single larva was found in each. This suggests that *Sycoscapter* sp. is not an ecto-parasitoid. It might be an endo-parasitoid that left the pollinator larvae to consume the contents of ovules before killing them, or an endo-parasitoid which also feeds on plant material after emerging from it. Whether *Sycoscapter* sp. are true parasitoids or inquilines that subsequently feed on plant tissues is still not clear, though no plant material remained inside the galled ovules after the adults emerged.

7.5. Discussion

Our results showed that *Sycoscapter* sp. oviposits 2-3 weeks after pollinator entry, when host larvae are present in the figs. In the general population, no correlation was found between the numbers of progeny of the two wasp species, which might have indicated that they were using different resources within the figs. However, in experiments where the numbers of pollinators entering a fig were controlled, *Sycoscapter* sp. significantly reduced the numbers of pollinators, suggesting that *Sycoscapter* sp. is a parasitoid, or inquiline. It is unlikely to be an ectoparasite.

Non-pollinating wasps are ubiquitous associates of figs. Their biology has received little attention so far, even though some authors have looked for the impact they have on the fig pollinator mutualism (Bronstein, 1991; Cook and Power, 1996; Kerdelhue and Rasplus, 1996). Weiblen *et al.* (2000) were the first to study the rates of parasitism in functionally dioecious figs, showing that the non-pollinator community had a negative effect on pollinator numbers. They will also have an indirect negative effect on fig trees, as gallers compete with pollinators for female flower resources and parasitoids attack the larvae of pollinators.

The study of oviposition behavior and feeding habits is quite important in developing an understanding the role of non-pollinators in natural fig wasp communities (Morris *et al.*, 2003). Pollinating and non pollinating wasps oviposit at specific stages of fig maturation, but regardless of the timing of oviposition, all fig wasp species usually emerge from the galls at the same time as the pollinators and often use the same exit hole, made by male pollinators, to escape (Weiblen, 2002). Because the timings of oviposition were not exactly determined most previous studies only highlighted qualitative relationships between fig wasps (West and Herre, 1994; West *et al.*, 1996). Non pollinators can be classified according to the time when female wasps arrive to oviposit on the figs (Kerdelhue *et al.*, 2000) and the diameters of figs and thickness of walls of figs are often used to quantify the characters of ovipositing wasps, but exact oviposition times of the fig wasps have rarely been investigated (Kerdelhue and Rasplus, 1996). An exception is Peng *et al.* (2005) with the non pollinating fig wasps of *F. hispida*. They divided non pollinators into gallers and parasitoids on the basis of their oviposition timings. The oviposition time determined for *Sycoscapter* sp. in our experiments suggests that it targets larvae of the pollinators.

Non-pollinator wasps that mature within figs are highly specialized and are not associated with any other plants. Hill (1967) mentioned that most non pollinators can escape without the aid of pollinator males. According to him the non pollinators that require the aid of agonids to escape by chewing the exit hole are mostly species specific. If such non-pollinators eclosed well before the pollinator's adults they would be trapped within the figs, if they developed more slowly they would be still present in ripened syconia, which in monoecious fig species will be eaten by animals (Galil *et al.*, 1970). *Sycoscapter* sp. males can chew an exit hole in *F. montana* (Compton Pers Comm), so they are independent of their host.

The evolution of dioecy in figs appears to have reduced the incidence of non pollinators. Assuming that non pollinators compete for fig ovaries, Kerdelhue and Rasplus (1996) attributed lower numbers of non pollinating species in dioecious figs compared with monoecious figs to the absence of multiple ovary layers in gall figs. The absence of multiple ovary layers is hypothesized to have allowed pollinators to exclude non-pollinators competitively, thereby resulting in lower species richness and abundance in functionally dioecious figs. Non-pollinators may waste time by probing female figs, one advantage of dioecy may also be that this will reduce their rate of parasitism in male figs (Weiblen *et al.*, 2000).

Kerdelue *et al.* (2000) found a significant negative relationship between the numbers of the pollinator *Agaon cicatiferens* and another *Sycoscapter* sp. in *Ficus sagittifolia*. They concluded that this *Sycoscapter* is likely to be a parasitoid or an inquiline. We found no such relationship until we controlled for foundress number, which equates to the number of hosts available, perhaps because of difference in parasitism rates. Patel (1998) found no correlation between pollinating and non pollinating wasp number per fig, which is similar to our results. Her results were interpreted by suggesting that they were not competing for limited fig resources, but she also argued that the deleterious effects of non pollinators might have not been seen because as the total flower numbers per fig increased so did the numbers of pollinators and this could have confounded the results.

Peng *et al.* (2005) even found a positive correlation between the numbers of non pollinators and pollinators in figs of *F. hispida*. In some large collections of *F.*

montana figs at Leeds a similar positive relationship is sometimes detected (pers. observation).

Our results highlight that simple correlations can fail to provide accurate information on the impact of non-pollinators on hosts. Only by controlling for initial host number can a more accurate picture be obtained.

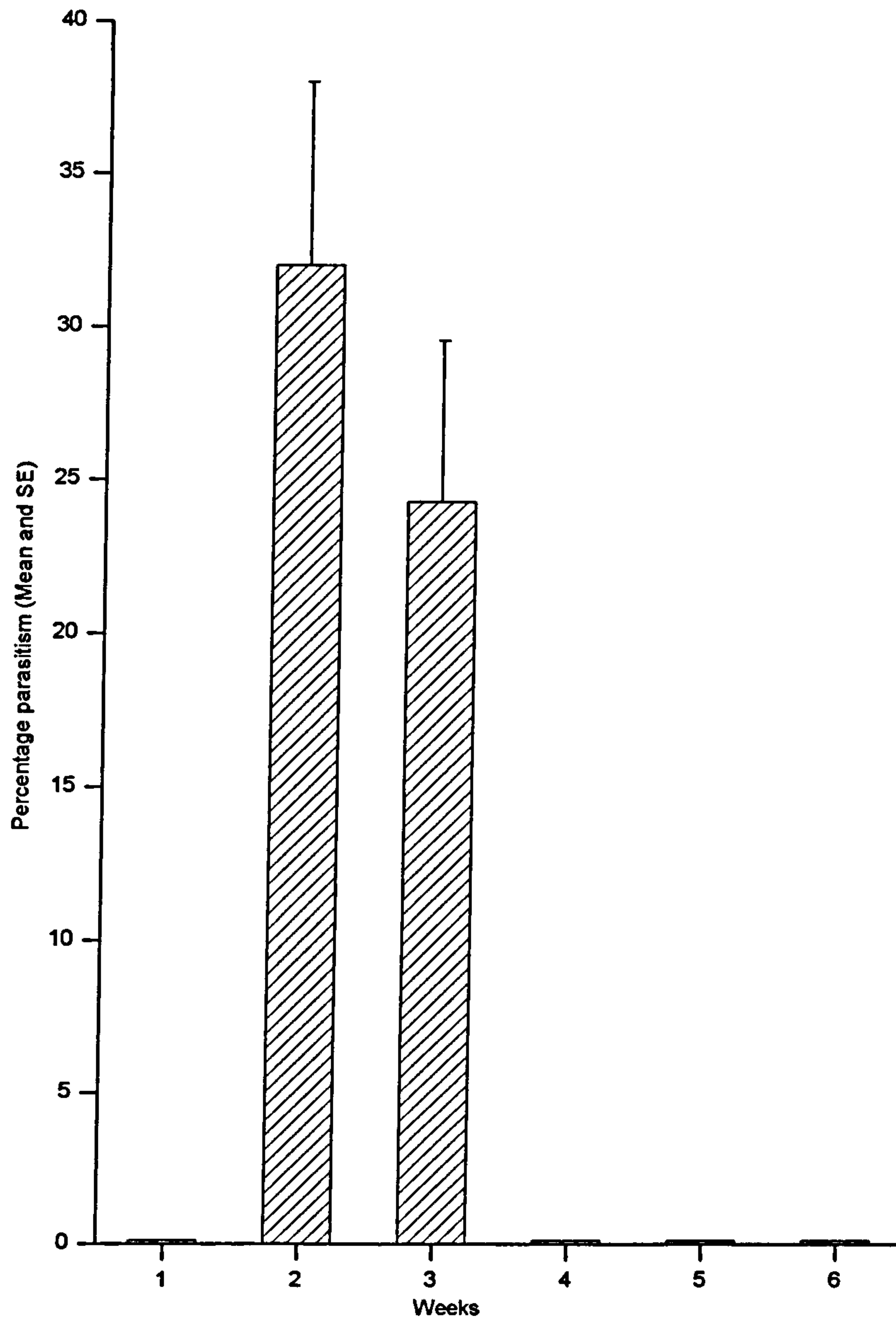


Fig. 7.1 Parasitism in figs exposed to *Sycoscapter* sp. for different time intervals after pollinator entry.

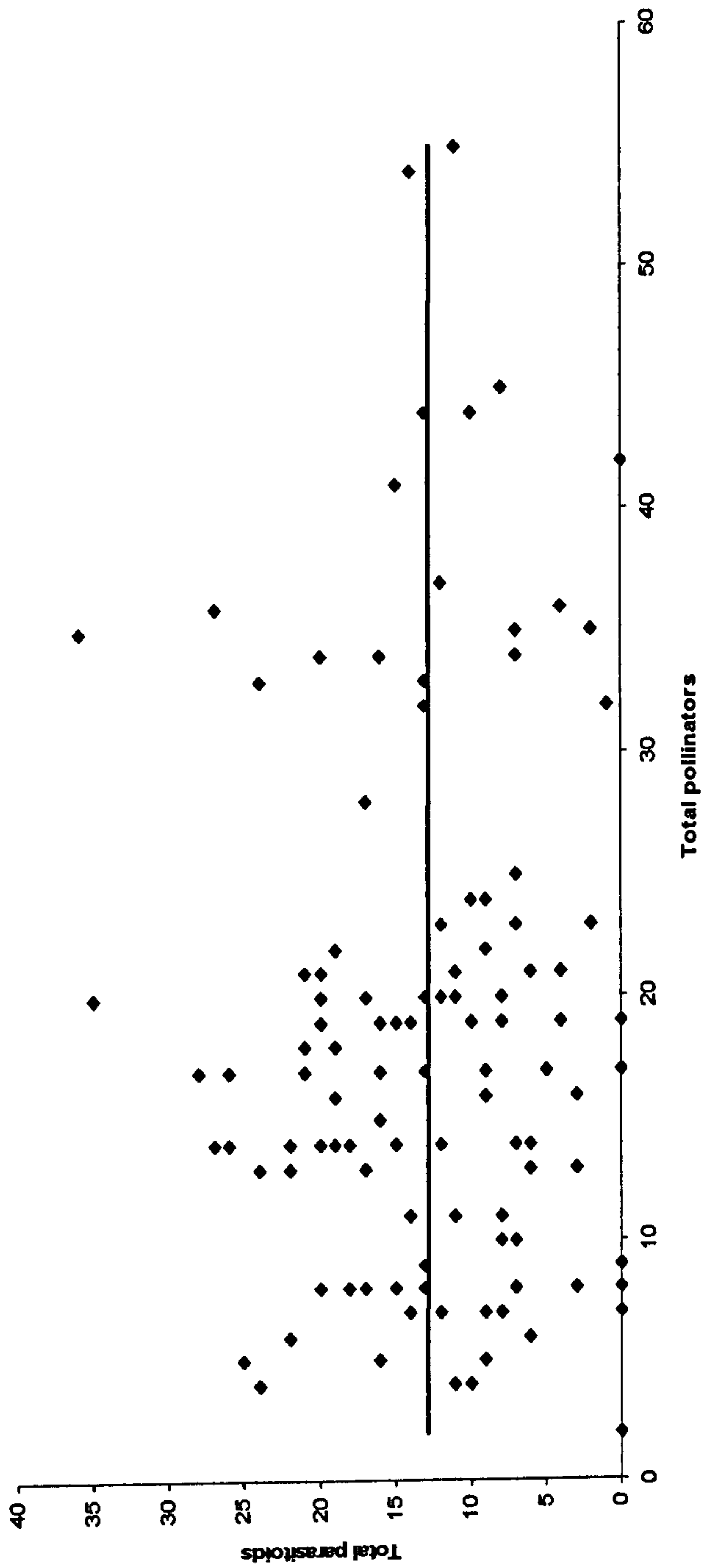


Fig. 7.2 The relationship between the number of pollinators and *Sycoscapter* sp. emerging from figs of *Ficus montana*.

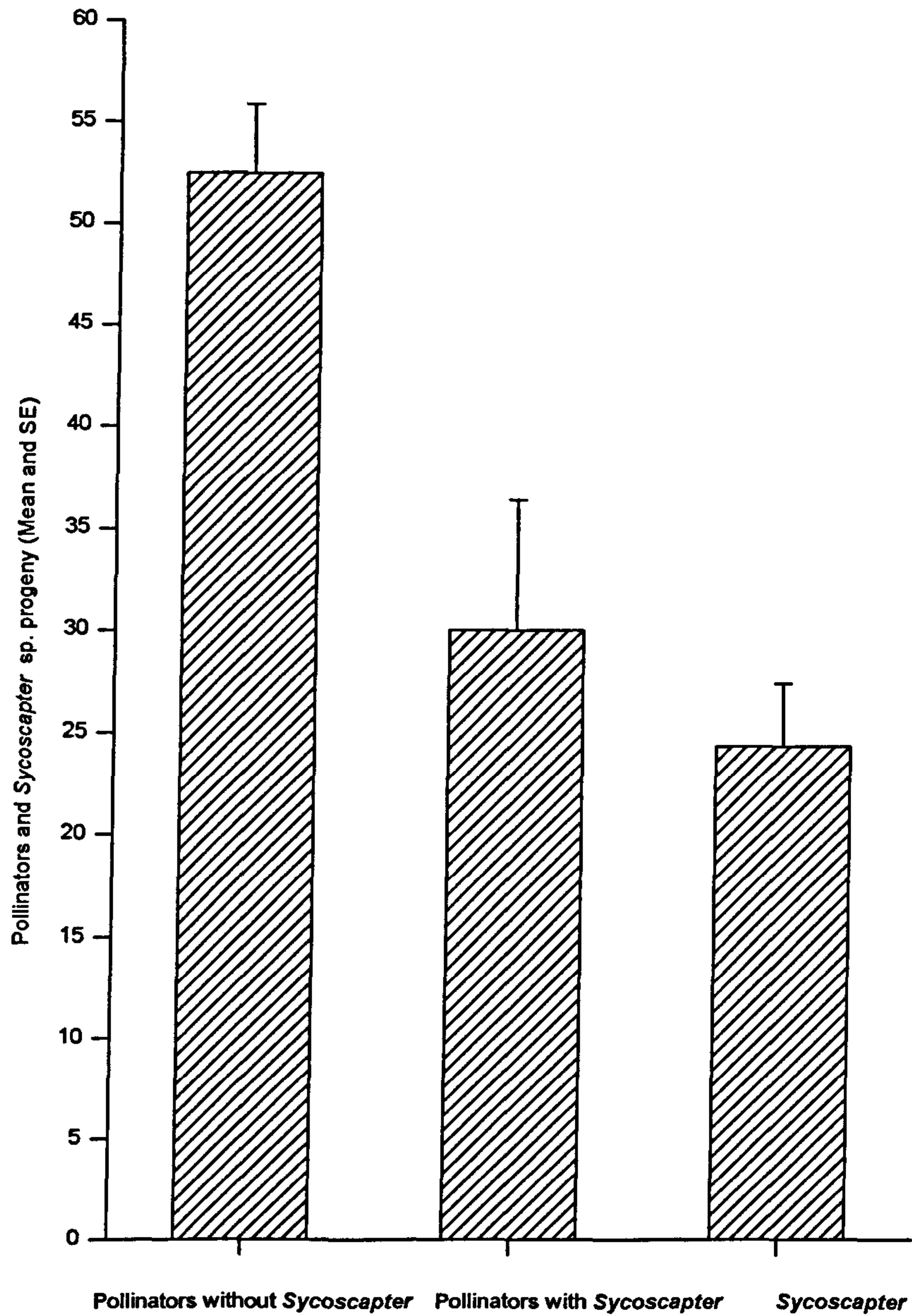


Fig. 7.3 The total numbers of pollinator progeny in control figs and those exposed to *Sycoscapter sp.* along with the number of *Sycoscapter sp.* that emerged from these figs. Each fig had been entered by a single foundress.

Table 7.1 Summary of the contents of figs with a single foundress with and without *Sycoscapter* sp.

	With <i>Sycoscapter</i> sp.		Without <i>Sycoscapter</i> sp.	
	Mean	SE	Mean	SE
Male pollinators	7.35	0.62	8.30	0.76
Female pollinators	26.22	5.87	44.20	3.31
Total pollinators	33.57	6.45	52.50	3.46
Male parasitoids	4.55	0.74	---	---
Female parasitoids	22.00	1.69	---	---
Total parasitoids	26.55	3.05	---	---
Total wasps	60.12	7.62	52.50	3.46
Un-pollinated flowers	13.83	3.83	16.40	4.11
Total bladders	31.50	3.33	27.60	5.72
Total female flowers	105.45	9.47	96.50	9.26
Total male flowers	17.11	2.15	15.40	1.68
Total flowers	122.56	10.30	111.90	9.60

8. Parasitoids do not change the realized sex ratio of a fig wasp that pollinates a dioecious fig tree

8.1. Abstract

Pollinator fig wasps (Hymenoptera, Agaonidae) are one of the most widely utilized model systems for studies of sex ratio evolution. In these fig wasps, mating occurs inside the figs, with brothers routinely competing against each other for matings. As a result of this local mate competition, and inbreeding, mothers (foundresses) invest more in daughters and less in sons, resulting in female biased sex ratios. The optimal sex ratio varies according to the numbers of foundresses entering a fig and empirical studies have confirmed that sex ratio adjustment takes place. Non-pollinating fig wasps include parasitoids that target pollinator larvae. A previous study has found that parasitoids in South America can preferentially destroy female pollinators, generating less female biased secondary sex ratios. I utilized laboratory populations of the south East Asian fig wasp *Liporrhopalum tentacularis*, its host the dioecious fig tree *Ficus montana* and the parasitoid *Sycoscapter* sp. indesc to study the influence of non-pollinators on fig wasp sex ratio adjustment under controlled conditions.

I compared pollinator sex ratios in figs entered by varying numbers of *L. tentacularis* in the subsequent presence or absence of its parasitoid. *L. tentacularis* lays most of its male eggs at the start of an oviposition sequence. I also manipulated its primary sex ratio by allowing foundresses to oviposit for different length of times before killing them with insecticide and than exposed half the figs to the parasitoid. In neither situation did *Sycoscapter* have an effect on the sex ratio of the pollinator. I suggest that because of differences in anatomy, alteration of the realized sex ratios of pollinators by

parasitoids is more likely to occur with pollinators of monoecious fig species, and is also more likely to occur with pollinators of tree species that produce larger figs.

8.2. Introduction

Sex allocation theory has been hailed as one of the successes of modern evolutionary biology (Charnov, 1982; Godfray, 1994; Herre *et al.*, 1997). The sex ratio of a parent's offspring is tightly linked to fitness and Fisher (1930) was the first to show that natural selection would favour equal investment in the sexes in a random-mating population. Exceptions to 50:50 sex ratios provide an opportunity to test theory and an area that has received particular attention has been sex ratio evolution in structured mating populations. When mating occurs between the offspring of a subset of the population, sibling males compete for mates. Hamilton (1967) considered the case in which a single foundress colonizes a patch, where her offspring then develop and mate amongst themselves before dispersing. Selection favours a highly female biased sex ratio, with just enough males to ensure the fertilization of females. This phenomenon is known as local mate competition (LMC) because brothers compete with each other for mating with their own sisters. If the offspring in the patch originate from two foundresses, the marginal value of sons increases because sons have the potential to also mate with the daughters of the other female. Consequently the progeny sex ratio (defined as the proportion of males) will increase with the number of foundress females ovipositing in the patch (Charnov, 1982; Taylor, 1981). Assuming foundresses have knowledge of how many others are making equal contributions to the mating population (or brood), the predicted sex ratio becomes less female biased with increasing density (Hamilton, 1967). In haplodiploid species, such as hymenopterans, structured mating populations also increase the level of inbreeding (Hamilton, 1979, Taylor and Bulmer, 1980; Herre, 1987), which also skews optimal sex ratios.

In hymenopterans, control of fertilization allows sex ratios to be adjusted by ovipositing females, but the mechanisms involved in the generation of sex ratio vary. Sex ratio adjustment can be obtained by sequential oviposition of male, and then female eggs, but the adult female needs to assure that enough males are present to inseminate all the female progeny. The egg parasitoid *Trichogramma evanescens* (Trichogrammatidae) has been shown to lay male eggs first, followed by female eggs (Waage and Lane, 1984). Oviposition sequences can be extremely precise. *Gryon japonicum* (Scelionidae), a solitary egg parasitoid, lays a male egg in the second host egg of an oviposition sequence and female eggs in the others. The sequence is reset after a three hour interval, allowing *G. japonicum* to closely align sex ratios to the size of its host egg batches (Noda and Hirose, 1989).

The genus *Ficus* offers a useful model system for studies of ecology and evolution due to its diversity of biotic interactions involving diverse groups of organisms (Bawa and Beach, 1981; Compton and Hawkins, 1992). *Ficus* and its fig wasp pollinators of the family Agaonidae exhibit a highly specific plant-insect mutualism. One or more female fig wasps enter the figs, closed inflorescence containing large numbers of flowers on the inner surface and a closed apically by numerous bracts forming the ostiole (Verkerke, 1989). The females lose their wings and antennae while penetrating through the ostiole. The females deposit pollen while laying the eggs into, and galling, the ovules. The fig was considered as a trap for pollinators because after pollination and oviposition they often die inside the figs without emerging from them. However, it has now been reported that some pollinator females can remerge and lay eggs in other figs (Gibernau *et al.*, 1996; Moore, 2003a).

Fig trees occupy two functional reproductive categories, monoecious and dioecious, although the later are anatomically gynodioecious (Compton *et al.*, 1996). Monoecious fig trees have one type of tree and one type of fig having both male and female flowers. The male flowers produce pollen and the female flowers develop into seeds or become galled by fig wasps and provide food for their larvae. Male flowers develop at the same rate as the wasp progeny, so that the next generations of wasps are supplied with pollen before the adult females fly away to another fig (Nefdt and Compton, 1996; Kerdelhue *et al.*, 2000). Dioecious fig trees have separate male and female plants. The female flowers prevent the wasps from laying eggs, so a wasp entering a female fig dies without reproducing even if she re-emerges, as she loses her wings on entry. Male trees are anatomically hermaphrodite (Patel, 1998). The female flowers of the male fig are for wasp oviposition and development (Kameyama *et al.*, 1999).

Not all fig wasps are pollinators, and some fig tree species support twenty or more species of non-pollinating fig wasps (Boucek *et al.*, 1981, Compton and Hawkins, 1992). Non-pollinating wasps probably have extremely diverse trophic relationships (gallers, kleptoparasites, inquilines and parasitoids) but little is known about the details of their biology. All pollinator and some non-pollinator species lay their eggs from inside the figs, but most non-pollinators oviposit from outside, inserting their long ovipositors through the walls of the figs to lay eggs inside the flowers. Pollinating and non pollinating fig wasps may oviposit at different stages of fig maturation, but regardless of this they emerge from the figs at about the same time, often using a shared exit hole to escape from the figs (Weiblen, 2002). The morphologies and mating strategies of non-pollinators are quite variable and the details of their impact on the mutualism are poorly understood (Hamilton, 1979; Bronstein, 1991; Compton and Hawkins, 1992; West and Herre, 1994).

Non-pollinating fig wasps, whether parasitoids or gallers, have a negative impact on the mutualism through predation of pollinator larvae and through competition with pollinators for resources (Compton *et al.*, 1994; West and Herre, 1994; Kerdelhue and Rasplus, 1996), as well as destroying seeds.

Because the early stages of the life cycle of fig wasp take place in a closed environment, it is easy to study their sex ratios, especially in species where foundresses do not re-emerge, making fig wasps a useful model system for testing sex ratio theory (Hamilton, 1979; Frank, 1985; Greef and Compton, 1996). Moore *et al.*, (2002) showed that sex ratios of the *Liporrhopalum tentacularis*, one of the most intensely studied species, become less female biased with increasing foundress numbers and later that progeny sex ratios are not directly related to foundress density, but rather to the size of clutch laid by a particular female (Moore *et al.*, 2005). Current work (chapter 6) has shown that the link between sex ratios and clutch sizes reflects a strategy of laying mostly male eggs early in an oviposition sequence. Sex ratio adjustment is not necessarily optimal for the animals. Distortion of sex ratios towards females has been reported in many species of insects and crustaceans (Dunn *et al.*, 1995). Causes of sex ratio distortion include genetic factors and parasites. Some of these parasites are transmitted maternally and distort sex ratios by host feminization, primary sex ratio bias and parthenogenesis (Dunn *et al.*, 1995). Male killing has been found in a number of insects, with male embryos produced from a female infected with a sex ratio distorting agent showing a significantly higher rate of mortality (Hurst, 1993). In mosquitoes which are infected with microsporidians, death of males occurs during the fourth instar larvae (Hurst, 1991). In Lepidoptera, all- female broods are known from wild populations of at least 20 species (Hurst, 1993). The causative agents

of sex ratio distortion have not been identified in these species, except that *Wolbachia* kills early instar larvae of males (Jiggins *et al.*, 2000). Loss of pollinator males would be particularly significant in figs, where if only a single foundress lays eggs, already produces few males. Cook (1993) postulated that sex ratios may be more male biased than expected by models, in order to reflect this eventuality. Patel (1998) found a dioecious fig species where 90% of the figs contained dead adult pollinating and non-pollinating wasps that had been trapped, presumably because too many non-pollinating wasps had reduced the number of pollinator males to the point where there were too few to chew an exit hole.

Periera and Prado (2005) have argued that sex ratio studies of fig wasps have been based on counts of adults (secondary sex ratios) rather than primary sex ratios (the sex ratios of the eggs laid), and that secondary sex ratios may not precisely reflect the primary sex ratios when non-pollinators are numerous. They examined the effect of non pollinating wasps on the sex ratio of the pollinator wasp *Pegoscapus tonduzi* that pollinates the monoecious *F. citrifolia*. They found a positive relationship between the pollinator's realized sex ratio (as percentage males) of *P. tonduzi* and the densities of non-pollinating wasps, which was independent of foundress number and brood size. The change in sex ratio was because the non-pollinating wasps killed significant numbers of female pollinators, but had no affect on male pollinator numbers. Therefore in studies of fig wasps we should distinguish between primary sex ratios (before any mortality factor occurs) and secondary sex ratios observed (after any mortality factors have taken effect) (Godfray, 1994).

In this paper, we examine the effect of a parasitoid fig wasp on the realized (secondary) sex ratio of an Asian pollinator fig wasp. We compared pollinator sex

ratios in the presence and absence of parasitoids in two situations where we also varied putative primary sex ratios by varying the number of foundresses contributing to combined broods or by varying the length of time that a pollinator had available to oviposit in a species where most male eggs are laid first.

8.3. Materials and Methods

Ficus montana is a small shrub which is placed in Subgenus *Sycidium* Section *Sycidium* by Berg and Corner (2005). This species is functionally dioecious, with male trees bearing figs that have both male and female flowers, but produces only pollen and pollinators and female trees bearing figs containing only female flowers that produce seeds. Like many other dioecious figs this species has asynchronous fruiting within plants, with pollinators routinely able to cycle on a single male plant (Moore, 2001).

The pollinator of *F. montana* is the diurnally active fig wasp *Liporrhopalum tentacularis*. Adult female *L. tentacularis* that enter figs (foundresses) lose their wings and most of their antennae when passing through the ostiole, but are nonetheless capable of re-emerging. These foundresses can enter other figs within walking distances (Moore *et al.*, 2003a). *Sycoscapter* species *indesc* (Sycorytinae, Agaonidae) is a non-pollinating species associated with *F. montana*. The winged female oviposits through the walls of the fig from the outside. Males are apterous. *Sycoscapter* sp. is the most common and widespread parasitoid associated with *F. montana*, and is often the only other wasp species in figs along with the pollinator (Zavonda, 2004). We have shown that *Sycoscapter* sp. develops as a parasitoid, killing pollinator larvae (Chapter 7). Studies of other *Sycoscapter* species also indicate that they are parasites of fig wasp pollinators (Cook and Power, 1996; Kerdelhue and Rasplus, 1996).

The experiments used glasshouse population of *F. montana*, along with its associated wasps *L. tentacularis* and *Sycoscapter* sp. housed at the Experimental Gardens, University of Leeds (U. K). They originated from the Centre for International Forestry Research (CIFOR) plantation, West Java, Indonesia and from Rakata (Krakatau), Indonesia and have been maintained in culture since 1995 (Moore *et al.*, 2002).

8.3.1. Figs with varying foundress numbers

Foundresses were introduced into 90 receptive male figs by placing the wasps at the ostiole. One third had only one foundress introduced, another third had a second foundress introduced after a one-hour interval and the remainder had a third foundress introduced after another hour. A fine mesh bag was then placed over the figs to prevent entry by unwanted foundresses. After three days half the experimental figs from each group had the bags removed to allow oviposition by free-ranging *Sycoscapter* sp. which occur in about two weeks. Later, when the figs had ripened, they were removed from the plants, and their contents recorded.

8.3.2. Figs where foundresses were allowed to oviposit for limited periods of time

Preliminary experiments with the contact insecticide pyrethrum found that exposure to one micro-litre for less than one minute was sufficient to kill the pollinator females (chapter 6). This quantity of pyrethrum is effective at killing ovipositing females quickly and does not affect the eggs and larvae of the wasps (chapter 6).

Single foundresses were introduced into male figs as before. After periods of 5, 15, 30, 60, 120 and 240 minute, we halted further oviposition by gently introducing a syringe through the ostiole and injected one micro liter of pyrethrum to kill the females. The figs were then bagged to prevent the entry of other females. After 3-4 days half of the figs had the bags removed to allow exposure to *Sycoscapter* while the remainders were left inside bags until the end of experiment. When the figs had ripened they were removed and the contents were counted as before.

8.4. Results

8.4.1. Figs with varying foundress numbers

The characteristics of the experimental figs are shown in Table 8.1. Parasitism rates (based on counts of adult pollinators and parasitoids) were not significantly different between treatments (ANOVA, $F_{(2, 29)} = 0.54$, $P = 0.56$). The mean percentage parasitism for one, two and three foundress was $18.49 \% \pm 1.82$ (Mean \pm SE), $17.85 \% \pm 1.81$ (Mean \pm SE) and $19.98 \% \pm 1.36$ (Mean \pm SE) respectively. The effects of parasitoids and foundress numbers on the number of pollinator progeny were analyzed using a generalized linear model in the 'R' statistical package. Foundress number (1, 2 or 3) did not affect the number of pollinator progeny (Chi sq, $X^2 = 1.54$, $df = 2$, $P = 0.46$, Fig 8.1), but parasitoids significantly affected the numbers of pollinator progeny (Chi sq, $X^2 = 3.99$, $df = 1$, $P = 0.04$).

The data were also analyzed GLM to assess the effect of foundresses and parasitoids on the sex ratios of the pollinator progeny. It was found that brood sex ratio was significantly affected by the density of foundresses (Chi sq, $X^2 = 65.46$, $df = 2$, $P >$

0.001, Fig 8.2) but the number of parasitoids did not significantly influence the sex ratios (Chi sq, $X^2 = 0.523$, $df = 1$, $P = 0.46$).

8.4.2. Figs where foundresses were allowed to oviposit for limited periods of time

Percentage parasitism ranged from $7\% \pm 4.52$ (Mean \pm SE) to $15\% \pm 5.13$ (Mean \pm SE), with no significant difference between oviposition durations (Kruskal Wallis, $X^2 = 7.19$, $df = 5$, $P = 0.206$).

The brood sizes achieved varied considerably for different oviposition times ranging from 7.11 ± 1.34 (Mean \pm SE) to 54.44 ± 2.49 (Mean \pm SE) in the without parasitoid group and from 5.50 ± 0.65 (Mean \pm SE) to 51.11 ± 4.37 (Mean \pm SE) in with parasitoid group (Fig 8.3).

The effect of parasitoids and varying time for oviposition on the number of pollinator progeny were analyzed by using GLM (Binomial link function) in R statistical package and it was found that both the time (Chi sq, $X^2 = 696.95$, $df = 1$, $P < 0.001$) and the presence of parasitoids (Chi sq, $X^2 = 9.53$, $df = 1$, $P < 0.001$) had significant effects on pollinator progeny numbers.

Similar analysis was done separately for the effect of time and parasitoids on the sex ratio of the pollinator progeny. For the time available for oviposition, we found a significant effect on sex ratio depending on the time available for oviposition, with more male biased sex ratios early on during laying (Chi sq, $X^2 = 22.13$, $df = 1$, $P < 0.001$, Fig 8.4). However, as in the varying foundress experiment parasitism did not

effect the sex ratio of pollinator progeny (Chi sq, $X^2 = 0.0019$, $df = 1$, $P = 0.965$).

There is thus no evidence that parasitoids differentiate between different sexes.

Power analysis by simulation was done by Y. Wang to determine the level of parasitism which can distort the sex ratio of the pollinating wasps. Simulated data based on actual data (10 figs each with and without parasitoids) were selected randomly and the numbers of males were based on a 0.1 sex ratio. The results are shown as a contour plot (Fig 8.5). Even when parasitoids attack only females then the levels of parasitism need to be 50 % to detect a sex ratio distortion.

8.5. Discussion

Although characteristics that influence the susceptibility of insects to parasitoids have received considerable attention (Hawkins, 1994), sex dependent parasitism is reviewed very rarely. Female biased sex ratios have been reported in many species of insects (Green *et al.*, 1982; Werren, 1984). These biases are generally explained through arguments based on the local mate competition (LMC) model constructed by Hamilton (1967). Assuming that females have knowledge of the numbers of ovipositing conspecifics and they make equal contributions to the brood, predicted sex ratios become less female biased with increasing density (Hamilton, 1967). Such sex ratios are advantageous under conditions where a few males can inseminate all of their sisters. To assure that a sufficient number of males to inseminate all females are present, the sequence of oviposition is very important. In our experiment we varied the putative primary sex ratios in two different ways, but we did not find any change in the sex ratios of *L. tentacularis* when they were subject to attack by the parasitoid *Sycoscapter* sp.

Non-pollinators typically show a negative impact on the populations of pollinators (Kerdhue and Rasplus, 1996). Previous studies have predicted that the persistence of the mutualism in functionally dioecious figs might also be affected by the stability of host parasitoid interactions, as in monoecious figs (Compton and van Noort, 1992; Nefdt and Compton, 1996) but there have been few tests in functionally dioecious figs (Kjellberg *et al.*, 1987; Grafen and Godfray, 1991). Weiblen *et al.* (2000) were the first to study the rates of parasitism in functionally dioecious figs, highlighting the fact that non-pollinators had a direct negative impact on pollinator numbers.

Concealment of endophytic herbivores may protect them from attack by predators, but their immobility may also make them highly vulnerable to parasitoids (Hawkins, 1994). It is therefore possible that ecological and biological differences among herbivores could result in attacks by different types of enemies, even if overall rates are similar. All forms of endophagy reduce mortality by pathogens and many predators, but protection from parasitoids requires that endophages develop in tough plant tissues like stems and galls (Hawkins, 1994).

Periera and Prado (2005) found that the sex ratio of *Pegoscapus tonduzi*, a pollinator of the monoecious *Ficus citrifolia*, was distorted by non-pollinator wasps. This is contrary to our results. According to them the sex ratio was distorted due to the differential killing of females by the non pollinators, as the cleptoparasitic species *Idarnes carne* laid eggs preferentially in the galls containing female larvae of the pollinating wasp. We did find that *Sycoscapter* sp. reduced the number of pollinators, but we did not find any distortion of sex ratios in our data. We also have a 15 month data set from the glasshouse population of *L. tentacularis* (N. Suleman in prep) which

showed that there was a seasonal effect on the size of the pollinator and parasitoid populations, but in spite of a clear cyclic pattern and variation in the numbers of pollinators and parasitoids, we did not find sex ratio distortion.

Periera and Prado (2005) also inspected the data for other species besides *F. citrifolia* and showed that the sex ratio distortion ability of non-pollinating wasps was stronger for species with smaller flower numbers (*F. citrifolia* = 325, n = 55, *F. obtusifolia* = 976, n = 34 and *F. popenoei* = 1124, n = 78). According to them it was easier to detect sex ratio distortion in small figs with fewer flowers, but in spite of the small size of *F. montana* figs, with flower numbers ranging from 60-130, we did not find any sex ratio distortion in the pollinating wasp. The percentage parasitism in both of our experiments remained low. It would have been easier to detect sex ratio distortion if percentage parasitism were higher.

In monoecious figs there is typically a larger range in style lengths than in dioecious figs. Presumably, sex ratio effects are generated by differential parasitism at different depths in the figs, linked to female wasp eggs being laid closer to the outer ovary layers. Foundresses of *L. tentacularis*, and probably other species, lay mostly male eggs first (chapter 6), and there is evidence from other species that shorter styled flowers are used first (Compton *et al.*, 1994). These are furthest from the outer surface of a fig. Larvae towards the periphery are likely to be female and more prone to parasitism by non pollinators which oviposit from the outside of the fig. Parasitoid wasps nonetheless often have ovipositors that easily reach the ovules of shorter styled flowers (Compton and Nefdt, 1988) and measurements of the styles of flowers occupied by wasps in *Ficus sur* and *Ficus burtt-davyi* showed that attacks on pollinator

larvae by parasitoids did not vary according to style length, nor distance from the outer surface of figs (Nefdt, 1989).

Sex ratio distortion may be common in many arthropods, with far reaching consequences for the evolution of their host organisms (Jiggins *et al.*, 2000). Mechanisms for primary sex ratio distortion in animals were reviewed by Hurst (1993) and Dunn *et al.* (1995). Major mechanisms for over production of female offspring caused by cytoplasmic infection can be classified into three kinds: microbe induced parthenogenesis that mainly produces males, selective death of males during the immature stages and feminization of males.

There are other mechanisms by which primary sex ratios are distorted. A female biased sex ratio has been found in a population of the oriental tea tortrix moth, *Homona magnanima* (Tortricidae) in Japan. Greater than 50 % mortality was observed in all female strain larvae, suggesting that female-only broods are produced as a result of late male killing.

Sex chromosome meiotic drive was suspected to cause female biased sex ratios in two species of butterfly, *Acraea encedon* (Nymphalidae) and *Danaus chrysippus*, (Nymphalidae) but the death of male embryos is now known to be the cause of sex ratio distortion in both these species (Owen and Smith, 1991). Male death may benefit female siblings by preventing inbreeding, but Owen *et al.* (1973) pointed out that in female biased populations most of the females must remain un-mated and will in turn lay infertile eggs. It is unlikely under these conditions that inbreeding will be disadvantageous, as uninfected females will get better chances of mating.

Given this variable impact, the ecological and biological factors that influence insect sex ratios need to be identified in order to predict when and where they are likely to be significant and to test conventional wisdom about the factors influencing insect population dynamics.

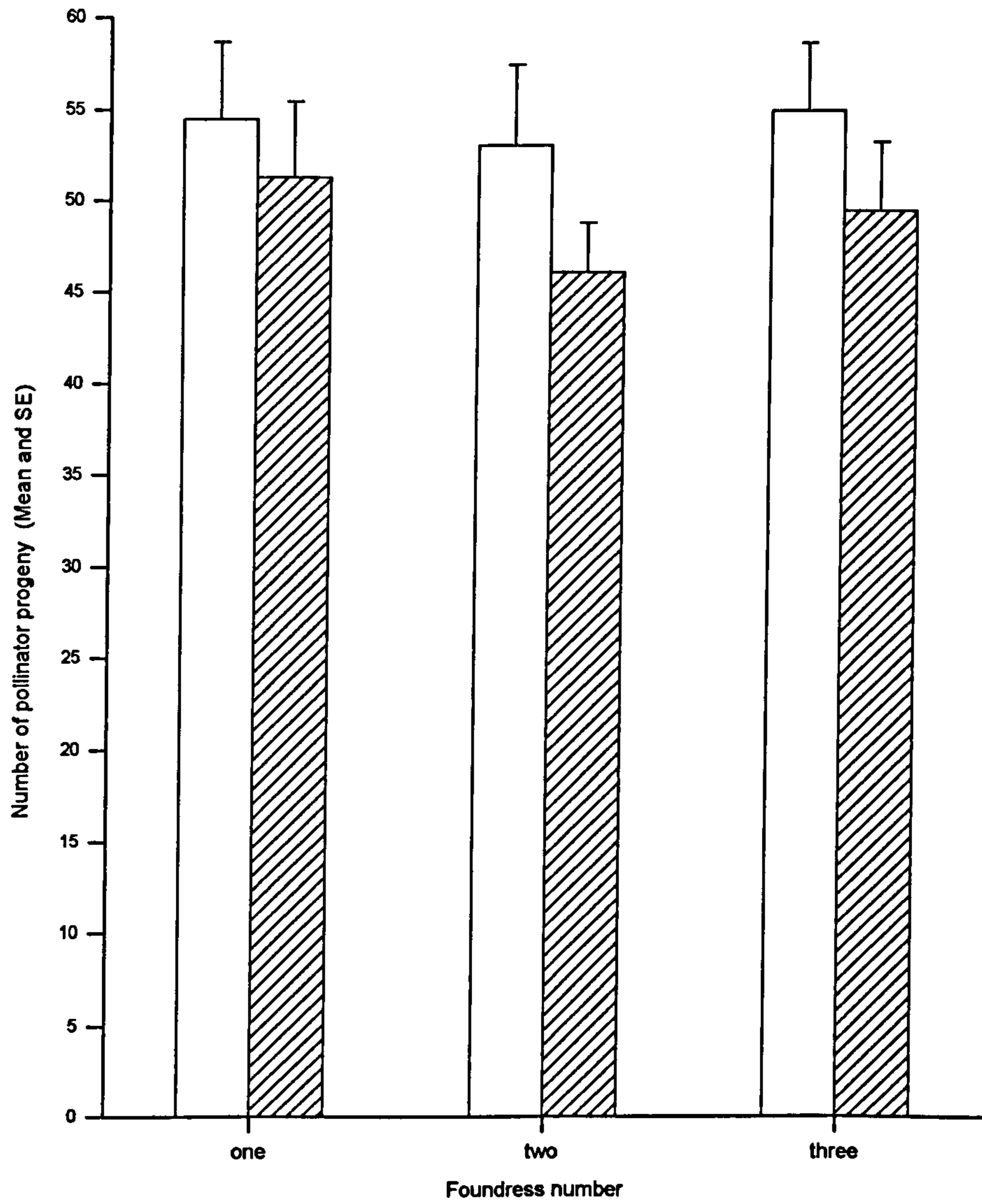


Fig. 8.1 The number of pollinator progeny in figs entered by different numbers of foundresses in the absence (open bars) and presence (shaded bars) of *Sycoscapter*.

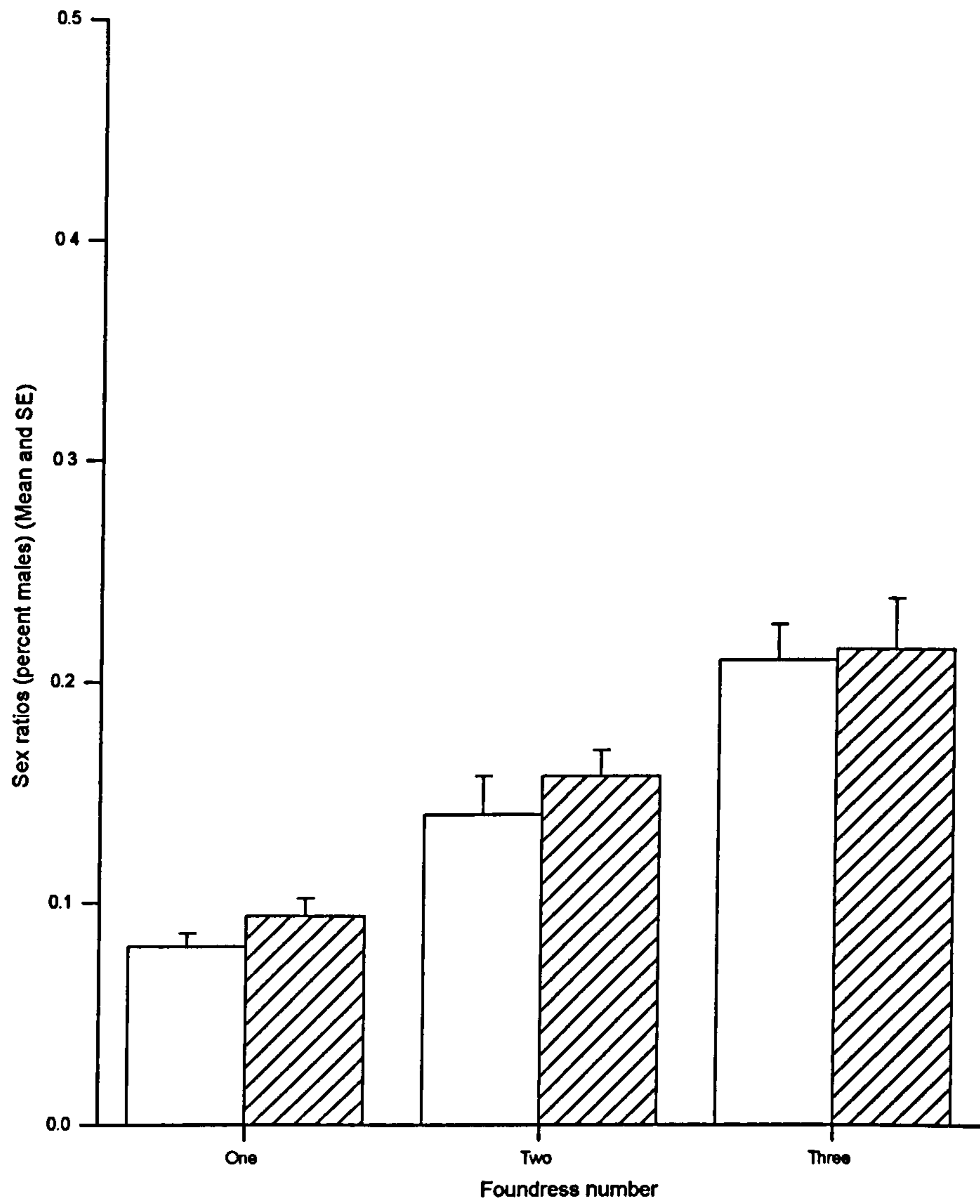


Fig. 8.2 The sex ratio of pollinator progeny in figs entered by different numbers of foundresses in the absence (open bars) and presence (shaded bars) of *Sycoscapter sp.*

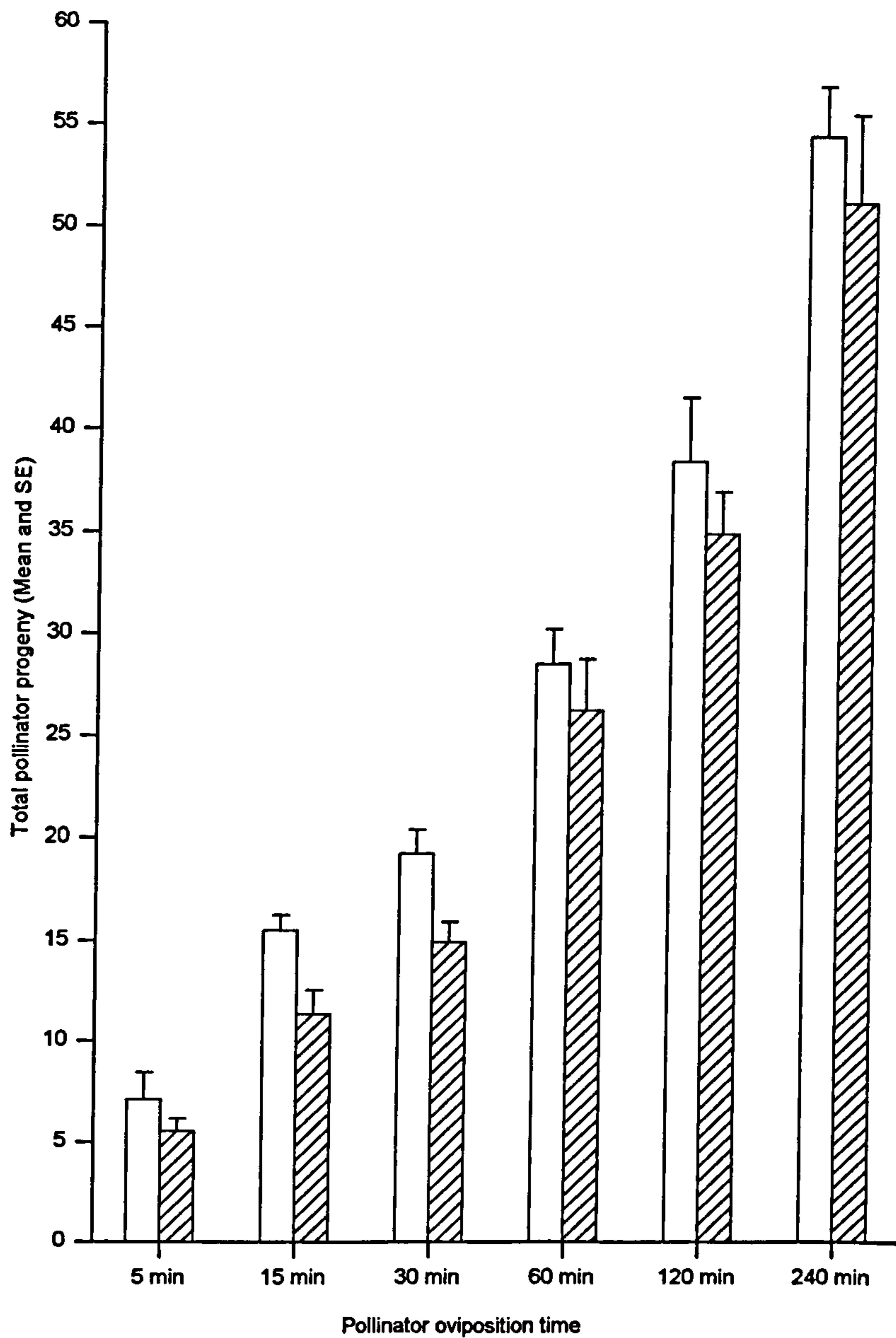


Fig. 8.3 The total number of pollinator progeny in figs where pollinator females were allowed to oviposit for different periods of time in the absence (open bars) and presence (solid bars) of *Sycosapter sp.*

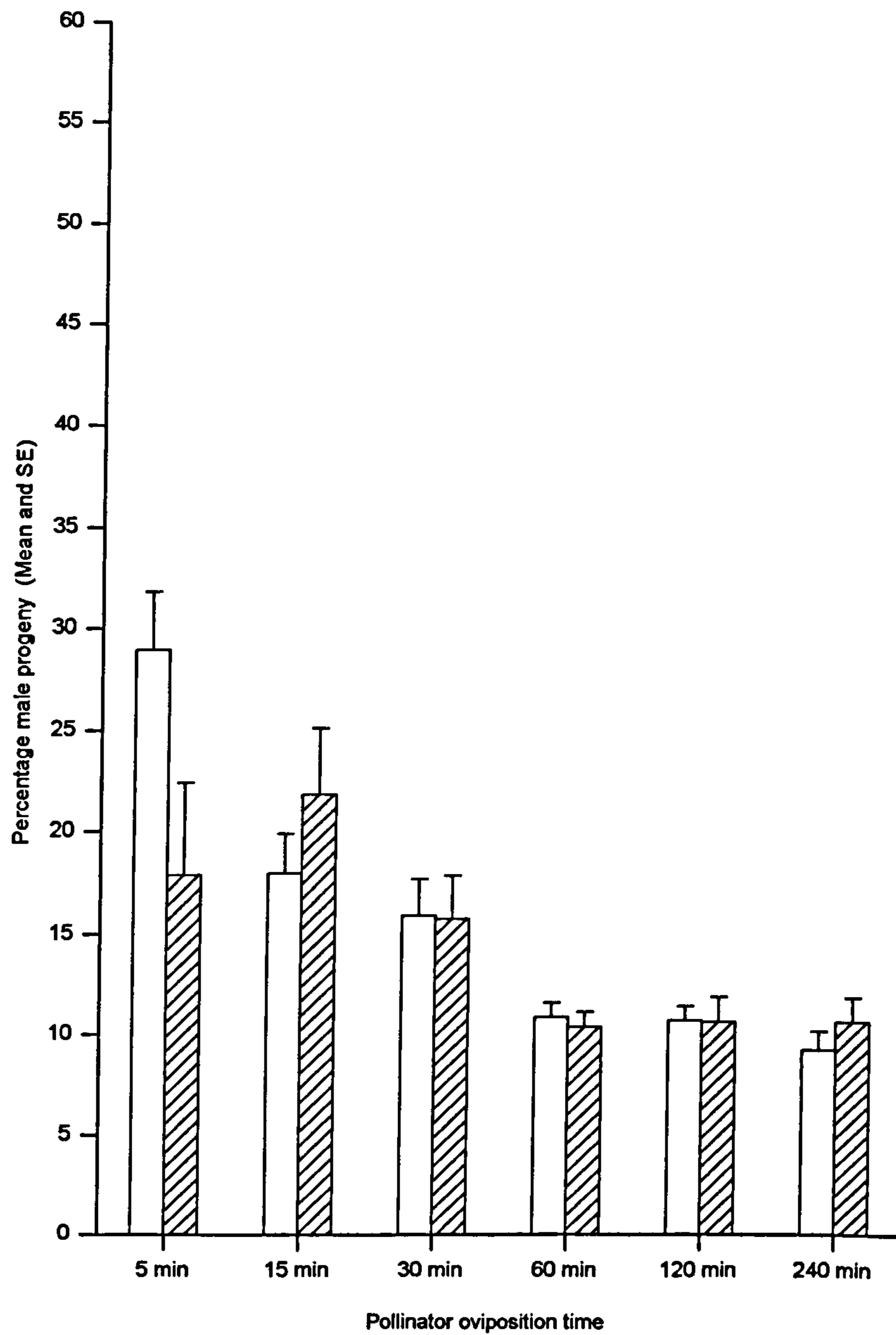


Fig. 8.4 Progeny sex ratios (% males) of pollinator wasps in figs where females were allowed to oviposit for different periods of time in the absence (open bars) and presence (solid bars) of *Sycosceptor sp.*

Power to detect sex ratio differences between parasitised and unparasitised figs (at $\alpha = 0.05$)

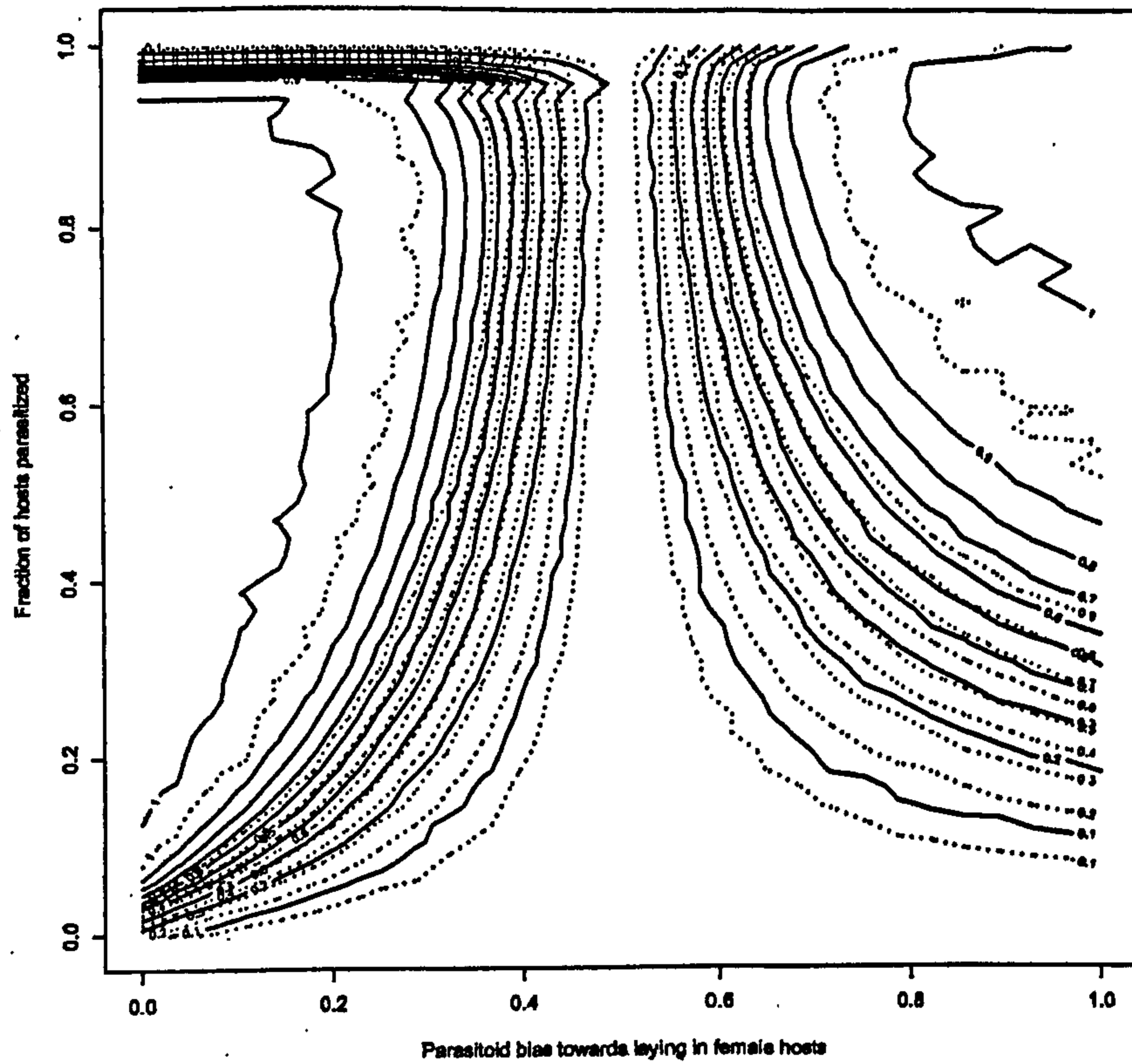


Fig. 8.5 Power analysis to detect potential sex ratio distortion between parasitized and un-parasitized figs. Supplied by Dr Yan Wong.

Table 8.1 A comparison of the contents of figs with and without exposure to parasitoids when foundresses were allowed to oviposit for different periods of time. Bladders are galled flowers that failed to produce any wasps.

Pollinator oviposition time (minutes)	Male pollinators		Female pollinators		Total pollinators		Bladders		Pollinator sex ratio (% males)	
	X ²	P	X ²	P	X ²	P	X ²	P	X ²	P
5	2.08	0.14 NS	0.04	0.83 NS	0.02	0.88 NS	0.09	0.09 NS	3.09	0.07 NS
15	0.001	1.00 NS	3.01	0.08 NS	3.44	0.06 NS	0.72	0.39 NS	0.37	0.53 NS
30	1.58	0.20 NS	3.25	0.07 NS	3.3	0.06 NS	2.29	0.13 NS	0.01	0.91 NS
60	1.21	0.27 NS	0.51	0.47 NS	0.54	0.46 NS	8.97	0.001 ***	0.30	0.57 NS
120	1.73	0.18 NS	2.11	0.14 NS	2.40	0.12 NS	2.93	0.08 NS	2.22	0.13 NS
240	0.006	0.93 NS	0.63	0.42 NS	0.92	0.33 NS	13.15	0.001 ***	0.86	0.35 NS

* P < 0.05

** P < 0.01

*** P < 0.001, NS Non significant

9. General Discussion

Understanding the ecological and evolutionary dynamics of any mutualism requires knowledge of the factors affecting the reproductive success of the participants. However, so far little is known about the dynamics of pollinator and seed production in dioecious species of fig trees. In this thesis I investigated factors affecting glasshouse populations of the dioecious *F. montana*, its pollinator *L. tentacularis*, and the non-pollinating wasp the *Sycoscapter* sp. I now briefly review the key findings and discuss their implications for our understanding of ecology and evolution of this mutualism.

I examined how the male and female plants of *F. montana* respond to nutrient manipulation (Chapter 2). It was found that with enhanced soil nutrition both of the sexes behaved similarly in vegetative growth, producing more leaves, more stems and more figs. The higher densities of figs on male as well as female plants would help flightless females emerging from one fig and trying to find another. This is beneficial for female plants as foundresses will bring pollen which will result in higher seed set, but the higher densities of figs may be less beneficial for male plants as we found that in situations where foundresses never shared figs, the clutch sizes of foundresses which failed to re-emerge were not significantly different from those who re-emerged and laid a second or third clutch (N. Suleman in prep). Inflorescence size (the numbers of female flowers) was found to be the most interesting response of the plants to enhanced nutrition (Chapter 3). Female plants not only produced figs with more flowers when given enhanced nutrition, but they also produced more seeds, which although smaller in size were not less efficient at germination. In contrast, in the male plants the size of figs remained unaltered and they also did not produce more

pollinators. A possible reason for the difference in response of the sexes may be the aggregation of parasitoids on high nutrition plants, as when we controlled parasitoid access we did find that pollinators had laid more eggs in high nutrition figs. Male trees may therefore fail to benefit from making larger figs.

Central to the mutualism between dioecious fig trees and their pollinators is the conflict that exists over the pollination of female figs. Pollinator foundresses are unable to oviposit in female figs, so should be selected to avoid them (Kjellberg, 1987; Grafen and Godfray, 1991). However, the evolution of such discriminatory abilities could be fatal to the mutualism. It has already been shown that females usually do not discriminate between male and female figs (Patel, 1995; Moore *et al.*, 2003b). We took this study a little further with our controlled experiments (Chapter 4) and showed that females behave in a similar manner inside both sexes and their frequency and timing of re-emergence is also not different. When foundresses discover that they cannot oviposit inside female figs they might be expected to come out as quickly as possible, but this is not the case. Male trees may be selected to allow foundresses to re-emerge from their figs because it results in more efficient use of pollinator resources on female trees, where pollinator numbers may often be limited. I suggest that they behave in the same manner in both tree sexes because foundresses are selected to do so in male figs. In order to gain the advantage of multiple fig entry on female trees, male trees have to allow foundresses to re-emerge from their own figs. This phenomenon also explains the lack of differences in foundress re-emergence rates from male and female figs.

I also showed that re-emergence rates of foundresses from male figs are not affected by the number of female flowers or wasp age and we also did not find any change in the likelihood of re-emergence by foundresses that entered together or an hour apart

(Chapter 5). The only factor that was found to influence the likelihood of re-emergence was the age of the figs, with wasps more likely to vacate older figs.

Sex ratio studies of fig wasps have largely remained separate from studies relating to the mutualism between fig wasps and their figs. However, aspects of the interaction between the wasps and their figs may provide explanations of the proximate mechanisms of sex ratio adjustment. LMC theory predicts that more sons are produced when more foundresses contribute to an isolated brood (Hamilton, 1967; Herre, 1987). If some foundresses are unable to lay their total egg complement, what would be the advantage of producing sons when many foundresses enter a fig? A mother that produces a son inside a fig with many female progeny is likely to have greater fitness than a mother that produces a daughter. This is because a son has a potential to inseminate many female progeny. In contrast, a daughter will only produce one clutch. Therefore, under oviposition site limitation it is a selective advantage to produce proportionately more sons than daughters. The sequence of egg laying in relation to clutch size has been shown to be important when considering sex ratios adjustments (Nefdt, 1989; Kinoshita *et al.*, 2002). The strategy of laying male eggs early, but not necessarily first, and female eggs later is a common way of achieving sex ratios adjustment. The poisoning experiments showed that sex ratio change in *L. tentacularis* at least, is a result of laying mostly male eggs first, followed by female eggs, under conditions of variable oviposition site limitation (Chapter 6). When several winged foundresses entered a fig there was a competition for oviposition sites and sex ratio adjustment, but this was not seen in wingless foundresses. The underlying cause of this difference was that wingless foundresses laid smaller clutches than winged foundresses. They were independent of foundress number because oviposition sites were not limiting.

Non-pollinating fig wasp communities vary greatly between fig tree species (Compton and Hawkins, 1992) both in terms of wasp species number and the structure of the communities. In addition to *L. tentacularis*, the only non-pollinator wasp species present in our glasshouse population is *Sycoscapter* sp. *Sycoscapter* sp. was found to oviposit 2-3 weeks after pollinator entry, but I did not find any negative correlation between densities of the two wasp species inside the figs (Chapter 7). This might have indicated that they were using different resources within the figs. However, when we controlled the number of foundresses entering a fig, *Sycoscapter* sp. significantly reduced pollinator numbers so we suggest that *Sycoscapter* sp. is a parasitoid, or an inquiline that kills its host.

Although characteristics that influence the susceptibility of insects to parasitoids have received considerable attention (Hawkins, 1994), sex dependent parasitism is reviewed rarely. Periera and Prado (2005) showed that non-pollinators distort the primary sex ratios of pollinators in *F. citrifolia*, and mentioned that the sex ratio distortion ability of non pollinating wasps may be stronger for species with smaller figs. In spite of the small size of *F. montana* figs we found that *Sycoscapter* sp. do not distort the sex ratio of the pollinating wasp. *L. tentacularis* secondary sex ratios therefore appear to accurately reflect primary sex ratios (Chapter 8).

I have focussed on a number of factors that contribute to the fig wasp: fig tree mutualism: soil quality, plant characters, pollinator abundance and sex ratios and a parasitoid. Interactions between pollinators with members of their own species and natural enemies, and all of these interactions, have the potential to affect the performance of and stability of the mutualism. The results illustrate the complex

relationship between plant nutrition and reproductive success in fig trees and show that in dioecious species the sexes may differ in their responses to better soil conditions. *F. montana* has been recorded growing on different soil types ranging from very fertile to very poor soils, so the effects that I showed in our experiments are likely to be present in field. They are also likely to be present in other species as well. Global environmental changes provide exciting long-term experimental systems to investigate the responses of insects to gradual changes in the nutritional quality of their host plants (Awmack and Leather, 2002). A key challenge for the future is to link knowledge of the effects of host plant quality to the performance of pollinators and in turn non-pollinators to the larger scale processes that influence the populations in natural systems. The results presented in this thesis should encourage plant-insect researchers to devote more attention to quantitative and qualitative variation in primary nutrient compounds.

Foundress re-emergence behaviour appears to differ between different pollinator wasp species, suggesting that it may be adaptive for one or other partner in the mutualism. Further work on a variety of fig species and their pollinators, both to delineate which species re-emerge and to examine the costs and benefits of such behaviour for each partner, is required before any general conclusion can be drawn as to the selective advantages of re-emergence to male figs, female figs and wasps. Further comparative studies of dioecious species might be usefully aimed at those population attributes that are general to all dioecious plants that distinguish them from monoecious figs. Within dioecious species differences that reflect habitat or other environmental variables need to be considered.

My results confirmed the hypothesis that sex ratios in fig wasps are regulated by the sequential laying of male and female eggs in combination with oviposition site limitation. There is much left to understand about sex ratio strategies of pollinating wasps, not least whether the mechanism of sex ratio adjustment in *L. tentacularis* is typical of fig wasps in general. It seems as if foundresses are responding to changes in LMC under conditions other than those originally envisaged. For future work it will be useful to use the poisoning technique along with micro satellite markers in figs with different numbers of foundresses, to determine the interference effect of foundresses on oviposition, if any.

In the case of non-pollinators, further comparisons of fig wasp systems will be of interest to understand general features of community development and functioning. In future work it may be possible to determine the dietary habits of non-pollinators by using stable isotopes, which may solve the problem of being parasitoid or inquiline (Gratton and Forbes, 2006). It is important from both ecological and evolutionary points of view to take into account the presence of non-pollinator fig wasps while studying the fig tree-fig pollinator system. Ignoring the non-pollinator's impact in co-evolution models misses out an important selective pressure on the mutualist partners. Even though we did not detect an impact of the parasitoid on pollinator sex ratios, clearly sex ratio studies in future must distinguish between primary and secondary sex ratios in fig wasps.

Understanding the ecological and evolutionary dynamics of mutualisms requires knowledge of the factors affecting the reproductive success of both participants. A basic factor by which fig tree reproductive success can be determined is the number of female pollen-dispersing wasps, and the number of seeds, produced in figs. However,

so far little is known about the factors affecting pollinator and seed production in dioecious fig species. Comparing the findings of this study with those of fig trees and fig wasps it is quite clear that the reproductive strategies of the participants in this mutualism differ between species, which is also typical of other specialised mutualisms (Bronstein, 1994). It is also the most fascinating thing about fig trees because it will allow comparative studies to be made. Our results indicate that a consideration of the environmental context may be important for understanding of the ecological and evolutionary significance of sex related differences in dioecious plants. The relationship between reproductive allocation and reproductive success in males and females needs to be established for a number of species.

Mutualisms are reciprocal exploitations which nonetheless result in an overall benefit for each species and the sustainability of mutualisms depends upon endogenous and exogenous factors regulating the costs and benefits of the interaction (Anstett *et al.*, 1998). Thus, even within a restricted taxonomic sphere there may be great variation in the functioning of species interactions. Comparative studies of such variations could lead to new generalizations about the evolutionary mechanisms involved in species interactions and has implications for the ecology and evolution of their interactions. Fig trees are often found to be key-stone resources for frugivorous vertebrates in many forests (Borges, 1993), but in this they depend on their obligate pollinators. The viability of pollinator populations is affected by many factors and the mating system of the fig trees is one of them. In monoecious fig trees all the trees can help to maintain a pollinator population, but in dioecious fig species the female trees are not helpful, but at the same time they provide food to vertebrates. Differences in the behaviour of pollinators, by adjusting their sex ratios or re-emerging from figs, presumably reflect

benefits for the wasp as more trees that are seen in female trees and ultimately influence how many fig vertebrates frugivores can utilise.

In conclusion, we can say that the genus *Ficus* is very ancient (Machado *et al.*, 2001) and this very long span time has enabled evolution to mould the complex pattern of interrelationships that are encountered today. According to Galil (1977), Corner was right to conclude that “fig biology reveals the incredible ability of natural evolution”.

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