TR 90 - 53

INTERACTIONS BETWEEN FIG WASPS AND THEIR HOST FIGS

Thesis submitted in fulfilment of the requirements
for the degree of MASTER OF SCIENCE
of Rhodes University

by

RORY JOHN CHARLTON NEFDT

DECEMBER 1989

CONTENTS

Acknowledgements

I would like to thank my supervisor, Dr. Steve Compton for introducing me to the world of fig wasps. Many of his suggestions and constructive comments lead to substantial improvements in this thesis. I am also extremely grateful for his encouragement and time so generously given throughout this study.

My sincere thanks are also due to Professor Patrick Hulley, Mr Tony Ware, Miss Stephanie Vincent, and Miss Nikite Muller and my father, for proof - reading, valuable comments and moral support. Mr Paul Magnuson generously contributed his skills towards statistics and computer management and Mr. Casper de Villiers helped towards the preparation of Figures. Mr Alan Gardiner allowed me to use his unpublished data.

I am also am indebted to the CSIR, the Dr Kendall Scholarship, and the William Waddel Trust for financial assistance.

Abstract

Fig trees (*Ficus* spp.) and fig wasps (Hymenoptera: Agaonidae) are partners in an intimate mutualism. The trees provide ovules in which wasp larvae develop while the wasps pollinate the flowers and are therefore indispensible for fig seed production. Agaonid fig wasps oviposit down the styles of fig flowers and it has generally been accepted that they were unable to reach the ovules of "long" styled flowers, which would produce seeds, thus maintaining an evolutionary stable mutualism. 11 African fig species were found to have unimodal style length frequencies,with no separation into long and short styled flowers. In several species the ovipositors of their associated agaonids were long enough to reach the majority of ovules. The number of foundress agaonids entering a fig influenced fig seed set and therefore was an important factor regulating the proportion of flowers producing seeds or pollinators. In the two *Ficus* species that were studied, entry of more than three agaonid foundresses into one fig resulted in competition for limited oviposition sites and less female - biased offspring sex ratios. It is hypothesised that sequential laying of male eggs followed by female eggs, under variable oviposition site limitation, results in sex ratio adjustment, as predicted by local mate competition theory. Evidence in support of this hypothesis is presented.

A number of non - pollinating torymid and pteromalid fig wasps also oviposit into each fig species. The sycophagines and sycoecines oviposit down the styles from inside the fig inflorescences like their agaonid

counterparts, while other species insert their ovipositors through the wall of the fig from the outside. Like the agaonids, sycophagines were characterised by being pro - ovigenic, with numerous fully developed eggs at emergence. Sycoecines were able to re - emerge from figs they had oviposited in and lay their eggs in more than one fig. They had short ovipositors, allowing access to a smaller proportion of flowers than agaonids or sycophagines. Externally ovipositing fig wasps were syn - ovigenic, able to develop eggs as adults and invested more energy and time during each oviposition event. Differences in the ovipositor lengths of these species did not segregate their oviposition sites spatially, and therefore does not reduce competition between species. Attack by parasitoids and inquiline fig wasps from the exterior did not constitute a selection pressure against agaonids ovipositing in ovules closer to the periphery of the fig's surface, as predicted by Michaloud's enemy-free-space hypothesis. It cannot therefore explain the preference shown by ovipositing agaonids for shorter styled flowers.

CHAPTER 1

Introduction

1.1 Fig trees

Fig trees (*Ficus* spp.) belong to the Mulberry family Moraceae which has five other genera in southern Africa. Worldwide there are about 750 species of *Ficus* distributed mostly in the tropical and subtropical regions. Approximately 500 species occur in the Asian and Australasian areas, with the rest equally divided between the Americas and Africa (Wiebes, 1986; Berg, in press). About 30 species are found in the southern part of the African continent (Palgrave, 1983).

Botanically the fig is a spurious fruit consisting entirely of vegetative penduncular tissue, but pomologically the ripe fruit has been called a succulent syconium (Galil & Eisikowitch, 1968a; 1971; Storey, 1975; Faegri & van der Pijl, 1979). The *Ficus* inflorescence is in the form of a hollow ball lined on its inner surface with hundreds of unisexual flowers. Entrance into the fig lumen is possible only through the ostiole, which is tightly packed with bracts. Female fig flowers consist of a single ovule surrounded by two to five perianth lobes, depending on the species (Verkerke, 1988a). Each flower has a single style that in most species has a bilobed papillate stigma. The stigmata generally unite to form the synstigma (Galil, 1977; Verkerke, 1986; 1988a; 1988b; 1989). Pedicel lengths of the flowers vary; those with long pedicels have shorter styles and those that have short pedicels, or that are sessile, have longer styles.

1.2 Coevolution of Ficus and Agaonidae

Plant-insect mutualisms encompass numerous species and many books have devoted large sections to mutualistic interactions (Faegri & van der Pijl, 1979; Price, 1984 and others). Examples of mutualisms vary from loosely related associations, as in Apoidea and flowering plants (Faegri & van der Pijl, 1979), to obligatory intimate relationships between coevolved species, such as the relationship between yucca moths (*Tegeticula spp.*, Prodoxinae, Incurvariidae) and yuccas (*Yucca spp.*, Agavaceae) (Addicott, 1986). Coevolution has been defined in several ways, but is generally understood to involve the reciprocal evolutionary change between two partners (Strong *et al*, 1984).

Nearly all species from the family Agaonidae pollinate fig flowers and thus ensure seed set. The figs and the agaonid fig wasps have been described as partners in a symbiosis (Galil & Eisikowitch, 1968a) or mutualism (Janzen, 1979a; Kjellberg *et al*, 1987a; 1987b), an example of an intimate system where the two partners appear to be mutually adapted in morphological, behavioural and physiological ways. Figs and agaonids are totally dependent on one another for sexual reproduction: the wasps pollinate the flowers, while the fig sacrifices a proportion of its seeds as feeding sites for wasp larval development (Galil & Eisikowitch, 1968a; 1968b; 1968c; 1971; 1973; 1974; Ramirez, 1969; Galil, 1977; Ramirez, 1978; Janzen, 1979a; 1979b; Newton & Lomo, 1979; Wiebes, 1984; Corner, 1985).

Agaonids probably evolved from pollen feeding gall-makers on pre-*Ficus* plants (Ramirez, 1976). By evolving a flask like inflorescence, the inflorescences of pre-*Ficus* may have reduced the number of insect species visiting the flowers, many of which were probably injurious to the plant. Eventually a true fig appeared, with spherical inflorescences that are completely enclosed, except for a small pore which allows a specific pollinator to enter. Concurrently, a species-specific pollinating system evolved which was condusive to the radiation of figs and their associated fig wasps (Ramirez 1976; Wiebes, 1989). Wiebes (1987) devised an evolutionary scheme indicating possible routes of selection leading to the partnership between agaonids and fig trees. Basically, the closure of the inflorescence provided a selective entrance (the ostiole) which is said to aid the survivorship of the seeds and pollinator progeny. In response, the Agaonidae have become specialy adapted to the characteristics of the figs, particularly with regard to gaining entry through the ostiole.

Ficus is probably unique among plants in that almost every fig species is totally dependent on just one species of pollinator (Ramirez, 1970; 1974; Wiebes, 1973; Bronstein, 1987), in contrast even to the relationship between yucca moths and yucca plants where one pollinator species can pollinate a number of yucca species (Addicott, 1986). Only a few rare exceptions have been reported where two species of agaonids pollinate figs of the same tree species (Ramirez, 1970; Michaloud *et al*, 1985).

1.3 The developmental cycle of figs and their pollinators

Fig development within trees is usually highly synchronised. Nearly all figs ripen together on the same tree and consequently self-pollination is absent. Exceptions are provided by a few cases of crop asynchrony which allow wasps to pollinate young figs on their natal trees (Wharton *et al*, 1980; Baijnath & Ramcharan, 1983). A single tree can produce a number of crops each year and as trees fruit at different times, populations usually contain crops at all stages of development throughout the year (Compton & Nefdt, in press).

Galil & Eisikowitch (1968a) divided the developmental cycle of the fig into five stages. During phase A (the pre-female phase), while the flowers are still developing, the ostiole remains closed and only at phase B (female phase) does it open to allow the entrance of pollinators. At this stage female flowers are mature, but the male flowers are still undeveloped. Impregnated female agaonids foundresses from other trees force their way between the bracts lining the ostiole and enter the fig, often losing their antennae and wings in the process. Once inside, they cannot leave because the bracts block any exit. The female wasp then begins to probe rapidly down the styles of the flowers and in some will deposit an egg. In many species pollen grains, which were collected from the natal fig before emergence, are actively removed from specially adapted invaginations in the mesothorax , the pollen pockets, and deposited onto the stigmata via the tarsi (ethnodynamic pollination - Galil & Eisikowitch, 1973; Ramirez, 1969). The exact sequences of pollination and oviposition differ, depending on the species (Frank, 1984). After pollinating the flowers and depositing her eggs the female wasp dies within the lumen.

At the time of oviposition the female agaonids are thought to inject an acid gland secretion near the egg which stimulates galling and the parthenocarpic development of the endosperm (Joseph & Aburahiman, 1981; Joseph, 1984; Verkerke, 1986; 1989). The wasp larvae feed on this endosperm and probably on other ovular tissues such as the nucellar tissue and the aborted embryo.

During phase C, the interfloral stage, the agaonid larvae and seeds develop side by side in the same fig. At pupation all the contents of the gall are consumed leaving a hard dry casing, the sclerotised pericarp, that envelops the pupa. With rare exceptions, only a single larva develops in each gall (Galil & Eisikowitch, 1968a; 1971). If pollination is experimentally prevented by introduction of pollenless wasps, there is reported to be a high mortality of the resulting agaonid progeny, suggesting that pollination has a direct benefit to the wasps (Galil & Eisikowitch, 1971). Furthermore, figs that do not receive wasps abscise and fall to the ground. Such abortion seems to be prevented by the act of oviposition *per. se.*, perhaps through the action of the acid gland secretion (Berg, 1983; Verkerke, 1988a; 1988b; 1988c; 1989).

Just before the fig ripens (phase D), the male agaonids bite their way out into the lumen. They then locate and make a small incision in galls containing females, through which they insert their telescopic (solenogastric) abdomens and copulate. Agaonids exhibit striking sexual dimorphism, with males adapted for living inside the enclosed environment of the fig. They are apterous, with reduced eyes, and some have enlarged spiracular peritremata that have water repellent properties (Compton & McLaren, 1989). The well-developed mandibles of agaonid males help to perforate the galls containing females and to subsequently tunnel through the wall of the fig to the exterior, usually near the vicinity of the ostiole, so that the female wasps can leave. Males of *Ceratosolen* spp. also chew through the male flowers, apparently to make the pollen more accessible to females (Galil & Eisikowitch, 1973). Most agaonid males do not use their mandibles for fighting, although *Alfonsiella* spp. are an exception (Michaloud, 1982).

Before male individuals of *Blastophaga quadraticeps* Mayr tunnel out of the figs of *F. religiosa* L., the internal atmosphere is rich in carbon dioxide. This is thought to inhibit both ripening of the fig and female emergence from their galls (Galil, *et al*, 1973b). After the exit tunnel is completed by the males the carbon dioxide escapes from the fig and the females are stimulated to emerge from their galls. In some species the females proceed to actively collect pollen from the anthers and scrape the pollen grains into their pollen pockets by using their tarsi (Galil & Eisikowitch, 1973; Galil, 1977; Galil & Neeman, 1977). In other species, the pollinators are simply dusted with pollen (Galil & Eisikowitch, 1968a). Once pollen-loaded, the wasps leave the fig and seek out other trees with figs at the receptive stage (phase B). Recent experiments have confirmed that in at least one species, a chemical attractant emanates from the interior of the figs and that these chemicals are species - specific (van Noort *et al*, 1989).

Subsequently (phase E) the figs become soft and succulent and are then eaten by birds and mammals that disperse the seeds (Janzen, 1979a;

Bronstein, 1988a). Baijnath (in press) has shown that seeds from the faeces of bats have the same germination success rate as seeds obtained directly from figs while Waters, Craig & Compton (in prep.) have found that germination rates of seeds of *F. burtt-davyi* Hutch. are enhanced by the passage through the guts of birds.

1.4 Style and ovipositor lengths

The biology of *Ficus* described previously applies only to monoecious species of the genus. However, there are also 300 or so dioecious species, of which the best known is *F. carica* L. These are characterised by having two types of trees (Valderyon & Lloyd, 1979; Ramirez, 1981; Kjellberg, *et al*, 1987b). Caprifig trees produce 'male' figs that contain short styled female flowers which are said to allow the pollinators to lay eggs into all of the ovules. The figs also contain staminate flowers which mature at phase D, some time after the female flowers have been pollinated. Because few, if any seeds are produced, the main function of 'male' figs is that of pollen production and dispersal. Female trees produce 'female' figs which contain flowers with styles that are said to be longer than the wasp's ovipositor. They can be pollinated, but remain free from wasp progeny. Instead of pollen dispersal, the main function of 'female' figs is seed production (Galil & Neeman, 1977; Valderyon & Lloyd, 1979). Berg (1983) has suggested that dioecious figs evolved as a way for figs to produce large numbers of seeds at certain times of the year, a feature of particular benefit for trees growing in seasonal climates.

In monoecious figs the relative lengths of agaonid ovipositors and styles have also been seen as the main factor controlling the proportion of flowers that produce either seeds or wasps (Murray, 1985a; Galil & Eisikowitch, 1968a; 1968b; 1968c; 1974; Ramirez; 1969; Janzen, 1979a; 1979b; Wiebes, 1984). Agaonid ovipositors have been said to be long enough to reach flowers with short styles, but not those with long styles. Thus fig biology dogma has held that (1) monoecious figs contain two types of flowers: long styled flowers and short styled flowers and (2) the long styled flowers produce mainly seeds because their ovules cannot be reached by the ovipositors of the agaonid wasps (Berg, 1983; Faegri & van der Pijl, 1979; Galil, 1977; Galil & Eisikowitch, 1968a; Janzen, 1979a; Kjellberg *et al*, 1987a; Ramirez, 1970; 1976; Weibes, 1977; 1979a; 1982; 1984; 1986). The ratio of flowers producing either seeds or wasps and therefore the relative lengths of ovipositors and styles has thus been considered as critical in maintaining the evolutionary stability of the fig-fig wasp mutualism by preventing all the seeds of the host plant from being destroyed (Kjellberg *et al*, 1987a; 1987b).

By having a longer ovipositor, individual females would be expected to be at a selective advantage inside figs because they would gain more sites for oviposition. Various ideas have been proposed to explain why fig wasps have not evolved longer ovipositors, but most are unsatisfactory or have not been adequately tested (Murray 1985a). Murray (*loc. cit.*) concluded that ovipositor length is constrained by the selective abortion of figs with high densities of agaonid larvae and low numbers of seeds.

To try and predict the proportion of accessible ovaries, Newton and Lomo

(1979), who studied *F. lutea* Vahl in Ghana, and Galil & Eisikowitch (1968c) working with *F. sycomorus* L. in East Africa, compared ovipositor sheath lengths (taken as a measure of the true ovipositor length) with style lengths of their respective figs. They found that at full insertion less than half the flowers would be accessible, and yet some pupae could be found in flowers with long styles. More recent observations (Kjellberg *et al*, 1987a; Verkerke, 1987) have suggested that the ovipositor lengths of some fig wasps are long enough to reach the majority of ovules and Bronstein (1988a) estimated that the ovipositors of the pollinator from *F. pertusa* Linn. F. are long enough to reach 82% of the ovules. She also noted that because measurements of fig wasp ovipositor lengths in the literature had been of the ovipositor sheaths, they had underestimated the true ovipositor length.

Style length frequencies of the figs studied by Galil & Eisikowitch (1968c) and Newton & Lomo (1979) were found to be unimodal, with most flowers of intermediate style lengths, although the authors continued to talk of long and short styled flowers. Bronstein (1988a) also found a unimodal distribution, although the styles were skewed towards the shorter style lengths. These findings were in contrast to older ideas that style lengths had a bimodal distribution (Faegri & van der Pijl, 1979) and suggested that there may be no clear cut division between long and short styled flowers.

1.5 Sex ratios in agaonids

Female animals produce fewer and larger gametes than males. One can expect then, given equal capacities in acquiring resources, that males can produce many more gametes than females, and can fertilise the eggs produced by many females. Therefore theoretically a population with more females than males should produce larger numbers of progeny. Darwin (1899) had problems in explaining why this prediction was incorrect, with most species in practice producing equal numbers of males and females.

It was Fisher (1930) who first explained why most species have a 50:50 sex ratio. He predicted that in panmictic (randomly mating) populations there should be equal investment in the production of males and females, because in a population with an excess of females, individuals which produce males would be at a selective advantage. This is because there are many more females for their sons to mate with. Hence, individuals in a population with an excess of one sex would invest more in the rarer sex and the evolutionary stable strategy would be a 50:50 sex ratio (Maynard Smith, 1978; 1984).

However, this 50:50 sex ratio does not fit the observed sex ratios found in many hymenopterans. In bisexual hymenopterans this can be related to their haplodiploid sex determination system. Males are produced from unfertilised eggs (males are therefore haploid) while females arise from fertilised eggs and are therefore diploid. Thus a female wasp may be able to control the primary sex ratio of her offspring via the release or retention of sperm from the spermatheca (Flanders, 1939). This method of sex determination therefore provides a mechanism whereby sex ratios can be adaptive and deviate from the 50:50 ratio.

Under certain circumstances, the assumptions of Fisher's (1930) model may be relaxed, as for example where mating is non-random and frequency-dependent selection at the population level is unable to operate (Maynard Smith, 1978). This occurs where a single or a few foundress females produce broods that are reproductively isolated from other populations, as is the case with agaonids in figs (Figure 1.2 a). Under these conditions, Hamilton (1967) showed that brothers compete to inseminate their sisters (referred to as Local Mate Competition - abbreviated as LMC), and the evolutionary stable strategy for foundresses would be to invest more heavily in female progeny. This is because, by producing an excess number of daughters and just enough sons to mate them all, a female would maximise her number of grandchildren.

If more than one female contributes offspring to an isolated brood, the intensity of LMC decreases and a less biased optimal sex ratio would be expected (Hamilton, 1967; Maynard Smith, 1984; Werren, 1980). Thus, to optimise their reproductive success, haplodiploid organisms should exercise sex ratio adjustment by manipulating their sex ratio according to the number of foundresses participating in a given brood. Between 1 and over 40 agaonid foundresses may enter a single fig to oviposit (Compton, unpub. data). Hence fig wasps provide a useful model for testing ideas of sex ratio adjustment.

Hamilton (1967) expressed the optimal sex ratio *p* in the following equation:

$$p = (n-1)(2n-1)/n(4n-1)$$

Where n is the number of foundresses colonising a patch. Hence in figs with many foundresses (n is large), the optimal sex ratio approaches 0.5, while as n decreases, the sex ratio approaches zero. When there is only one foundress ($n = 1$) the female should produce only enough sons to mate all her daughters.

Average levels of inbreeding at the population level are also thought to complement LMC in influencing the sex ratio adjustment in fig wasps (Herre, 1985; 1987). The figs of certain species tend to contain, on average, greater numbers of foundresses than others (Herre, 1985). Thus sex ratio biases in agaonids may reflect both average levels of inbreeding and sib-competition (Green *et al*, 1982; Frank, 1985a; Nunney, 1985). A combination of the LMC model and the inbreeding model gave rise to Herre's (1985) equation, which was shown to accurately predict sex ratio adjustment in the fig wasps he studied. He predicted the sex ratio of progeny using the equation:

$$p = (1-m)(2n-1)/n(4n-1)$$

where p is the expected optimal sex ratio and n is the harmonic mean of the number of foundresses per fig, that is, the overall population level of inbreeding through sib-mating. As n decreases inbreeding increases. m is the proportion of individuals contributed to a given brood by a single mother, that is, the reciprocal of the number of foundresses (Herre, 1985). As *m* increases the effects of LMC will increase among the progeny, thus leading to a more female-biased sex ratio. This equation predicts that as the number of foundresses increases, the optimum sex ratio of their progeny will tend towards a 50:50 sex ratio and that more highly inbred species will have relatively more biased sex ratios, for a given number of foundresses.

1.6 Proximate mechanisms of sex ratio adjustment

One of the main problems that has beset sex ratio studies is the inability to distinguish between 'primary' sex ratios (that is the sex ratio at conception, or the total sex ratio of all the eggs deposited by a wasp) (Suzuki, *et al*, 1984) and the 'secondary' sex ratio based on the adults that emerge from a brood (Wellings *et al*, 1986). Up to eight factors, many of which may interact, are known to affect either 'primary' and 'secondary' offspring sex ratios (King, 1987). Some of these operate through females manipulating the fertilisation of their eggs, or through other mechanisms, such as differential mortality (Clausen, 1939; Grosch, 1948; Wellings *et al*, 1986), or changes in sperm availability and polymorphic spermatozoa (Lee & Wilkes, 1965).

Little work has been directed at the actual mechanisms of sex ratio adjustment in Hymenoptera. The developmental advantages of males under conditions of superparasitism have been shown to cause secondary sex ratio variation (Grosch, 1948; Wilkes, 1963; Wylie, 1966; Suzuki, *et al*, 1984) and differential mortality of the sexes has been proposed as an explanation of sex ratio adjustment. (Cees, *et al*, 1987).

Superparasitism seems unlikely to occur in fig wasps because normally only a single egg is deposited in each flower (Galil & Eisikowitch, 1971; Joseph & Abdurahiman, 1981; Verkerke, 1986; 1989). However, Galil & Eisikowitch (1971) provided evidence that under certain conditions female larvae of *C. arabicus* were more prone to death than males, and consequently the adult sex ratio would become less female biased. One could further hypothesize that if oviposition sites become limited in supply (for example when many foundresses enter a fig), competition between sibs for nutrients could result in differential mortality. This could then cause the secondary sex ratio to change with an increase in foundress numbers.

Sex ratio adjustment may also occur indirectly due to the sequential oviposition of male and female eggs. The egg parasitoid, *Trichogramma evanescens* has been shown to lay male eggs first, followed by female eggs (Waage & Lane, 1984; Waage & Ng, 1984). If disturbed, females may re-start the sequence and lay male eggs again. Because they tend to avoid superparasitism, a female may not be able to lay all her eggs when several females share a fig. Since the first few eggs she lays produce mainly males, her offspring sex ratio at that site will be less female - biased than if she had been able to lay her total clutch. The parasitic wasp, *Telenomus remus* Nixon., which attacks the egg batches of *Spodoptera* spp. (Lepidoptera, Noctuidae), was shown to achieve sex ratio adjustment in this way (Cees *et al*, 1987). However, in addition to this mechanism, LMC also appeared to increase the sex ratio in the presence of other wasps. Hence, more than a single mechanism may operate to bring about sex ratio adjustment.

1.7 The non-pollinating fig wasps

Agaonids are not the only chalcidoid wasps associated with figs, but they are the only ones that actively pollinate them. The non-pollinating fig wasps are referred to as *secondary sycophiles* (Galil & Eisikowitch, 1974) *mess-mates* (Weibes, 1977) or *interlopers* (Bronstein, 1988a). Consequently, each species of *Ficus* supports several species of fig wasps, an extreme example being *F. thonningii* Bl. where about 25 species of wasps have been reported (Boucek *et al*, 1981).

Non-pollinating species belong to the families Torymidae, Ormyridae, Pteromalidae and Eurytomidae (Joseph, 1954; 1955; 1956; 1958; 1959; 1964; 1966; Abdurahiman & Joseph, 1978a; 1978b; 1978c; 1979) and may be divided on the basis of their trophic relationships and oviposition sites into the internal phytophages, external phytophages, inquilines and parasitoids. Most of these species oviposit through the wall of the fig from the outside (Ansari, 1967; Ulenberg, 1985: Joseph, 1954). Some, however, namely the sycoecines (Newton & Lomo, 1979) and the sycophagines (Galil *et al*, 1970; Baijnath & Ramcharan, 1983) have evolved the same ability as agaonids and are able to enter figs via the ostioles. They also oviposit by inserting their ovipositors down the lengths of the styles. They are purely seed predators and therefore can only be detrimental to seed set, although pollen may be accidentally transported into the figs in a few rare cases (Newton & Lomo, 1979; Compton *et al*, unpub.).

Like the agaonids, these non-pollinating wasps are also highly synchronised with the developmental cycle of the fig tree. However, the stage at which they oviposit into the flowers varies, depending on the species. Those entering through the ostiole, obviously oviposit at the same time as the agaonids when the ostioles are open (Phase B). The time at which species probing through the wall from the outside oviposit is much more variable and may be at phase A, the pre-floral stage, or phase B, the female stage, or phase C, the interfloral stage.

The behaviour leading to copulation in the non-pollinating species varies. In some, copulation occurs outside the galls, but still inside the lumen of the fig, while others mate outside the fig. In many species there is marked aggression between males (Murray, 1987).

Boucek (1988) has recently combined the agaonids *sensu stricta* with the sycoryctine and sycophagine torymids, Otitesellinae and Epichrysomallinae in an enlarged Agaonidae. However, these wasps appear to be a polyphyletic assemblage and they probably evolved independently from pteromaloid ancestors (Ulenberg, pers. comm.). In this thesis the traditional classification is retained and the Agaonidae are regarded as distinct from the other chalcid groups.

1.8 Reproductive strategies of Fig wasps

Fig wasps have reproductive characteristics that adapt them to the structure and life cycles of the respective figs. Access to resources, clutch sizes, egg shapes and sizes and behavioural traits are some of the factors that can be studied (Frank, 1984). Studies of other insect communities have made ecological and phylogenetic inferences from morphological data (Hespenheide, 1973; Askew & Shaw, 1986; Gilbert *et* *al*, 1985). These data can also shed light on host utilisation patterns, such as in the communities of parasitoids attacking *Asteromyia carbonifera* (Diptera: Cecidomyiidae) (Weis, 1982), cynipids in oak galls (Askew, 1965; Askew & Shaw, 1986) and leaf-mining Lepidoptera (Askew & Shaw, 1986).

Studies concerning other chalcid communities have shown that ovipositor lengths are adaptive (Brandl & Vidal, 1987) and may be important when considering competition between species (Heatwole & Davis, 1965) and interactions between plants, their herbivores and parasitoids (Price & Clancy, 1986; Weis, 1982). Recent studies have suggested that selection pressures exerted at higher trophic levels can influence interactions between plants and herbivores (Price *et al.*, 1980; Weis & Abrahamson, 1986). Atsatt (1981) and Jefferies & Lawton (1984) have introduced the term 'enemy free space', which the latter define as "ways of living that reduce or eliminate a species' vulnerability to one or more natural enemies". They suggest that the niche of a species may be moulded through evolutionary time by interactions with parasitoids or predators, rather than competition for food or space.

Michaloud (in Kjellberg *et al*, 1984) has related the oviposition behaviour of agaonids to the 'enemy free space' hypothesis. He proposed that agaonids which lay their eggs in longer styled flowers may suffer from disproportionately high levels of parasitism. The ovules of longer styled flowers are closer to the periphery of the fig than those of shorter styled flowers and consequently they are more likely to be encountered first by parasitoids probing from the outside of the fig. Furthermore, the ovules of flowers with the shortest styles may be out of reach of some parasitoids. It was suggested that this differential mortality of agaonids developing in longer styled flowers negates any advantages gained by being able to oviposit there and this favours individuals with shorter ovipositors that can only reach the ovaries of shorter styled flowers.

Ovipositor lengths provide estimates of the number of ovules that are accessible to the wasps, while egg numbers give an indication of the relative reproductive outputs by different species of fig wasps. Reproductive strategies are likely to be related to the lifestyles of each wasp species. Pollinators and sycophagines both enter the fig and have to lay all their eggs into one fig. Therefore, they would be expected to have high oviposition rates and ovipositors and egg numbers that are adapted to the number of oviposition sites available in a single fig. They may exhibit reproductive characteristics described by McArthur & Wilson (1967) as r - strategists. In contrast, the torymids and pteromalids oviposit through the fig wall and would be expected to have low fecundities and invest more time and energy during each oviposition event. This strategy is K - selected (Pianka, 1970). Askew (1975) examined the reproductive strategies using the parasitoids which attack species of Cynipidae in oak galls, and found they fell into those that appeared to be aligned as r - strategists and those that were K - strategists, as described by Price (1973a; 1973b) for other insect communities.

1.9 Objectives

The aims of this thesis were to investigate both the mutualism between figs and fig wasps and the sex ratios of agaonids. Since sex ratios are influenced by the environment in which foundresses lay their eggs, it seems plausible that the interactions between the wasps and their figs may explain how fig wasps adjust their sex ratios. Because access to flowers as oviposition sites could influence the competition between foundresses for flowers, Chapter 3 describes the accessibilities of flowers to a number of African fig wasps. These were calculated by comparing ovipositor lengths of agaonids to the style lengths of their associated fig flowers. To check that this method was accurate in estimating accessibilities, the style lengths of flowers inhabited by fig wasp progeny were also recorded.

Once estimates of the proportion of flowers available to varying numbers of foundresses had been obtained, the possible effects of this on competition between foundresses and between larvae were investigated. Chapter 4 describes experiments which examined whether oviposition site limitation, differential survival, or just the presence of other females might cause sex ratios to vary with changes in the numbers of foundresses entering a given fig.

Studies were also undertaken on the secondary sycophiles inhabiting four southern African figs species. The accessibilities of flowers to members of the Sycophaginae and Sycoecinae and the torymids and pteromalids ovipositing from the outside are examined in Chapter 5. The objectives were to investigate certain aspects of the reproductive strategies of these wasps, and to examine style length - related parasitism of agaonids. The overall conclusions of the study are given in Chapter 6.

CHAPTER 2

Figs and their associated fig wasps in the eastern Cape of South Africa

2.1 Introduction

There are 105 species of *Ficus* described from Africa and Malagasy (Berg, 1989a). These are placed in four subgenera. Species from the subgenus *Ficus* are all dioecious, having 'male' trees with figs that produce mainly pollen and 'female' trees with figs which produce mainly seeds. Of the 11 dioecious species in Africa, only one species is found in southern Africa. The remainder are all monoecious. Of the 11 species belonging to the subgenus *Sycomorus*, only two, namely *F. sycomorus* and *F. capensis* are common in Southern Africa, south of the Zambezi river. The subgenus *Pharmacosycea* has only four African representatives, none of which are found in this region. *Urostigma* is the largest subgenus in Africa, with 79 species, 35 of which have been reported from southern Africa (Berg, 1986; 1989).

Only four *Ficus* species are native to the eastern Cape. These are *Ficus burtt-davyi* Hutch., *F. thonningii* Bl. (both Subgenus Urostigma, section Galoglychia), *F. sur* Forssk. (Subgenus *Sycomorus*) and *F. ingens* (Miq.) Miq. (Subgenus Urostigma, section Urostigma). The first three species were studied in the vicinity of Grahamstown (33 19 S 26 32 E). Grahamstown is situated at an altitude of approximately 550 metres with an average rainfall of 650mm per annum. Rainfall is sporadic and aseasonal.

2.2 The Veld Fig *F. burtt-davyi*

The veld fig, *F. burtt-davyi* is a shrub or small tree found in the eastern parts of southern Africa. In the eastern Cape it occurs mainly on rocky outcrops and cliff faces, while elsewhere it has also been recorded as a coloniser of sand dunes and as a strangler of other trees (von Breitenbach, 1985). Studies in Grahamstown were undertaken on a population of more than 100 trees found in the 1820 Settlers Botanical Gardens and an adjacent disused quarry. Most were growing along rocky outcrops, though a few were found growing on steep embankments.

The figs of *F. burtt-davyi* are relatively small, reaching a maximum diameter of about 15mm and are produced in the leaf axils. In Grahamstown six of the chalcidoid wasps which utilise these figs where studied (Table 2.1). Two of these, *Elisabethiella baijnathi* Wiebes (Agaonidae; Agaoninae) and *Phagoblastus* sp. indesc. (Sycoecinae) enter the figs and oviposit down the styles into the ovules. *E. baijnathi* pollinates the flowers, thus ensuring seed set, whereas *Phagoblastus* sp. does not actively pollinate the figs (Baijnath & Ramcharun, 1988). The four remaining species all oviposit into the ovules by inserting their ovipositors through the walls from the outside. They are *Sycoryctes sp.* indesc., *Philotrypesis sp.* indesc. (both Torymidae), *Otitesella uluzi* Compton and *O. sesquianellata* van Noort (Pteromalidae, Otitesellinae) (van Noort & Compton, 1988).

2.3 The Cape Fig *F. sur*

The Cape fig, *F. sur* has a wider distribution than *F. burtt-davyi*, and occurs from the eastern parts of southern Africa, through Zambia (Palgrave, 1983) as far north as Ethiopia (Baijnath & Ramcharun, 1983) and Cameroun (Compton & Baker, 1986). This is a large spreading tree, reaching about 12 m in height, which is commonly associated with riverine habitats. Studies of *F. sur* and its associated chalcid fauna were carried out using several trees located at Howieson's Poort, 10kms South East of Grahamstown, and other trees within a 1 Km radius of the Rhodes University campus.

The figs of *F. sur* are large (up to 4cm in diameter), and are produced on leafless branches growing from the trunk or the main branches. Six wasp species associated with these figs were studied, two of which enter through the ostiole (Table 2.1). One of these is the agaonid pollinator *C. capensis* (Agaonidae: Blastophaginae) while the other, *Sycophaga cyclostigma* (Torymidae: Sycophaginae), also oviposits down the styles, but does not pollinate the flowers. The remaining wasps recorded in the eastern Cape were *Apocrypta guineensis*, and three *Apocryptophagus* spp. (all Torymidae). These oviposit through the wall of the fig from the outside, and either lay their eggs in the ovules of flowers, or into galls already containing larvae of other wasp species (Table 2.1).

2.4 The common Wild Fig, *F. thonningii*

F. thonningii is a variable species, ranging from a medium to very large tree. It is distributed from the eastern parts of South Africa, through Zimbabwe and northern Botswana into Zambia and throughout most of sub-saharan Africa (Palgrave, 1983). In the eastern Cape it is commonly planted as an ornamental tree, along roads, in parks and in gardens. The figs are about 1-2 cms in diameter, hairy (in most eastern Cape trees), borne in the leaf axils and in contrast to the other species, tend to remain on the tree for more than a week after the wasps have already emerged. Studies were carried out on about 30 trees located on and around the Rhodes University campus, principally growing along Rhodes Avenue.

In Zimbabwe 25 species of chalcids have been recorded from the figs of *F. thonningii* (Boucek, *et. al.*, 1981). Five of these species were studied (Table 2.1). *Elisabethiella stuckenbergi* (Agaonidae: Agaoninae) is the pollinator. Like *F. burtt-davyi*, *F. thonningii* has a sycoecine, *Phagoblastus barbarus* (Torymidae: Sycoecinae) which also enters the figs via the ostiole, but does not pollinate the flowers. The remaining chalcids, which oviposit from the outside, are either phytophagous or develop in galls already containing wasp larvae. They are *Otitesella tsamvi* Wiebes (Pteromalidae: Otitesellinae), *Philotrypesis parca* (Torymidae) and *Camarothorax equicollis* (Pteromalidae: Epichrysomallinae).

Table 2.1 Classification and the larval biology of fig wasps associated with three species of *Ficus* that were studied in the eastern Cape, South Africa.

Fig tree/Chalcids	Family	Larval biology	Oviposition
F. burtt-davyi Hutch.			
Elisabethiella baijnathi Wiebes	Agaonidae	Phytophage (pollinator)	Internal
Phagoblastus sp. indesc.	Sycoecinae (unplaced)	Phytophage	Internal
Otitesella uluzi Compton	Pteromalidae	Phytophage	External
Otitesella sesquianellata van Noort	Pteromalidae	Phytophage	External
Sycoryctes sp. indesc.	Torymidae	Inquiline/ Parasitoid	External
Philotrypesis sp. indesc.	Torymidae	Inquiline Parasitoid	External
F. sur Forssk.			
Ceratosolen capensis Grandi	Agaonidae	Pollinator	Internal
Sycophaga cyclostigma Waterston	Torymidae	Phytophage	Internal
Apocryptophagus sp.1 (*Parakoebelia*-short ovipositor)	Torymidae	Phytophage	External
Apocryptophagus sp.2 (*Idarnes* 1-long ovipositor)	Torymidae	Phytophage	External
Apocryptophagus sp.2 (*Idarnes* 2-intermediate ovipositor).	Torymidae	Phytophage	External
Apocrypta longitarsus Grandi	Torymidae	Inquiline	External

Table 2.1 continued.

Fig tree/Chalcids	Family	Larval biology	Oviposition
F. thonningii Bl.			
Elisabethiella stuckenbergi Grandi	Agaonidae	Phytophage (pollinator)	Internal
Phagoblastus barbarus Grandi	Sycoecinae (unplaced)	Phytophage	Internal
Otitesella tsamvi Wiebes	Pteromalidae	Phytophage	External
Philotrypesis parca Wiebes	Torymidae	Inquiline	External
Camarothorax equicollis Boucek	Pteromalidae	Phytophage	External

CHAPTER 3

Access to oviposition sites for agaonid fig wasps

3.1 Introduction

Since the fig-fig wasp mutualism has been in existence since the Cretaceous period (Galil, 1977; Murray, 1985a), it is clearly evolutionarily stable with both partners able to produce enough offspring to prevent their extinction. The most widely accepted explanation for the maintenance of this stability has been the functional division of female fig flowers into two discrete groups with different style lengths. This idea seems to have developed as an extension of the situation in dioecious figs, where certain figs have long styled flowers, and others much shorter styles. Short styled flowers in monoecious figs were said to produce mainly wasps while long styled flowers produce mainly seeds. This was because the styles of the latter were longer than the agaonid's ovipositor (Galil & Eiskowitch, 1968a; Ramirez, 1970; 1976; Galil, 1977; Wiebes, 1977; 1984; 1986; Faegri & van der Pijl, 1979; Berg, 1983; Price, 1984; Kjellberg *et al*, 1987a). Recent studies have questioned whether discrete short and long styled flowers are present, and whether many of the ovules are inaccessible (Bronstein, 1988a).

I investigated the style lengths of eleven African fig species, representing three of the four subgenera of *Ficus*. Their style length frequency distributions were examined in order to verify whether the fig flowers could be divided into two discrete groups: short and long styled flowers. Style lengths were then compared with the ovipositor lengths of their respective pollinators to determine the proportion of flowers available for oviposition if the ovipositors could be fully inserted. In order to check if availability gave a good indication of actual utilisation, the proportion of seeds and wasps produced by flowers with different style lengths were recorded from three fig species. Style length frequency distributions and flower accessibilities were also examined in a dioecious fig tree and contrasted with the monoecious species.

The proportion of flowers which are accessible to a pollinator is also of importance in relation to the reproductive biology of the wasps. This, together with the number of wasps entering a fig and their egg loads, determines whether flowers are in limited supply, and thus whether there is competition for oviposition sites between foundresses. Moreover, various studies have shown that oviposition site limitation can lead to increased mortality among larvae, and if one sex is more prone to death (Charnov, 1982; Charnov *et al*, 1981; Putters & van den Assem, 1988), then this could influence the adult sex ratios (see Chapter 4). This chapter therefore also examines the clutch sizes of agaonid fig wasps and relates them to the number of available flowers in order to determine whether there is frequent competition for oviposition sites.

3.2 Methods

3.2.1 Measurements of wasp ovipositor and fig flower style lengths

Figs from 11 species of *Ficus* were collected at various localities in southern and central Africa (Table 3.1). Figs were collected at the female receptive stage (phase B), the stage when agaonids enter to oviposit down the styles and pollinate the flowers (Galil & Eisikowitch, 1971). For each species, the style lengths of 99 flowers from each of three individual B phase figs were measured to the nearest 0.1 mm from the tip of the stigma to where the style joins the ovary wall, using a measuring eye-piece in a dissecting microscope. The tip of the stigma was taken from where the lobes join together to form the beginning of the cylindrical style. To check for within-fig style length differences, the figs were divided into three sections; basal, middle and ostiolar, by cutting with a sharp razor. 33 flowers were removed at random from each section.

Collections were also made of the agaonids associated with the 11 fig species (Table 3.1). Ripening figs (Phase D) were removed and placed in containers which trapped the emerging wasps. The ovipositor lengths of 20 individuals of each species were measured by squashing the chalcids in a drop of water between a slide and a glass coverslip. Manipulation of the coverslip revealed the entire length of the functional ovipositor (1st and 2nd valvulae) and the sheaths (3rd valvulae) which were then measured using a measuring eyepiece in a compound microscope. The length of the sheath was taken from its tip to where it reached the last gastral segment, while the true ovipositor was measured from its tip to

Table 3.1 Classifications of African figs and agaonids that were investigated. The style lengths of the fig flowers were compared with the ovipositor lengths of the associated pollinating agaonid. The monoecious species are arranged in order of increasing fig size.

Fig species	Subgenus	Section	Collected	Agaonid	collected	Collectors
F. salicifolia Vahl.	Urostigma	Urostigma	Kruger Park South Africa	*Platyscapa awekei* Wiebes	Mazoe Zimbabwe	S.G. Compton & A. Gardiner
F. burtt-davyi Hutch.	Urostigma	Galoglychia	Grahamstown South Africa	*Elisabethiella baijnathi* Wiebes	Grahamstown South Africa	R.J.C. Nefdt
F. verruculosa	Urostigma	Urostigma	Lusaka Zambia	*Platyscapa binghami* Wiebes	Lusaka Zambia	R.J.C. Nefdt
F. lutea Vahl	Urostigma	Galoglychia	Umhlanga Rocks South Africa	*Allotriozoon heterandromorphum* Grandi	Umhlanga Rocks South Africa	S.G. Compton
F. thonningii Bl.	Urostigma	Galoglychia	Grahamstown South Africa	*Elisabethiella stuckenbergi* Grandi	Grahamstown South Africa	R.J.C. Nefdt
F. sycomorus L.	Sycomorus	-	Gobabeb Namibia	*Ceratosolen arabicus* Mayr	Lusaka Zambia	S.G. Compton & R.J.C. Nefdt
F. abutilifolia (Miq.) Miq.	Urostigma	Galoglychia	Mkuzi Park South Africa	*Elisabethiella comptoni* Wiebes	Giyani South Africa	S.G. Compton
F. ottoniifolia (Miq.) Miq.	Urostigma	Galoglychia	Lusaka Zambia	*Courtella camerunensis* Wiebes	Makokou Gabon	G. Michaloud & R.J.C. Nefdt
F. sur Forsk.	Sycomorus	-	Grahamstown South Africa	*Ceratosolen capensis* Grandi	Grahamstown South Africa	R.J.C. Nefdt
F. sansibarica Warb.	Urostigma	Galoglychia	Kruger Park South Africa	*Courtella armata* Wiebes	Kabompo Zambia	S.G. Compton & R.J.C. Nefdt
F. capraefolia Delil.	Ficus	Sycidium	(male) Giyani South Africa	*Kradibia gestroi* Grandi	Mazoe Zimbabwe	A. Gardiner & S.G. Compton
			(female) Mkuzi Park South Africa			S.G. Compton

where it joined the basal plates. The style lengths of each fig species were compared to the ovipositor lengths of their associated wasps, so that the proportion of potentially accessible flowers could be calculated. Estimates of the proportion of flowers in which wasps could reach the ovules were based on the number of flowers with style lengths less than or equal to the mean ovipositor length of their associated pollinator. Unfortunately it was not always possible to use flowers and fig wasps collected from the same locality.

3.2.2 Flower utilisation

Estimated flower accessibility was related to ovary utilisation in certain species. This was made possible by the persistence of intact styles through to phase D. Figs of *F. burtt - davyi*, *F. sur* and *F. thonningii* were collected just prior to the emergence of male wasps, a time when pupae are still inside their galls and are identifiable. After harvesting, the figs were preserved in 70% ethanol or were frozen prior to dissection. A subsample of flowers were removed from each fig by scraping out a large group of flowers with a scalpel, thus ensuring that there were no biased selections of flowers, based on gall/seed size or style length. 50 flowers were then separated out randomly and divided into the following categories; 1) intact, healthy seeds distinguished by their white cotyledons and hard testas. 2) galls that were opened to reveal the identity and sex of the insect inside. 3) 'bladders' (Galil & Eisikowitch, 1971) that had the same external appearance as galls, but were empty and dry inside. 4) Infertile / unpollinated flowers that were smaller than other flower categories and lacked a hard testa. To relate utilisation to style lengths, the styles were measured using a measuring eyepiece in a dissecting microscope.

3.2.3 Egg production and foundress numbers

The reproductive systems of *E. baijnathi*, *E. stuckenbergi* and *C. capensis* were examined by squashing wasps in a drop of water between a slide and a glass coverslip. 20 individuals of each species was examined in this way. In each case an estimate of body size was recorded by measuring the distance between the inner margins of the compound eyes, henceforth called the inter - ocular distance. By manipulating the slide and coverslip, the total number of eggs in each individual wasp was revealed and counted.

In order to estimate the potential collective clutch sizes of wasps entering figs, the numbers of foundresses entering figs was recorded. 183 *F. burtt-davyi* figs at phase C were collected from 12 different trees in Grahamstown between May 1985 and April 1988. 73 *F. sur* figs were also collected from 3 trees in Grahamstown between June 1985 and December 1988, and 15 figs were collected by S. van Noort from one tree of *F. thonningii* in February 1989. Each fig was cut open and the total number of dead foundresses inside the lumen was counted.

3.3 Results

3.3.1 Style length frequency distributions

The style length frequency distributions of all 11 species were found to be unimodal (Figure 3.1). Most figs had flowers which did not differ significantly from a normal distribution (Goodness of fit Kolmogorov-Smirnov test, Table 3.2). Three exceptions were present, but given the large number of tests, these results may represent type II errors. The Kolmogorov - Smirnov test involves specifying the cumulative distribution which would occur under a theoretical distribution (a normal distribution in this case) and comparing that with the observed cumulative frequency distribution (style length distribution) (Siegel, 1956). The Chi^2 test of goodness of fit was not used because the Chi^2 does not take into account the overall arrangement of classes whereas this test does.

Skewness and Standardised coefficients of skewness were calculated to examine any asymmetries in the frequency distributions. While skewness gives the degree of asymmetry of a set of observed values, standardised coefficients of skewness compare skewness of the observed distribution to that of the theoretical normal distribution. Standardised coefficients with values outside the range -2.0 to +2.0 are considered to be significantly different from a normal curve. Table 3.2 shows that in the majority of fig species there was a tendency for observed style lengths to be skewed towards short styles, i.e, they were positively skewed. However, only four species had standardised coefficients that were greater than 2.0. *F. sansibarica* and 'female' *F. capraefolia* were atypical and had negative skewness values, but their standardised coefficients were not significantly different from a normal distribution.

A two-way Analysis of Variance was carried out to determine whether

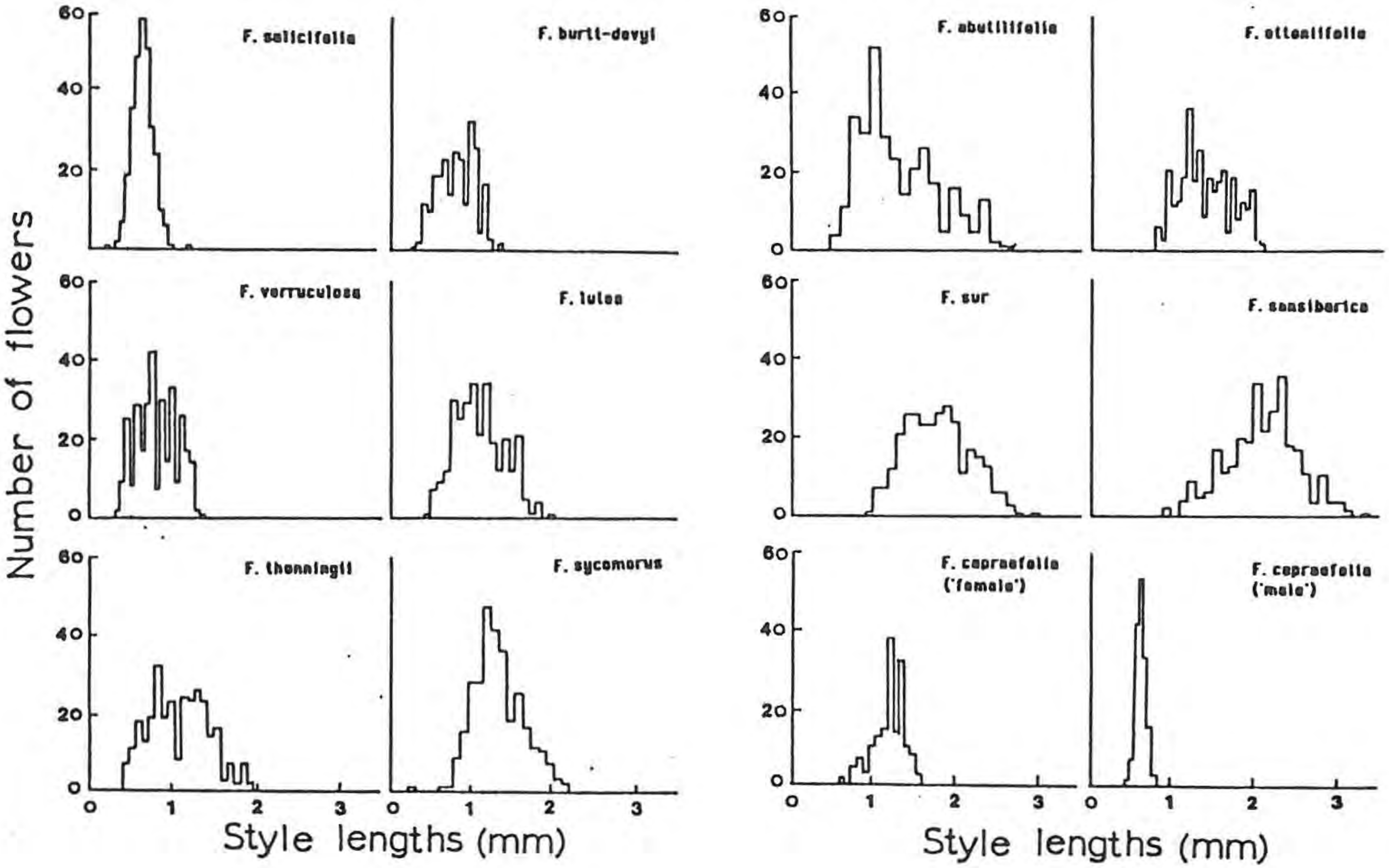

Figure 3.1 Style length frequency distributions of flowers from 11 species of African figs. The monoecious species are arranged by increasing size of the figs. 99 flowers were sampled from each of three figs for each *Ficus* species.

Table 3.2 Goodness of fit test of style length distributions from three figs of 11 *Ficus* species from southern Africa. The test compares the cumulative frequency distribution of style lengths with a normal frequency distribution. Numbers in brackets refer to the probabilities of the null hypotheses that there is no significant difference between the distributions. Skewness and standardised skewness were calculated from the combined counts of each species.

	Kolmogorov-Smirnov test statistic (Probability)				
Fig species	fig 1	fig 2	fig 3	Skewness	Standardised skewness
F salicifolia	0.088 (0.43)	0.048 (1.00)	0.061 (0.99)	+0.24	+1.69
F. burtt-davyi	0.097 (0.45)	0.110 (0.26)	0.099 (0.38)	+0.09	+0.68
F. verruculosa	0.122 (0.09)	0.104 (0.21)	0.099 (0.26)	+0.05	+0.36
F. lutea	0.139 (0.04)*	0.070 (0.99)	0.090 (0.40)	+0.34	+2.40
F. thonningii	0.116 (0.14)	0.094 (0.34)	0.127 (0.08)	+0.15	+1.04
F. sycomorus	0.104 (0.24)	0.114 (0.15)	0.097 (0.34)	+0.34	+2.41
F. abutilifolia	0.115 (0.13)	0.139 (0.04)*	0.131 (0.06)	+0.63	+4.50
F. ottoniifolia	0.101 (0.24)	0.106 (0.19)	0.112 (0.14)	+0.25	+1.79
F. sur	0.080 (0.99)	0.116 (0.14)	0.072 (0.99)	+0.28	+1.95
F. sansibarica	0.071 (0.99)	0.061 (0.99)	0.096 (0.33)	-0.14	-1.00
F. capraefolia (male)	0.132 (0.07)	0.111 (0.18)	0.176 (0.004)**	+0.38	+2.68
F. capraefolia (female)	0.075 (0.99)	0.091 (0.39)	0.130 (0.07)	-0.22	-1.53

* $P<0.05$
** $P<0.01$

mean style lengths varied in different sections of the figs and between figs (Table 3.3). This indicated that differences in style length means between the basal, middle and ostiolar sections of each fig, were generally not significant, though *F. burtt-davyi* and *F. ottoniifolia* were exceptions, with longest styles in the basal and ostiolar sections respectively. The mean style lengths of flowers from three separate figs were not significantly different in six of the 11 species examined (Table 3.3). In all the figs, as with the between-sections comparisons, the differences in means were minor compared with the overall variation in style lengths present in any one small area of each fig. Interactions between the two variables (figs and sections) were generally also insignificant.

3.3.2 Accessibilities of flowers to ovipositing agaonids

Measurements of the agaonids' ovipositor sheaths considerably underestimated the true lengths of their ovipositors, with sheath : ovipositor ratios varying from 0.64 to 0.24 (Table 3.4). Agaonids from monoecious *Ficus* had sheath/ovipositor ratios varying from 0.48 (*Allotriozoon heterandromorphum* and *Courtella camerunensis*) to 0.64 (*Elisabethiella stuckenbergi*). *Kradibia gestroi*, the only species collected from a dioecious fig, had a very short sheath length relative to its ovipositor length, with a sheath/ovipositor ratio of 0.23. Differences in ratios between species within the same genus were also evident. For example *Courtella camerunensis* had a ratio of 0.48 compared to 0.61 for *C. armata*. Although *E. baijnathi* and *E. stuckenbergi* had similar ratios of 0.63 and 0.64 respectively, the third species *E.*

Table 3.3 2-Way ANOVA of style lengths of flowers between figs from the same trees and between basal (B), middle (M), and ostiolar (O) sections from each fig.

Fig species	Fig	Between figs			Section	Between sections			Interaction	
		means (mm)	$F_{[2]}$	P		means (mm)	$F_{[2]}$	P	$F_{[4]}$	P
F. salicifolia	1	0.63	2.07	0.13	B	0.65	2.53	0.08	4.00	0.04^{*}
	2	0.63			M	0.62				
	3	0.67			O	0.66				
F. burtt-davyi	1	0.76	1.22	0.30	B	0.85	6.48	0.002^{**}	0.78	0.59
	2	0.83			M	0.80				
	3	0.80			O	0.75				
F. verruculosa	1	0.85	1.80	0.17	B	0.77	1.91	0.15	0.26	0.90
	2	0.80			M	0.83				
	3	0.79			O	0.83				
F. lutea	1	1.16	4.87	0.01^{*}	B	1.11	0.69	0.50	0.78	0.54
	2	1.07			M	1.06				
	3	1.03			O	1.10				
F. thonningii	1	1.20	6.59	0.002^{**}	B	1.11	0.31	0.73	0.83	0.51
	2	1.02			M	1.12				
	3	1.08			O	1.08				
F. sycomorus	1	1.25	5.35	0.05	B	1.24	1.27	0.28	3.18	0.01^{*}
	2	1.34			M	1.35				
	3	1.39			O	1.34				
F. abutilifolia	1	1.32	0.19	0.83	B	1.27	1.18	0.31	0.57	0.68
	2	1.32			M	1.30				
	3	1.28			O	1.36				
F. ottoniifolia	1	1.34	3.60	0.03^{*}	B	1.38	6.16	0.002^{**}	1.13	0.34
	2	1.42			M	1.34				
	3	1.45			O	1.49				
F. sur	1	1.76	0.60	0.55	B	1.77	2.44	0.09	1.76	0.14
	2	1.81			M	1.74				
	3	1.80			O	1.86				
F. sansibarica	1	1.94	15.93	0.00^{***}	B	2.11	2.07	0.13	1.58	0.18
	2	2.17			M	2.19				
	3	2.27			O	2.07				
F. capraefolia (male)	1	0.65	6.72	0.001^{**}	B	0.64	2.77	0.06	1.26	0.29
	2	0.62			M	0.64				
	3	0.63			O	0.62				
F. capraefolia (female)	1	1.29	23.40	0.00^{***}	B	1.20	0.53	0.59	1.31	0.27
	2	1.22			M	1.22				
	3	1.13			O	1.22				

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$

Table 3.4 Measurements of ovipositor, sheath and style lengths of 11 agaonid species and their host figs, and the percentage of flowers estimated to be accessible for oviposition.

Agaonid	Ovipositor length (mm) (mean + SD)		Sheath length (mm) (mean + SD)		Ratio Sheath/ Ovipositor	Style lengths (mm) Mean +	SD	Estimated accessible flowers (%)
Platyscapa awekei Wiebes	1.17	0.038	0.72	0.027	0.62	0.64	0.130	99
Elisabethiella baijnathi Wiebes	1.40	0.230	0.88	0.028	0.63	0.80	0.230	83
Platyscapa binghami Wiebes	0.88	0.027	0.50	0.037	0.57	0.81	0.240	60
Allotriozoon heterandromorphum Grandi	1.57	0.049	0.76	0.029	0.48	1.09	0.300	93
Elisabethiella stuckenbergi Grandi	1.71	0.108	1.09	0.049	0.64	1.10	0.360	95
Ceratosolen arabicus Mayr	1.95	0.122	1.08	0.051	0.55	1.33	0.300	98
Elisabethiella comptoni Wiebes	1.71	0.110	0.91	0.051	0.53	1.36	0.49	79
Courtella camerunensis (Wiebes)	1.67	0.060	0.78	0.010	0.48	1.40	0.320	76
Ceratosolen capensis Grandi	1.84	0.062	1.08	0.046	0.59	1.79	0.400	55
Courtella armata (Wiebes)	2.69	0.040	1.65	0.150	0.61	2.13	0.440	91
Kradibia gestroi (Grandi)	0.94	0.064	0.23	0.050	0.24 (male)	0.63	0.070	100
					(female)	1.21	0.180	8

comptoni had a sheath/ovipositor ratio of 0.53.

On the basis of the measurements of true ovipositor lengths, agaonid ovipositors were longer than the mean style lengths in all 10 monoecious fig species (Table 3.4 and Figure 3.2). It was estimated that between 55% and 99% of the flowers in monoecious species were accessible (Table 3.4). A highly significant correlation was obtained when mean style and ovipositor lengths from monoecious figs were compared (Figure 3.3) (r^2 = 79%, $P < 0.001$), even though in some cases figs and wasps were collected from separate localities. Exceptions to the general pattern were provided by the pollinators of *F. sur* and *F. verruculosa*, which had unusually short ovipositors, and r^2 rises to 92% if these are excluded.

Two species of agaonids utilise the figs of *F. sycomorus*, which is an exception to the rule. The first, *C. arabicus* is the legitimate pollinator while the second, *C. galili* does not undertake active pollination (Galil, 1968a; Kjellberg, 1987). The ovipositor of *C. arabicus* was long enough to reach 98% of the flowers ($n = 20$, $x = 1.95$mm, range = 1.78 - 2.40), while *C. galili* had a significantly shorter ovipositor, allowing access to 93% of the flowers ($n = 20$, $x = 1.81$mm $t = 4.99$, $P < 0.001$) (Figure 3.4).

It is not known, whether mean ovipositor lengths are the same throughout a species' range. Measurements of ovipositor lengths of *C. capensis* from Grahamstown, South Africa ($n = 20$, $x = 1.84$mm, range = 1.72 - 1.92) and individuals from Bambui in Cameroun ($n = 20$, $x = 1.67$mm, range = 1.50 - 1.79) were significantly different (Figure 3.4, $t = -6.06$, $P < 0.001$).

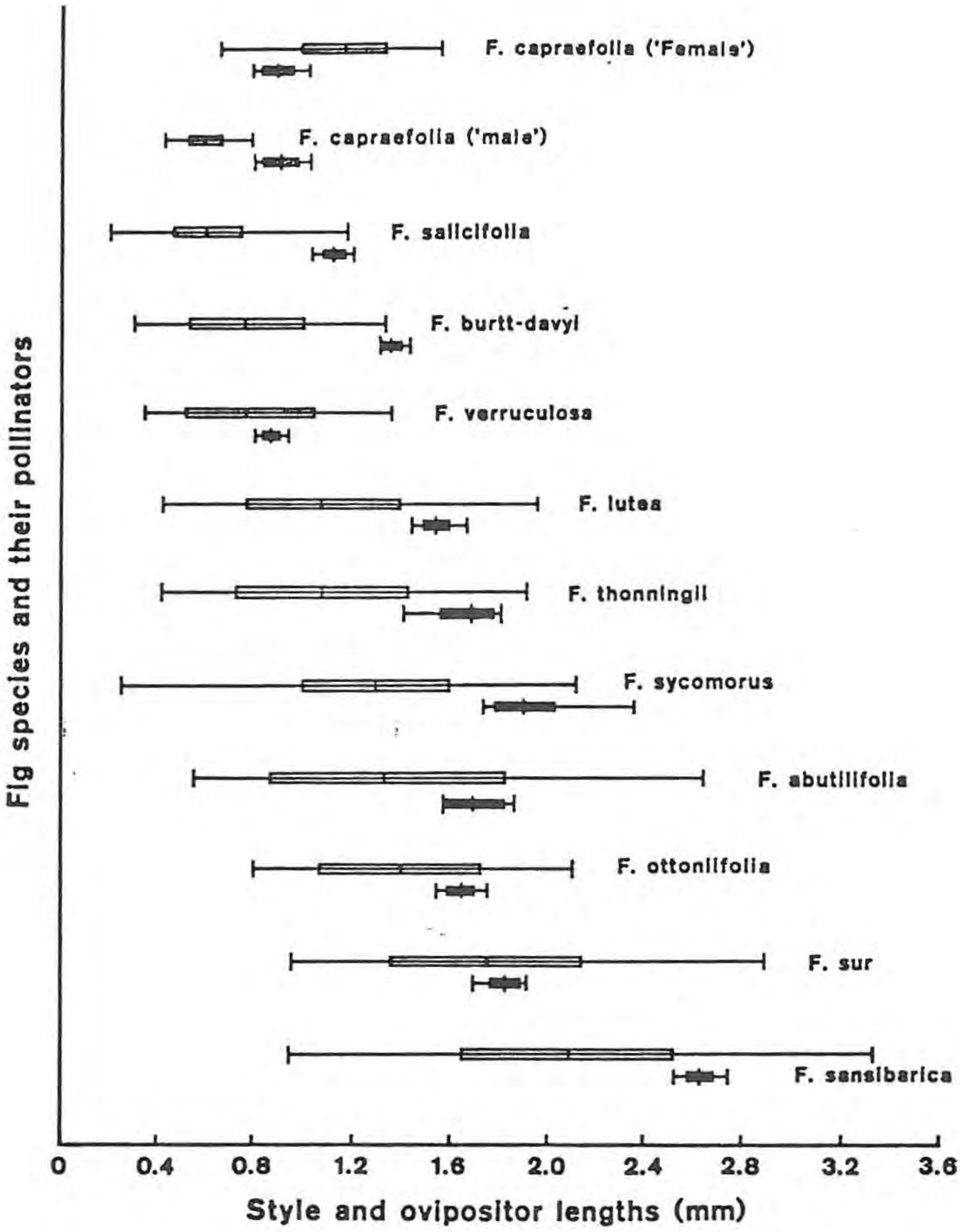

Figure 3.2 Comparisons of the style lengths (open bars) and ovipositor lengths (closed bars) of 11 fig-agaonid pairs. The means, standard deviations and range of lengths are illustrated.

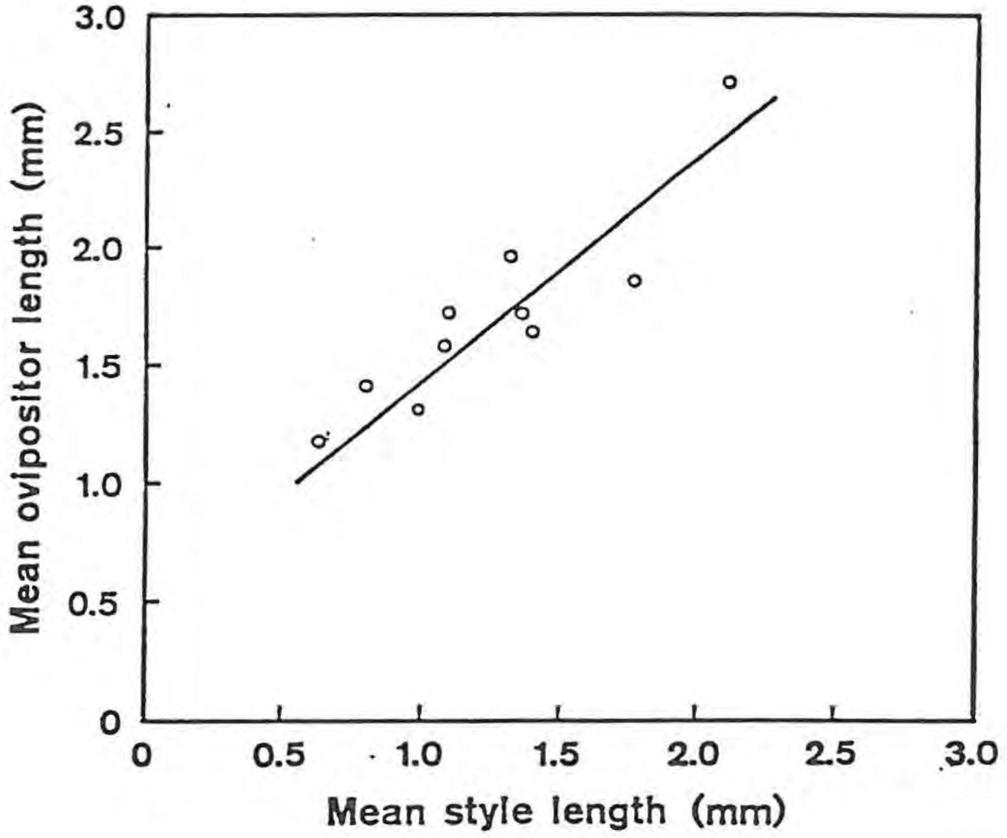

Figure 3.3 The relationship between mean ovipositor and style lengths and ten monoecious fig wasp-fig pairs. For each pair, 20 ovipositors and 300 styles were measured.

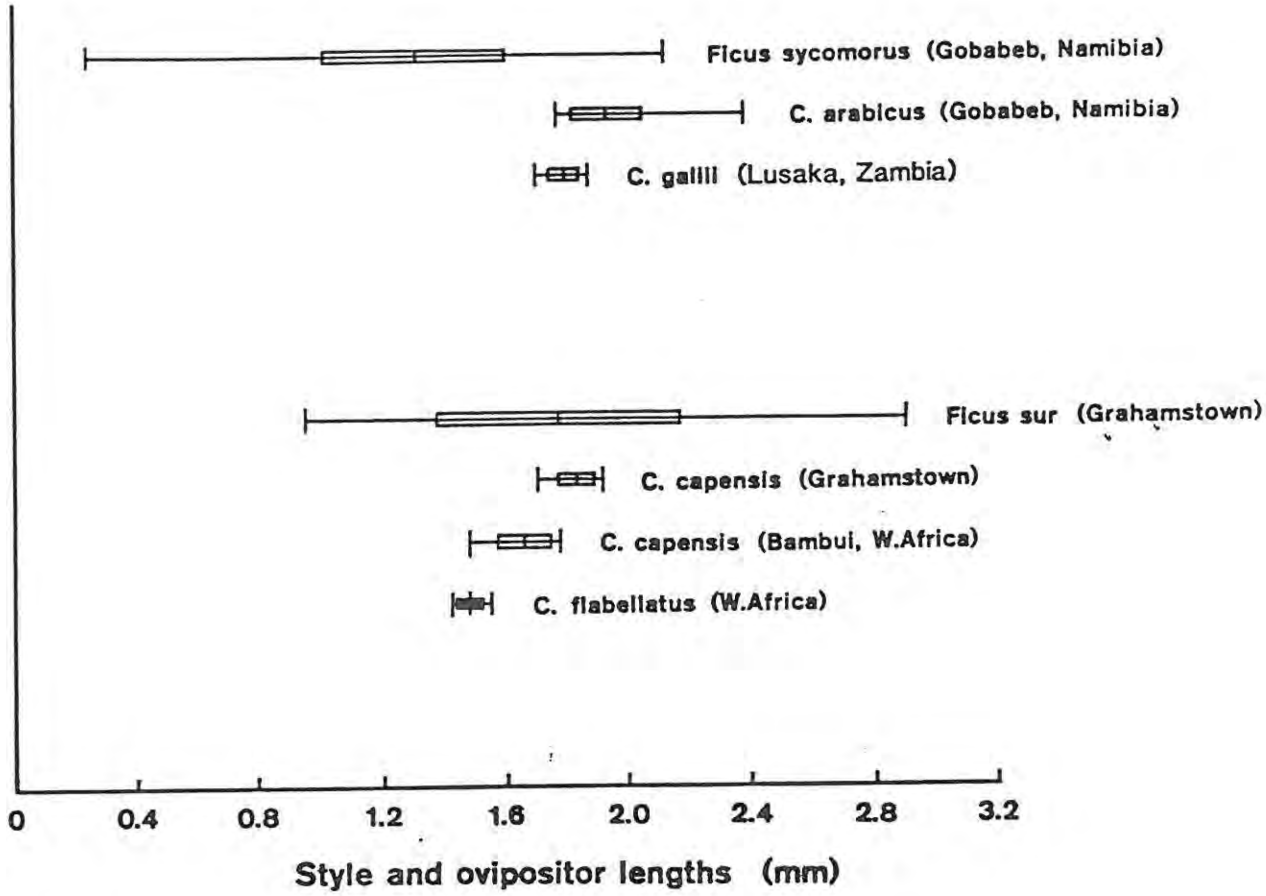

Figure 3.4 Comparison of means, standard deviations and ranges of styles of flowers from F. sycomorus and F. sur with the ovipositor lengths of agaonids from different geographical regions.

C. flabellatus, which is a second pollinator of *F. sur* in west Africa, has an even shorter ovipositor (Figure 3.4, n = 10, x = 1.49mm, range = 1.44 -1.56; t = -6.06, P < 0.001). The estimated proportion of accessible flowers (using measurements of figs from Grahamstown, South Africa), were 23% for *C. capensis* from Bambui and only 17% for *C. flabellatus*.

Figure 3.2 also illustrates the relationship between the mean ovipositor length and mean style length in *F. capraefolia*, a dioecious fig. Here certain trees (female trees) have figs with excessively long styled flowers which keep most flowers out of reach of the pollinator's ovipositor. Although the majority of the flowers in figs from a female tree had styles longer than the wasps mean ovipositor length, 8% of the flowers were estimated to be accessible (Table 3.4). A male tree produced figs with style:ovipositor length ratios more similar to those in monoecious species.

3.3.3 Observed utilisation of flowers by agaonid larvae

The comparisons between mean style and ovipositor lengths give only an estimate of the proportion of flowers that would be accessible, as they assume that the whole ovipositor could be inserted. By examining the utilisation of flowers of varying style lengths, the relative utilisation and maximum style lengths of occupied flowers could be recorded and related to the estimates. Figures 3.5 to 3.7 compare the frequency distributions of style and ovipositor lengths with the observed utilisation of flowers by the pollinators of three fig species.

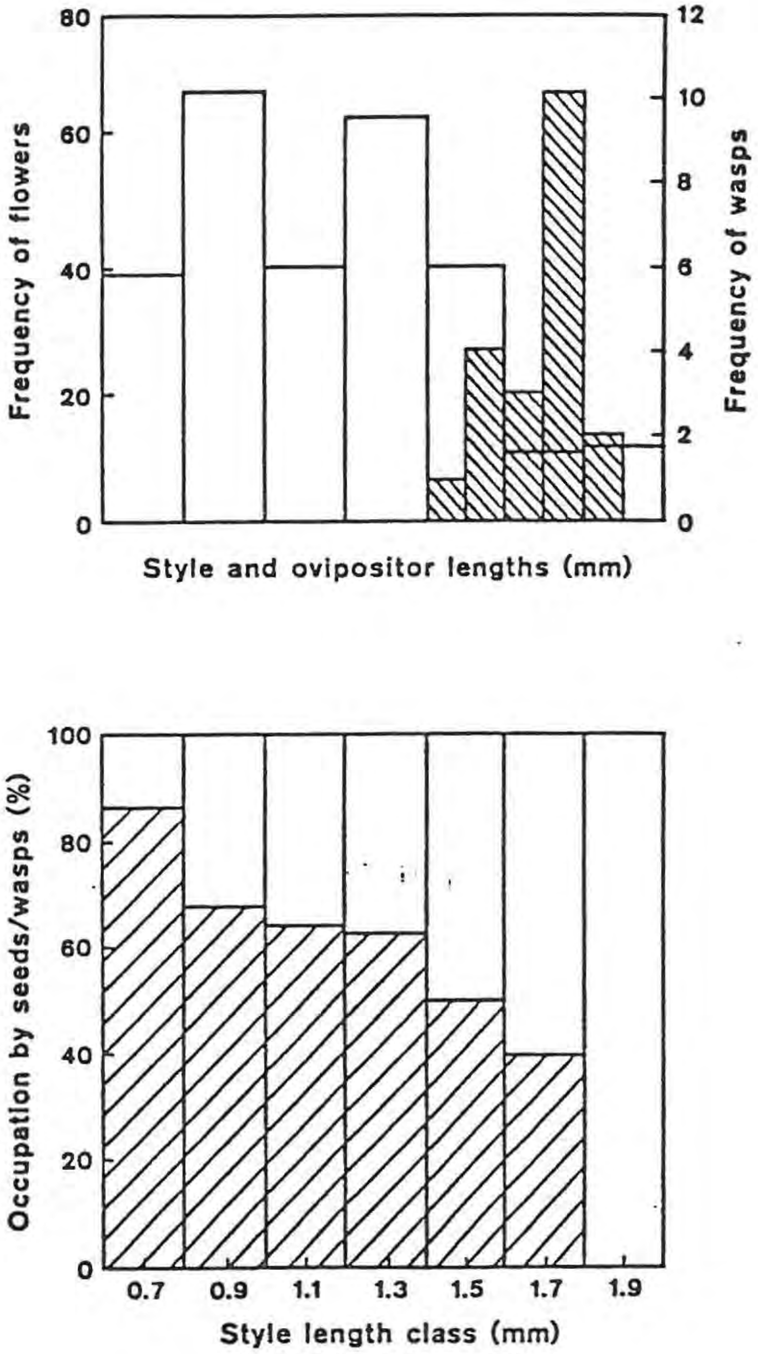

Figure 3.5 The relationship of style and ovipositor lengths to utilisation of F. thonningii flowers by E. stuckenbergi progeny. The histogram (top) compares style length (open bars) and ovipositor length (lined bars) frequency distributions. The pollinator is estimated to have access to 95% of the flowers. This is compared with the actual utilisation in the bottom histogram. Classes of flowers with longer style lengths produced proportionately fewer wasps (lined bars), but many more seeds (open bars).

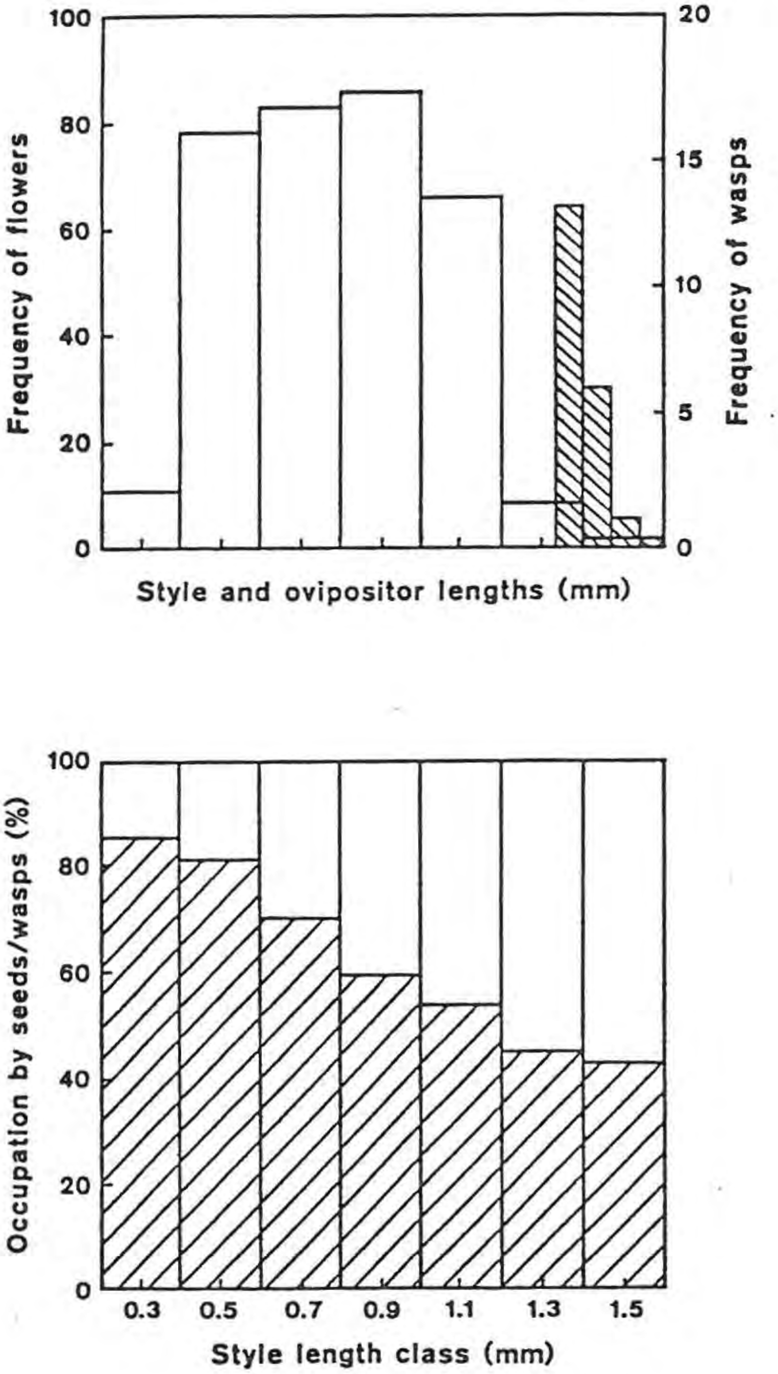

Figure 3.6 The relationship of style and ovipositor lengths to utilisation of *F. burtt-davyi* flowers by *E. baijnathi* progeny. The histogram (top) compares style length (open bars) and ovipositor length (lined bars) frequency distributions. The pollinator is estimated to have access to 83% of the flowers. This is compared with the actual utilisation in the bottom histogram.

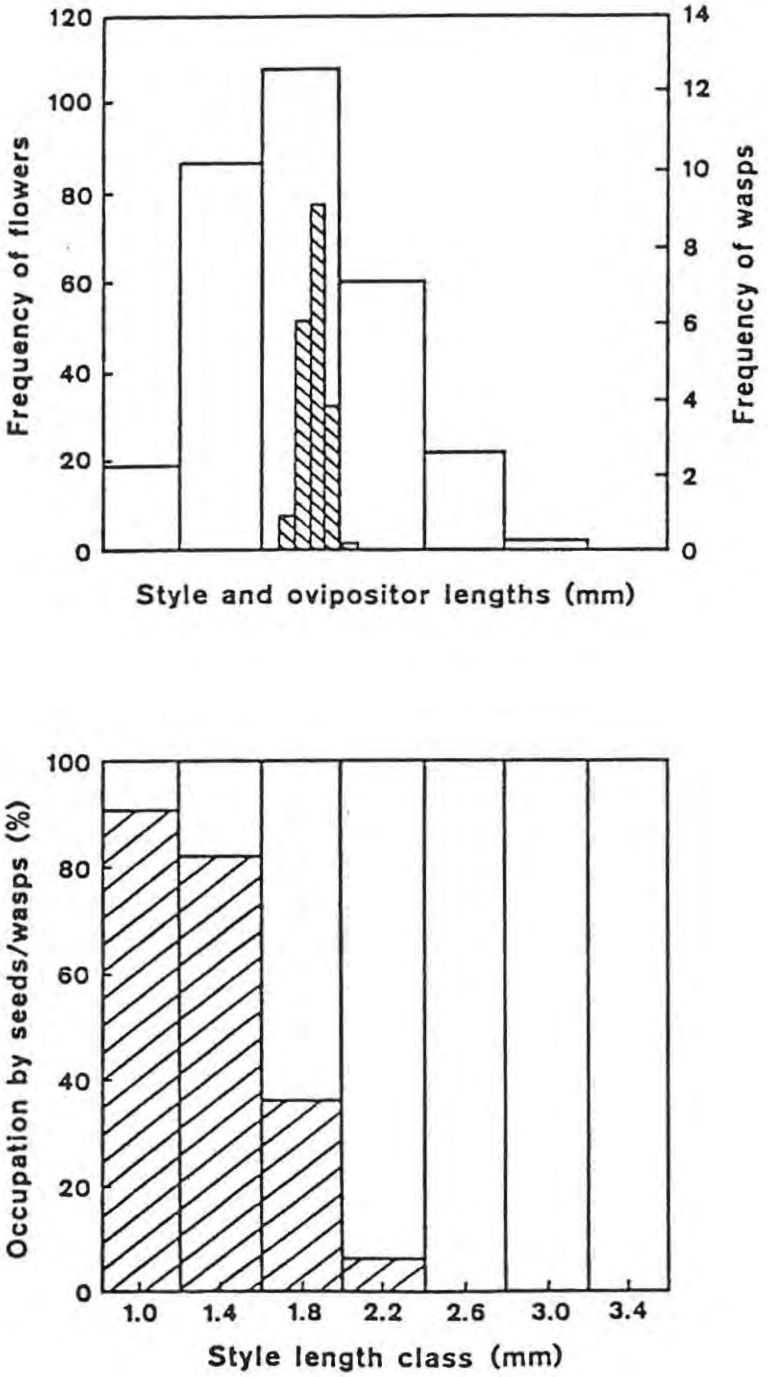

Figure 3.7 The relationship of style and ovipositor lengths to utilisation of F. sur flowers by C. capensis progeny. The histogram (top) compares style length (open bars) and ovipositor length (lined bars) frequency distributions. The pollinator is estimated to have access to 55% of the flowers. This is compared with the actual utilisation in the bottom histogram.

Ovipositor length proved to be a good indicator of the maximum style lengths that the pollinators utilised, but the ovipositing females appeared to use preferentially the shorter styled flowers and in all three species studied (*F. burtt-davyi*, *F. thonningii* and *F. sur*) the proportion of flowers utilised decreased with increase in style length. In *F. sur*, where the pollinator had a short ovipositor relative to mean style length, progeny were restricted to the shorter styled flowers (Figure 3.7). The pollinators of *F. burtt-davyi*, and *F. thonningii* had relatively longer ovipositors, and this was reflected in their flower utilisation, which included even the longest styled flowers (Figures 3.5 and 3.6 respectively).

3.3.4 Effects of progeny density on style length utilisation

In all three fig species the mean style length of flowers containing agaonid pupae was found to be related to the density of pupae within a given fig (Figure 3.8). A significant positive correlation was obtained between mean style length of occupied flowers and agaonid density (*F. burtt-davyi*: $y = 112x - 64$, $r^2 = 65\%$, $P < 0.01$; *F. thonningii*: $y = 83x - 36$, $r^2 = 94\%$, $P < 0.01$; and *F. sur*: $y = 103x - 96$, $r^2 = 62\%$, $P < 0.01$). Similar regressions using all style length measurements, rather than using the means, gave a significantly positive correlation from *F. thonningii* ($y = 0.004x + 0.68$, $r^2 = 3\%$, $P = 0.03$) , but not from *F. burtt-davyi* nor *F. sur* ($P > 0.05$). These results nonetheless imply that as more mother fig wasps enter a fig, oviposition tends to shift towards flowers with longer styled flowers. However, care must be taken when evaluating these correlations. In *F. burtt-davyi*, and perhaps in

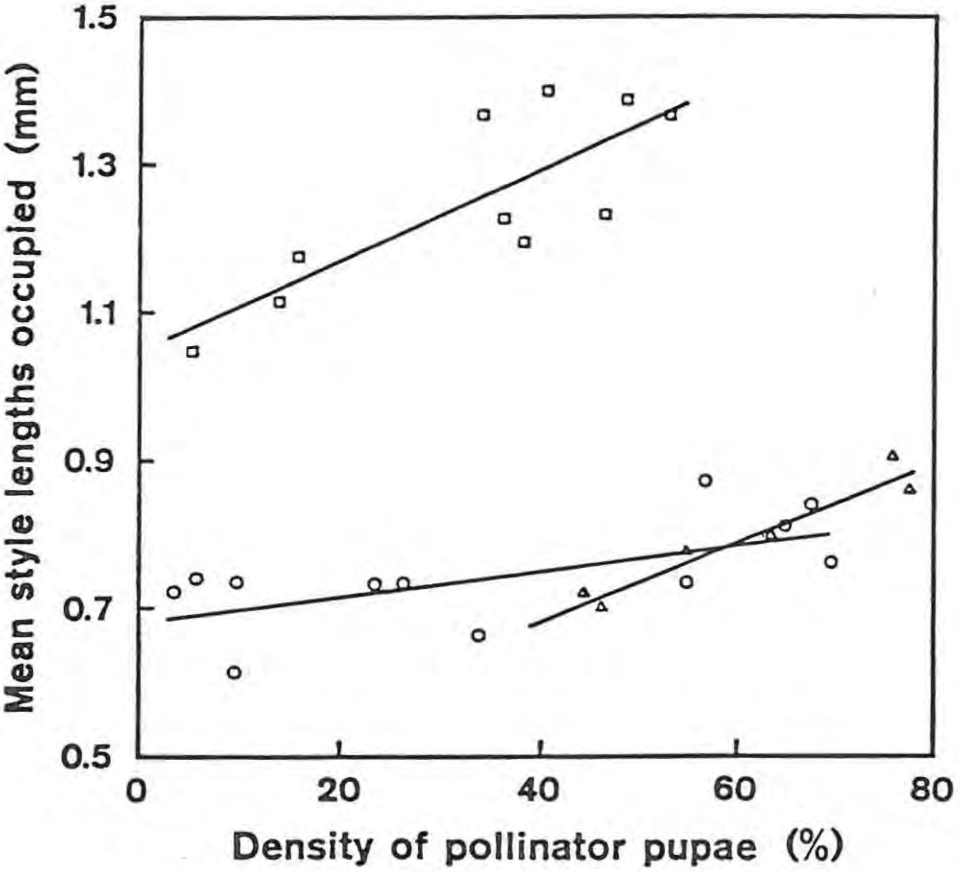

Figure 3.8 Regression lines computed to show the effects of agaonid density (indicated by the density of pupae inside each fig) on the mean style lengths occupied by wasp progeny. F. burtt-davyi and E. baijnathi (circles); F. thonningii and E. stuckenbergi (triangles); F. sur and C. capensis (squares).

general, as the density of wasp progeny increases, so does the range of style lengths utilised, with the upper limit increasing with progeny density.

3.3.5 Egg production and flower utilisation

The eggs of newly emerged agaonids were mature and ready to be laid. Agaonids are therefore pro-ovigenic (Flanders, 1939; Copland & King, 1973; Donaldson & Walter, 1988). Numerous ovarioles were present and Iwata (1962) described agaonid ovaries as having multiple ovarioles. Each egg had an ellipsoid basal body and a long pedicel that widened a little terminally. The basal ends of the bodies protruded into the single oviduct. Nearby was the spermatheca which was partially sclerotised in *C. capensis*, while in species of *Elisabethiella*, they were clear and the spermatozoa were clearly visible.

Since agaonids are pro-ovigenic, a count of the eggs from one wasp represents the total lifetime egg production for that individual. Table 3.5 gives the mean number of eggs found in the three pollinator species. Larger species had many more eggs. For example, the largest species, *C. capensis*, had on average three times as many eggs as *E. baijnathi*. The larger number of eggs in *C. capensis* may be related to there being 3.7 times as many accessible flowers in its associated fig, *F sur* than in *F. burtt-davyi*. Figs of *F. sur* had an estimated average of 895 accessible flowers (Table 3.5). Hence there were enough oviposition sites for the total egg complement of up to three female *C. capensis*. Similarly in *F.*

Table 3.5 Body sizes (measured as the inter-ocular distance), egg load and number of accessible flowers from three fig-agaonid associations.

Species of Pollinator	Inter-ocular distance (mm)		Number of eggs		Number of accessible flowers			Mean number of foundresses		
	mean	range	mean	range	mean	range	n	mean	n figs	n trees
E. baijnathi	0.24	0.20-0.27	79	67-94	237	141	37	1.37	183	12
E. stuckenbergi	0.32	0.27-0.36	103	85-135	177	10	3	1.07	25	1
C. capensis	0.44	0.39-0.51	238	180-370	895	669	20	1.89	73	3

burtt-davyi and *F. thonningii* the presence of three and two foundresses respectively could completely utilise all the accessible flowers (Table 3.5). Increasing numbers of foundresses in a fig will reduce the number of oviposition sites available to any one female. Figure 3.9 shows that out of a total of 183 *F. burtt-davyi* figs, 140 (77%) contained a single foundress and 27 (15%) contained two foundresses. The foundress numbers in figs of *F. sur* and *F. thonningii* indicate that a similar pattern is present in these species (Table 3.6). Therefore in a large majority of figs oviposition sites did not appear to be limiting. However, the actual number of eggs laid per female in figs with two or more foundresses is much less than when only one female is present (Chapter 4).

3.4 Discussion

It is clearly evident from the measurements of styles from ten African species that monoecious figs have not evolved two discrete forms of flowers. In all figs examined, the style length frequency distributions were unimodal, and seldom deviated from a normal curve. Bronstein (1988) found that the frequency distribution of styles from *F. pertusa* were positively skewed towards shorter styles. A similar trend was found amongst the African species examined in this study, but the asymmetries were minor in comparison with those recorded by Bronstein. Rather than an adaptation to control wasp and seed production, style length variation may be an effect of packing the maximum number of flowers in a spherically shaped inflorescence, as proposed by Janzen (1979a).

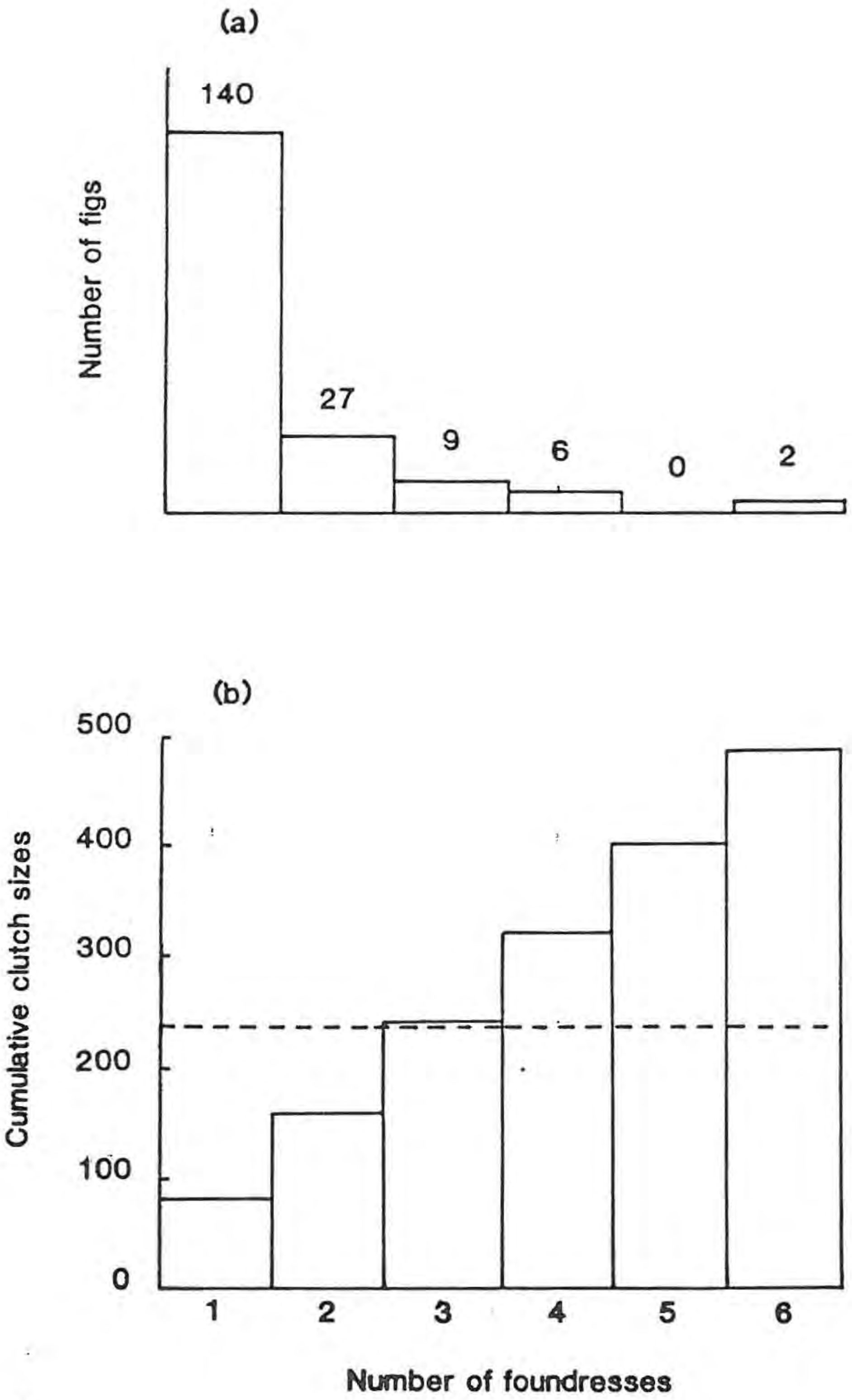

Figure 3.9 The observed frequency distribution of figs containing different numbers of *E. baijnathi* foundresses (top histogram) and the theoretical maximum combined clutch sizes (bottom graph), assuming foundresses lay all their eggs. The horizontal dashed line indicates the estimated number of accessible flowers.

Table 3.6 Mean numbers of foundresses in figs of F. burtt-davyi and F. sur from Grahamstown.

Tree No	Date	Sample size	Nos of wasps per fig 1	2	3	4	5	6	Arithmetic Mean wasps per fig	Harmonic Mean wasps per fig
F. burtt-davyi										
1	8.5.85	16	11	0	3	0	0	1	1.87	1.32
2	8.5.85	19	10	5	1	3	0	0	1.84	1.40
3	21.5.85	18	7	6	3	2	0	0	2.00	1.57
4	27.5.86	30	27	3	0	0	0	0	1.10	1.05
5	27.5.86	30	26	4	0	0	0	0	1.13	1.07
6	13.6.86	30	26	4	0	0	0	0	1.13	1.07
7	23.4.87	5	2	1	2	0	0	0	2.00	1.58
8	7.5.87	3	3	0	0	0	0	0	1.00	1.00
9	10.5.87	4	4	0	0	0	0	0	1.00	1.00
10	10.5.87	5	3	2	0	0	0	0	1.40	1.25
11	23.5.87	5	4	1	0	0	0	0	1.20	1.11
12	6.4.88	18	17	1	0	0	0	0	1.06	1.03
t		183	140	27	9	6	0	1	1.47	1.17
F. sur										
1	13.6.85	22	15	4	1	2	0	0	1.55	1.17
2	18.6.87	18	8	4	0	5	0	1	2.33	1.58
3	17.8.88	33	20	5	2	5	0	1	1.88	1.34
t		73	43	13	3	12	0	2	1.92	1.36
F. thonningii										
1	24.02.89	14	13	1	0	0	0	0	1.07	0.96

Examination of the styles of flowers from different sections of each fig showed that, if present at all, style length differences within figs were trivial in comparison with the range of styles available to an agaonid. In *F. burtt-davyi*, the differences in mean style length between sections were only around 0.1mm, compared with the 1.0mm difference in lengths between the longest and shortest individual styles. Thus localised variation in mean style lengths within one fig is unlikely to influence the distribution of accessible oviposition sites.

Different figs on the same tree were sometimes found to have significantly different style lengths. However, these differences were again minor in comparison with the range of style lengths available to agaonids. Hence, variation in style lengths between figs also appears insignificent as an influence on the distribution and proportion of flowers which are accessible as oviposition sites. Between - tree and regional differences in mean style lengths are also likely, but were not investigated. The mean ovipositor lengths of *C. capensis* collected from different geographical locations were found to vary. However, without the relevent style lengths to compare the ovipositors against, it is not known whether this would effect the proportion of accessible flowers. That *C. capensis* from Cameroun had such a short ovipositor is surprising since the style lengths of *F. sur* from west Africa (Verkerke, 1988b) have a similar frequency distribution to those from Grahamstown. This suggests that in west Africa less than half of the flowers of *F. sur* are accessible to *C. capensis*, and fewer still to the alternative pollinator, *C. flabellatus*. Thus the situation in *F. sur* approaches that predicted by the 'traditional' view of fig flower availability.

These previous comparisons of ovipositor and style lengths suggested that the ovipositors of agaonids were long enough to reach less than half the available flowers (Galil and Eiskowitch, 1968c - *F. sycomorus*; Newton and Lomo, 1979 - *F. lutea*), yet they also described finding pupae in long styled flowers. In contrast, 82% of flowers in *F. pertusa* were considered to be accessible to its pollinator, *Pegoscapus silvestrii* Grandi (Bronstein, 1988a). The former authors took ovipositor length as a measure of the sheath length, which is an underestimate of the true ovipositor length. The degree to which this technique underestimates true ovipositor length depends on the species, with *Krabibia gestroi* having a sheath : ovipositor ratio of 0.23, a major underestimate, and *Elisabethiella stuckenbergi* having a ratio of 0.64, a less severe underestimate. Quite clearly then, sheath length cannot be used to estimate the proportion of flowers that are accessible as oviposition sites.

Since maximum style lengths of occupied flowers coincided with maximum ovipositor lengths, ovipositor and style length comparisons can be considered an accurate way of estimating accessibilities of flowers to agaonid oviposition. Comparisons of the mean lengths of the true ovipositors and style lengths in each fig-fig wasp association showed that in the majority of these associations, around 70% of the flowers appeared to be accessible, with the extreme example of *Platyscapa awekei*, estimated to have access to 99% of the flowers in figs of *F. salicifolia*.

Examination of the style length frequency distributions of the dioecious

F. capraefolia, showed that 'male' figs seem to be equivalent to monoecious figs with regard to their ovipositor:style ratios. However, this was the only species where all the flowers were estimated to be accessible. That any flowers from 'female' figs should appear to be accessible is surprising given that only seeds are normally produced by 'female' figs (Verkerke, 1987). Perhaps *F. capraefolia* is atypical, or other factors, in addition to style length, may prevent oviposition in these figs. Verkerke (1987, 1988b) has shown that there is ovule dimorphism in the related *F. asperifolia*. In female, but not male figs there is a circumvallating inner integument which hinders egg deposition next to the nucellar epidermis. These data nonetheless support the general idea that 'female' trees produce seeds because their flowers are inaccessible to agaonids. 'Male' trees have short styles and consequently all of their flowers can be utilised by developing agaonids, if sufficient foundresses enter the figs.

Although most agaonids have ovipositors capable of reaching a majority of the flowers in monoecious figs, ovipositing females prefer to lay eggs in shorter styled flowers. Observations during this study showed that *C. baijnathi* and *C. capensis* probe rapidly down styles at random and probably cannot discriminate between flowers before probing. These agaonids often probe a short distance down a style, and then proceed to remove the ovipositor and seek out another flower, without ovipositing. The cues that result in either ovipositor removal or oviposition may involve physical attributes within the fig flower. Possibly it is easier for a wasp to insert its ovipositor down shorter styles because there is a shorter distance to penetrate. As a result a wasp may initially prefer short styled flowers, but if these are all utilised, it will still be able to oviposit into longer styled flowers. This explains why more longer styled flowers are utilised in figs which receive many ovipositing females, and subsequently produce large numbers of wasp progeny (Chapter 4).

Where more than two pollinators enter a fig, the number of oviposition sites will become progressively depleted. Also, the fig flowers begin to change in structure once the initial female has laid her eggs or begun pollination and this eventually prevents further ovipositions. This is evident in figs which have contained an ovipositing wasp for several hours, because the styles of their flowers become brown and begin to wilt. At high foundress densities, females will therefore be unable to lay all their eggs into separate flowers because even if ovules remain available, reduced oviposition rates mean that flowers cease to be suitable for oviposition before the whole clutch is laid. The question arises as to what happens to the remaining eggs? There are two possibilities. Firstly the wasps may begin to lay eggs into flowers already containing fig wasp eggs, giving rise to superparasitism. Superparasitism in fig wasps has not been confirmed however, and studies to date have shown that only a single egg is laid into each flower (Galil & Eisikowitch, 1968a; Joseph & Abdurihiman, 1981; Verkerke, 1986; 1987; 1988a). Agaonids may avoid flowers which already contain eggs of conspecifics by reacting to chemical markers. This would help prevent females from repeatedly ovipositing in the same flower. Other chalcids are known to place markers in oviposition sites to avoid superparasitism (Roitberg & Prokopy, 1987; Waage, 1986). It therefore appears that female fig wasps which cannot find un-used oviposition sites within the short time period, fail to lay all their eggs. Dead *E. baijnathi* foundresses were found to contain eggs (pers. observ.), confirming that when many foundresses enter a fig, competition for oviposition sites is severe.

In conclusion, ovipositors are not short relative to style lengths in monoecious figs and it appears the ovipositors are adapted to reach the majority of ovules in most fig species. It therefore seems unlikely that the ratio between ovipositor and style lengths is responsible for regulating the relative production of seeds and pollinators. Instead, the critical factor involved in maintaining a stable coexistance between figs and their pollinators, appears to be the number of foundresses entering the figs. Consequently, the population dynamics of agaonids will play an important role in influencing the 'stability' of the mutualism.

CHAPTER 4

Sex Ratio adjustment in *E. baijnathi* and *C. capensis*

4.1 Introduction

This chapter examines the sex ratios of agaonids. Sex ratio theory has provided an ultimate explanation of *why* agaonids should adjust their sex ratio in relation to the number of foundresses that contribute to the offspring in one fig, but no proximate mechanism of *how* this actually takes place has been proposed. Fig wasps are useful models for the study of sex ratio theory because foundress mothers produce broods which are isolated within the fig. The structure of these isolated broods fulfils the conditions of Hamilton's (1967) model of Local Mate Competition (LMC), where a high level of sib-mating can be expected. This hypothesis predicts highly female - biased brood sex ratios. Herre (1985; 1987) found that fig wasps adjusted their offspring sex ratios in relation to the number of foundresses contributing to a brood inside one fig. Sex ratios became less female biased with increase in the number of foundresses. These studies and others (Frank, 1985a; Ramirez, 1987) were undertaken on fig wasps from Central America. It has not been established previously that Old World fig wasps also exhibit sex ratio adjustment.

Although observed sex ratios in chalcids have been found to support theoretical models (Griffiths & Godfray, 1988; Werren, 1980; 1984; Strand, 1988; Cees *et al*, 1987; Donaldson & Walter, 1984; May & Seger, 1985; Owen, 1983), proximate mechanisms of sex ratio adjustment are poorly understood. Some species of chalcids have been shown to respond to the presence of other females, and change their sequence of sex allocation (Waage, 1982a; 1986). This stimulus may have been contact traces of other wasps, physical jostling by other individuals, or encounters with already inhabited oviposition sites. It is not known whether any of these stimuli influence sex ratio adjustment in fig wasps.

Adult sex ratios can also be influenced by differential mortality of female larvae, especially when many foundresses are present and there may be superparasitism or indirect competition for food resources (Suzuki *et al*, 1984). If flowers are in short supply for fig wasps, then more than a single egg could be laid into an individual flower. Under these circumstances the mortality of wasp larvae would be expected to be high. Evidence for differential mortality of the sexes is considerable among parasitoids. Most studies indicate that males, probably because of their more rapid development or lower nutritional requirements, fare relatively better as total survival decreases (Wilkes, 1963; Suzuki *et al.*, 1984). In the fig - fig wasp system, assuming that female larvae are more susceptible to starvation than males, this mortality could cause the sex ratio to become less female biased when foundress numbers are high.

A third mechanism of sex ratio adjustment is based on the possibility that female fig wasps lay male eggs first and then proceed to fertilise the remainder, which produce females. A sequence of haploid and diploid egg deposition under variable ovipositional site limitation could thus give rise to regulative sex ratio adjustments. In figs containing many foundresses, oviposition sites are in short supply (Chapter 3). Thus additional foundresses may not lay their total complement of eggs, but still lay the first few, which could be male. This would then cause increasingly less biased female sex ratios with any increase in the number of foundresses. The pattern of sex allocation by fig wasps can be examined indirectly by recording the style lengths of flowers producing either males or females. In chapter 3 it was shown that the first eggs appear to be laid into the shorter styled flowers. If male eggs are laid first, then one would expect there to be proportionately more male progeny arising from short styled flowers than longer styled flowers. Here I examine whether two southern African agaonids exhibit sex ratio changes in accordance with sex ratio theory, and examine possible factors that might contribute to these patterns, particularly those related to accessibilities of fig flowers.

4.2 Methods

4.2.1 Experiment 1 - The effect of differing foundress numbers on seed set, mortality and sex ratios of agaonids

Before their ostioles had opened, up to 10 figs of *F. burtt-davyi* were bagged in each of six nylon mesh bags. As soon as the figs were ready to receive wasps, one wasp was introduced into each fig in bag 1, two wasps per fig in bag 2, three wasps per fig in bag 3, and four wasps per fig in bag 4. This was achieved by placing individual pollinators (which had emerged in the laboratory) near the vicinity of the ostiole with a paint brush. More than 100 wasps were placed into each of the two additional bags, 5 and 6. In these bags large numbers of wasps attempted to enter each fig, but many became stuck in the ostioles. The number of foundresses which entered to oviposit could, however, still be recorded by counting the remains of individuals inside the lumens of figs at the end of the experiment.

The bagged figs were then left until they ripened seven months later. The presence of the bags prevented oviposition by other fig wasps. Figs were collected at the beginning of phase D, usually just prior to the emergence of the first male progeny from their galls, although in some cases males were already inside the lumen. Any figs with exit holes were discarded, as some of the wasps had been lost. The influence of the number of foundresses on seed set, bladder and wasp numbers and sex ratios was examined by dissecting the figs and counting the total numbers of seeds, unpollinated flowers, bladders, and male and female progeny. The numbers of all male and female progeny of *E. baijnathi* were recorded in relation to the style lengths of the flowers they occupied. This was done by measuring the style of each flower under a dissecting microscope. Then the flower was broken open and the identity and sex of the wasp inside was recorded.

An attempt was made to repeat this experiment with *C. capensis*. Four bags, each with up to seven figs, were set up on a *F. sur* tree at St. George's Chambers in Grahamstown. As for *E. baijnathi*, each fig in bag 1 received one foundress, each fig in bag 2 two foundresses, each fig in bag 3 three foundresses and each fig in bag 4 received four foundresses. Prior to wasp emergence, the figs were dissected open. All the galls were removed, broken open and the sex of the *C. capensis* offspring was recorded. A sample of 50 flowers containing wasp progeny had their styles measured before being broken open. The wasps in each gall were then sexed in order to compare the style lengths of flowers utilised by male and female progeny. Unfortunately, some of the *C. capensis* foundresses introduced into these figs did not contain any pollen. Consequently the flowers from 13 out of the 20 figs were unpollinated and as a result the effects of foundress numbers on seed set and bladder production could not be analysed. However, the data was used to examine the effects of pollen absence on the mortalities of wasp progeny and therefore is included in Experiment 2 as a comparison with *E. baijnathi*.

The sex ratios and numbers of *C. capensis* progeny in relation to foundress number have been studied previously by A. Gardiner (Gardiner, 1987), and here I compare his results with those from *E. baijnathi*. He used a similar experimental design. 43 experimental bags were set up, each with one fig and between one and 12 foundresses were introduced into each fig. As soon as the figs were ripe, they were dissected and all the galls were removed and the identity and sex of the wasp in each was recorded.

4.2.2 Experiment 2 - Does experimentally induced starvation cause differential mortality of the sexes ?

In order to obtain pollen - free foundresses, galls containing inseminated female *E. baijnathi* were placed into petri dishes. Inseminated females were identified by the mating holes through their galls, made by the male prior to copulation. The females subsequently emerged from their galls, but were unable to collect any pollen due to the absence of staminate flowers. To confirm that they were mated and pollenless, a subsample of 10 females were squashed on a slide to check for the presence of pollen grains and a charged spermatheca. None were found to contain any pollen grains and all had spermathecae containing spermatozoa. One experimental female was then introduced into each of 15 figs by placing the wasps near the vicinity of the ostiole with a paint brush. They entered the ostiole on their own accord. These figs were then enclosed in a single bag to prevent entry of other wasps. A control bag was also set up on the same tree, with six figs that each received one wasp containing pollen in its pollen-pockets. Data from Experiment 1 was used to examine the effects of pollen absence in figs of *F. sur*. Here 13 figs received between one and four pollen - free *C. capensis*, while the remaining figs each received between one and four pollen - laden wasps.

After a few months, the figs were picked before any exit holes had been made, thus ensuring that no wasps had escaped. Each fig was then placed in an emergence vial. After emergence, any galls which still contained wasps were dissected open so that the total number of progeny and sex ratio from each fig could be recorded. In *F. burtt - davyi*, the total remaining seeds, infertile flowers, and bladders were removed and counted, while the same categories were counted from subsamples of 100 flowers from each *F. sur* fig.

4.2.3 Experiment 3 - Does physical contact between foundresses stimulate sex ratio adjustment ?

Adult female *E. baijnathi* were collected by placing phase D figs into emergence containers. The figs were not split open to aid wasp emergence because it was found that such a technique inhibited wasps from collecting pollen. The ovipositors of some wasps were then amputated at the point where they project beyond the gaster. During this operation the wasps were kept quiescent by keeping them in a container over ice.

Small undeveloped figs (phase A) were bagged three weeks before their ostioles opened, thus ensuring they were wasp-free. At phase B, when the ostioles opened, one intact wasp was introduced into each of eight unpollinated *F. burtt-davyi* figs inside one bag. Immediately after this, about 50 wasps with amputated ovipositors were placed in the bag and allowed to enter the figs on their own accord. Many amputated wasps were observed to penetrate the ostioles of the figs already containing foundresses. Control figs were provided by 6 figs in one bag, each containing one intact wasp, from experiment 1 on the same tree.

Just prior to ripening, the experimental figs were harvested and taken back to the laboratory. They were then split open and all the progeny were dissected out of the flowers and sexed. The results were then compared to the sex ratios recorded from figs containing one foundress (experiment 1, see above).

4.2.4 Calculation of the theoretical sex ratio curves

Because remains of foundresses can be counted within the same fig species from which the offspring have emerged, both the progeny sex ratio and level of inbreeding can be calculated. To calculate the theoretical sex ratio curves (p) for *E. baijnathi* and *C. capensis*, the reciprocal of the number of foundresses, (m) of each fig, and the inbreeding value, (n) was substituted into Herre's (1985) equation (Chapter 1):

$$p = (1-m)(2n-1)/n(4n-1)$$

The inbreeding value of *E. baijnathi* was calculated from the number of foundresses in figs from 12 crops spanning four consecutive years (Table 3.6). The harmonic mean number of foundresses (n) was calculated to be 1.17 foundresses per fig.

$$p = (1-m)((2 * 1.17)-1)/((4 * 1.17)-1)$$

giving: $p = 0.18$ for two foundresses (where $m = 1/2$)
$p = 0.24$ for three foundresses (where $m = 1/3$)
$p = 0.27$ for four foundresses (where $m = 1/4$)

C. capensis was more outbred than *E. baijnathi*, with a harmonic mean of 1.34 foundresses per fig (Table 3.6). The theoretical sex ratio curve was calculated as before. The observed sex ratios of both *C. capensis* and *E. baijnathi* were then compared to these theoretical sex ratio curves.

4.3 Results

4.3.1 Experiment 1 - The effects of differing foundress numbers

4.3.1.1 The influence of foundress numbers on seed production and pollination

The number of foundresses entering figs of *F. burtt-davyi* influenced the numbers of seeds produced (Table 4.1; Figure 4.1a). Figs which contained single foundresses produced many more seeds than figs containing more than one foundress. While on average about 44 seeds were found in figs with a single foundress (Table 4.1), a mean of less than 11 was recorded from figs which contained more than one foundress (Mann - Whitney test, Z = -3.18, P < 0.001). The number of seeds produced in 2, 3 and 4 foundress figs did not differ significantly (Kruskal - Wallis test statistic = 4.097, P = 0.130).

There were no significant differences in the numbers of unpollinated *F. burtt-davyi* flowers between the four separate treatments (Kruskal - Wallis Test statistic = 5.223, P = 0.156), though there appeared to be a slight trend towards fewer unpollinated flowers in figs with more foundresses (Figure 4.1b).

Table 4.1 Experiment 1: The effects of different numbers of *E. baijnathi* foundresses on seed set, bladders, progeny numbers and sex ratios inside figs of *F. burtt-davyi*.

	Number of foundresses							
	1		2		3		4	
	mean	SD	mean	SD	mean	SD	mean	SD
	(n = 6 figs)		(n = 5 figs)		(n = 4 figs)		(n = 5 figs)	
Seeds	44.17	18.82	6.20	3.70	11.25	11.64	15.60	5.86
Infertile flowers	24.00	10.22	11.00	12.08	14.50	16.54	9.60	7.67
Bladders	13.67	5.39	18.00	9.95	29.00	14.67	17.00	8.78
Total wasp numbers	65.60	15.82	154.80	18.39	128.67	48.48	171.80	7.26
Males	7.00	2.76	22.40	8.88	26.00	15.52	32.80	8.56
Females	58.50	15.12	132.40	17.74	102.75	29.58	139.00	14.20
Sex ratios	0.108	0.041	0.144	0.054	0.192	0.070	0.192	0.055

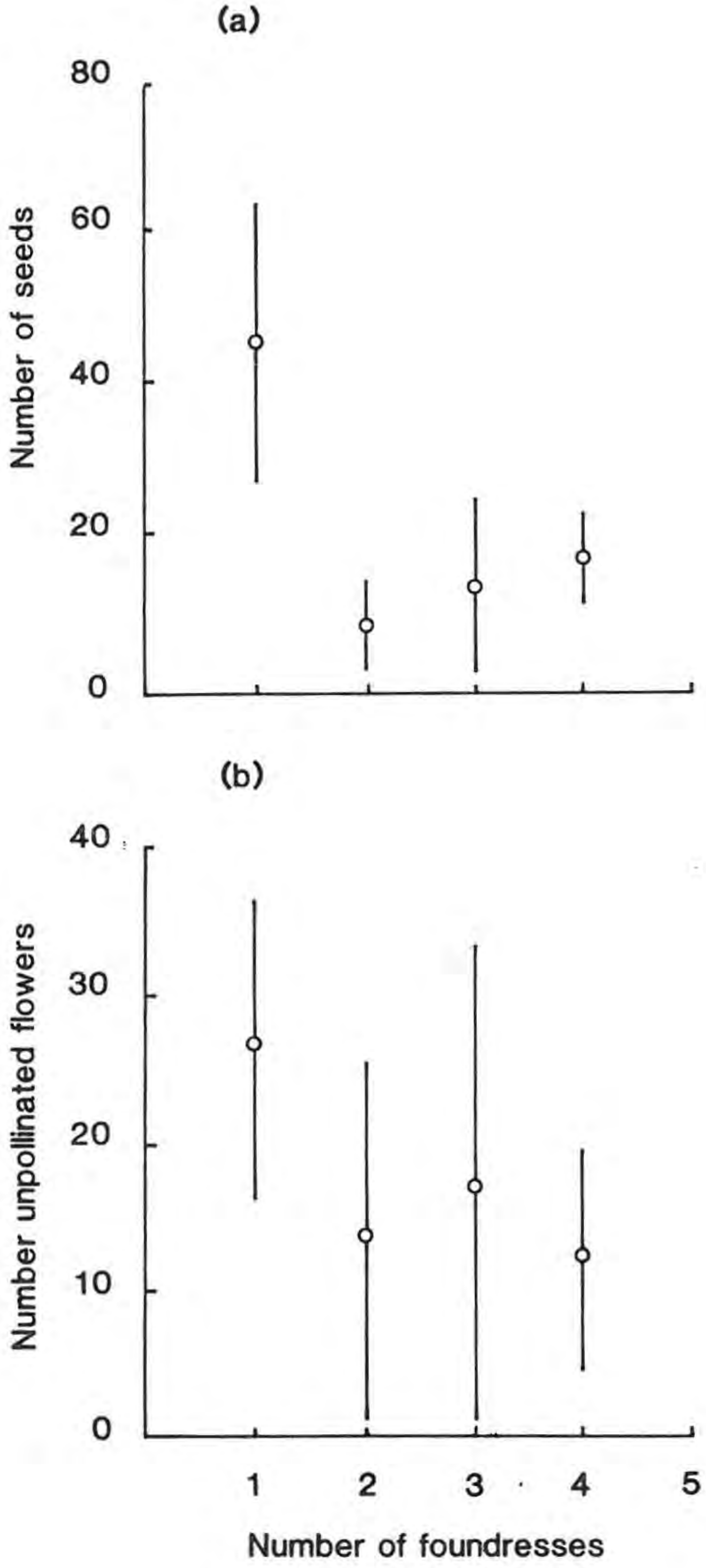

Figure 4.1 The effects of *E. baijnathi* foundress numbers on seed set in *F. burtt-davyi*. Means and standard deviations of numbers of seeds per fig (a) and unpollinated flowers per fig (b).

4.3.1.2 The effect of foundress numbers on agaonid offspring

i) *E. baijnathi*

Over twice as many wasp progeny were produced in *F. burtt-davyi* figs with two foundresses as in those with one foundress (Figure 4.2a). Addition of further foundresses however, did not increase the number of progeny emerging from a fig, which tended to fluctuate between 100 and 200 (Table 4.1), as expected given that an average of only 140 sites are estimated to be available (Chapter 3). Assuming an unlimited supply of oviposition sites, the hypothetical increase of progeny with number of foundresses was calculated by multiplication of the foundress number with the average number of progeny produced in figs with a single foundress (broken line in Figure 4.2a). This would be the total number of progeny produced if each foundress produced the same number of offspring, regardless of the number of foundresses present. Numbers of offspring from figs containing two foundresses did not deviate from the 'expected hypothetical' median (Wilcoxon signed ranks test, $n = 5$, $Z = 1.89$, $P = 0.06$). This test calculates the differences between the data values (the numbers of progeny in Figure 4.2a) and the hypothesised median (the 'expected' number - dotted line), and ranks the absolute values of the differences. Figs with more than two foundresses produced fewer offspring than expected (for three foundresses, $n = 4$, $Z = 2.08$, $P = 0.04$; for four foundresses, $n = 5$, $Z = 2.15$, $P = 0.03$), confirming

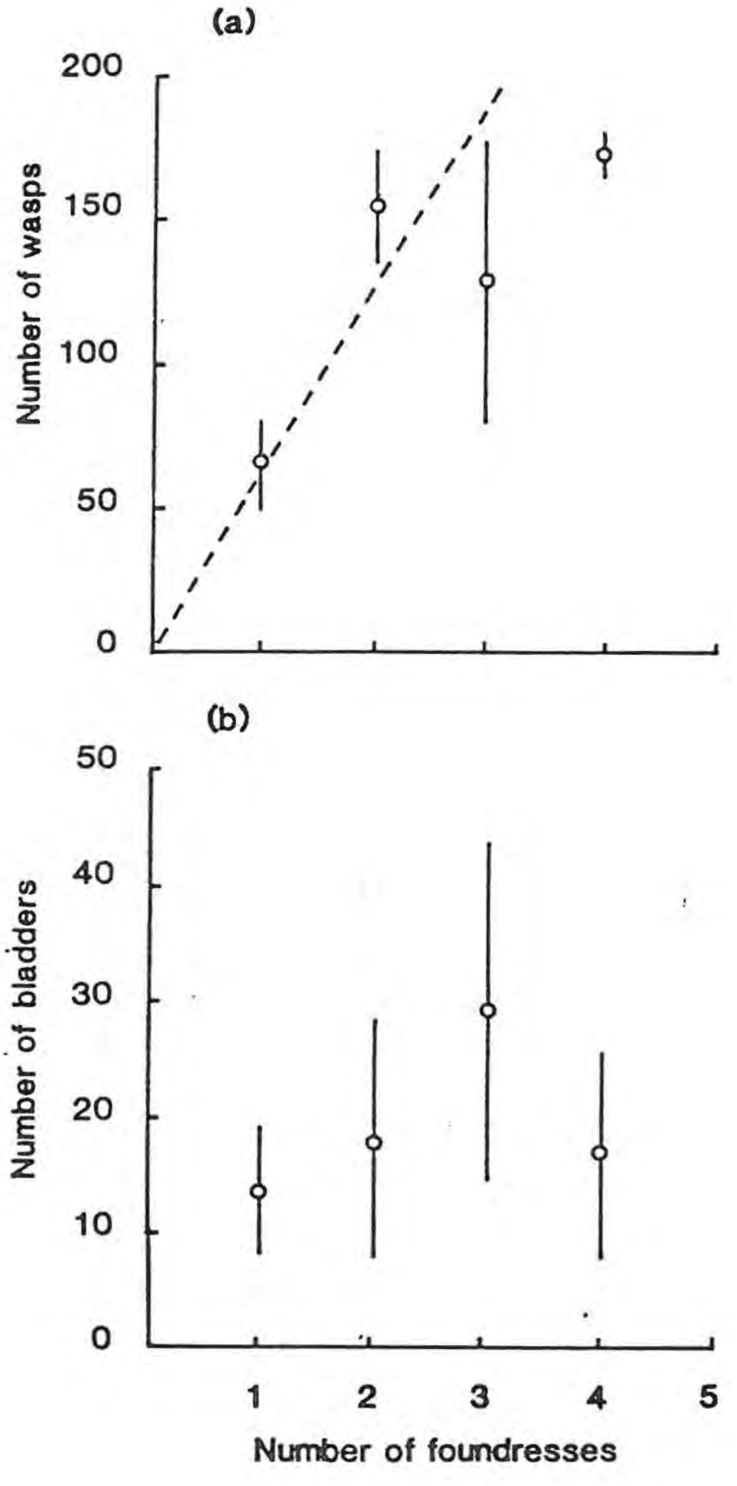

Figure 4.2 The effects of E. baijnathi foundress numbers on the total production of wasp progeny per fig (a) and number of bladders per fig (b) (means + S.D.). The dotted line represents the cumulative number of offspring, assuming 66 eggs are laid by all foundresses in each fig.

that the number of eggs laid per female declines after more than two females enter a fig. Figure 4.2b shows that there was no significant difference in bladder numbers in figs with different numbers of foundresses (Kruskal-Wallis test statistic = 4.75, $P = 0.19$).

A breakdown of wasp offspring production by sex showed that the number of male *E. baijnathi* progeny increased additively as the number of foundresses increased (Table 4.2; Figure 4.3a). A Wilcoxon signed ranks test showed that the 'expected' medians (dotted line in Figure 4.3a) did not differ from the numbers of males produced from each set of figs (Wilcoxon signed ranks test, for two foundresses, $n = 5$, $Z = 0.894$, $P = 0.37$; three foundresses, $n = 3$, $Z = 0.000$, $P = 1$; four foundresses, $n = 5$, $Z = 0.500$, $P = 0.62$). The number of males produced per female therefore stayed constant with changing foundress numbers.

Even though the production of male progeny per foundress remained constant, the sex ratio could still change if proportionately fewer female eggs were laid per foundress at increasing foundress densities. The numbers of female *E. baijnathi* progeny increased additively from one to two foundresses per fig, but in contrast to male progeny, fell significantly below the expected line in figs with more than two foundresses (Figure 4.3b) (Wilcoxon Signed ranks test, three foundresses: $Z = 2.0$, $P < 0.05$, four foundresses: $Z = 2.16$, $P < 0.04$). Sex ratios therefore become less female biased because fewer females were produced.

Table 4.2 Experiment 1: Offspring sex ratios of *E. baijnathi* from figs with different foundress numbers (total numbers of offspring from each fig in parentheses).

	Foundress number							
Fig No.	1	2	3	4	5	6	8	9
1	0.082	0.137	0.168	0.169	0.210	0.348	0.516	0.240
	(73)	(153)	(161)	(178)	(157)	(141)	(62)	(85)
2	0.106	0.219	0.270	0.133		0.170	0.250	0.260
	(66)	(151)	(152)	(181)		(106)	(68)	(70)
3	0.132	0.087	0.137	0.170		0.230		
	(68)	(173)	(73)	(165)		(70)		
4	0.079	0.176	0.250	0.213				
	(38)	(170)	(44)	(169)				
5	0.079	0.102	0.125	0.277				
	(86)	(127)	(72)	(66)				
6	0.177		0.130					
	(62)		(72)					
7			0.250					
			(42)					
Mean	0.109	0.144	0.190	0.192	0.210	0.249	0.383	0.250
SD	0.039	0.054	0.064	0.055	0.000	0.091	0.188	0.014
(Mean)	(65.50)	(154.80)	(88.00)	(151.80)	(157.00)	(105.67)	(65.00)	(77.50)
(SD)	(15.82)	(18.39)	(48.67)	(48.40)	(0.0)	(35.50)	(4.24)	(10.61)

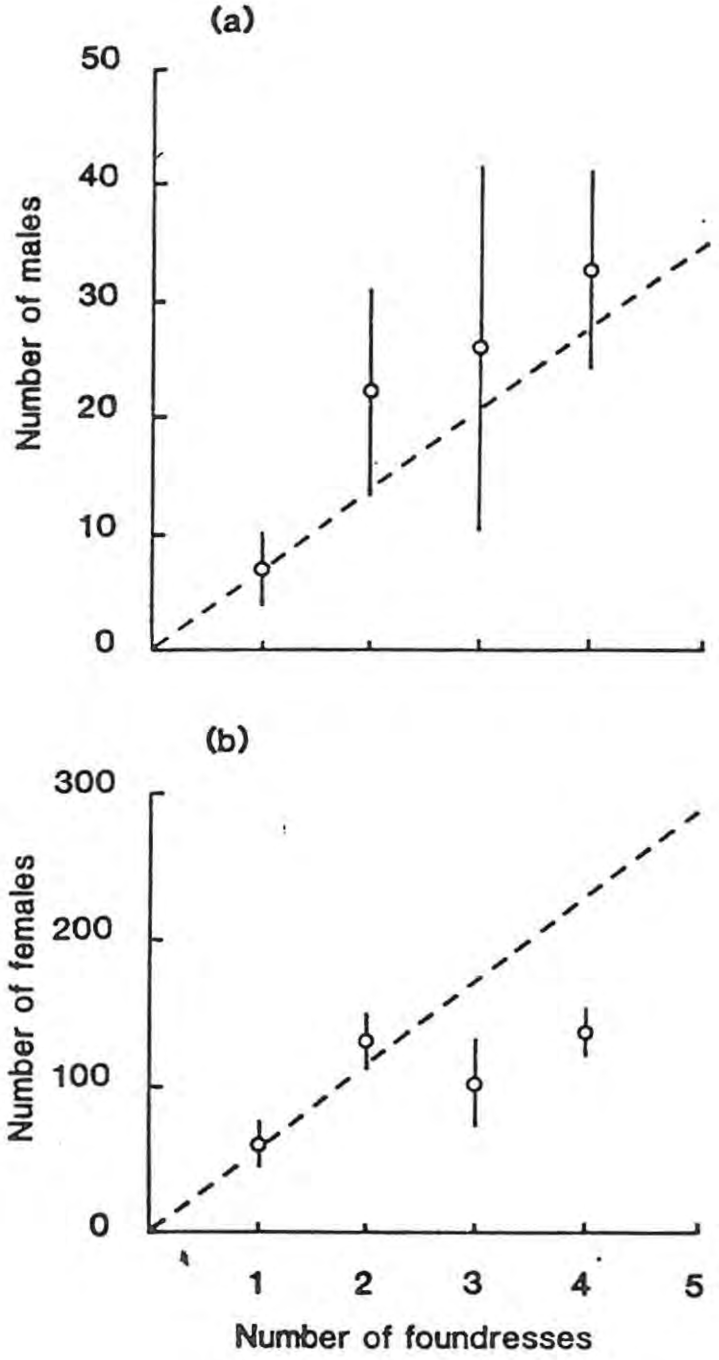

Figure 4.3 Male (a) and female (b) <u>E. baijnathi</u> progeny from figs which contained different numbers of foundresses (mean + S.D.). The dotted lines represent the total number of males and females that would be produced assuming seven male and 59 female eggs were laid.

ii) *C. capensis*

The sex ratios and numbers of offspring of *C. capensis* recorded by A. Gardiner (1987) are given in Table 4.3. *C. capensis* progeny that emerged from figs with more than two foundresses were well below the predicted offspring production line that assumes constant mortality and the laying of the maximum number of eggs by each female (dotted line in Figure 4.4). Nonetheless, there was a significant difference in the mean numbers of offspring between the experimental treatments (One - Way ANOVA, F = 2.44, d.f. = 7, P = 0.04), with more offspring in some figs that contained many foundresses.

There were also differences between the mean numbers of male *C. capensis* progeny produced. They generally increased with foundress numbers (One - Way ANOVA, F = 2.76, d.f. = 7, P = 0.023) (Table 4.3 and Figure 4.5a). Figs containing up to six foundresses produced mean numbers of males that did not differ from the 'expected' line (Wilcoxon signed ranks test, all P > 0.05), while means from figs containing more than six foundresses were below the predicted values (all P < 0.05).

The numbers of female *C. capensis* progeny exhibited a similar trend as the total production of offspring (compare Figure 4.4 with 4.5b), although a One - Way ANOVA comparing the mean numbers of female progeny across foundress numbers was marginal (F = 2.20, d.f. = 7, P = 0.60). Figs containing two or more foundresses had significantly less female progeny than the 'maximum' collective clutch sizes (Wilcoxon signed

Table 4.2 Experiment 1: Offspring sex ratios of *E. baijnathi* from figs with different foundress numbers (total numbers of offspring from each fig in parentheses).

	Foundress number							
Fig No.	1	2	3	4	5	6	8	9
1	0.082	0.137	0.168	0.169	0.210	0.348	0.516	0.240
	(73)	(153)	(161)	(178)	(157)	(141)	(62)	(85)
2	0.106	0.219	0.270	0.133		0.170	0.250	0.260
	(66)	(151)	(152)	(181)		(106)	(68)	(70)
3	0.132	0.087	0.137	0.170		0.230		
	(68)	(173)	(73)	(165)		(70)		
4	0.079	0.176	0.250	0.213				
	(38)	(170)	(44)	(169)				
5	0.079	0.102	0.125	0.277				
	(86)	(127)	(72)	(66)				
6	0.177		0.130					
	(62)		(72)					
7			0.250					
			(42)					
Mean	0.109	0.144	0.190	0.192	0.210	0.249	0.383	0.250
SD	0.039	0.054	0.064	0.055	0.000	0.091	0.188	0.014
(Mean)	(65.50)	(154.80)	(88.00)	(151.80)	(157.00)	(105.67)	(65.00)	(77.50)
(SD)	(15.82)	(18.39)	(48.67)	(48.40)	(0.0)	(35.50)	(4.24)	(10.61)

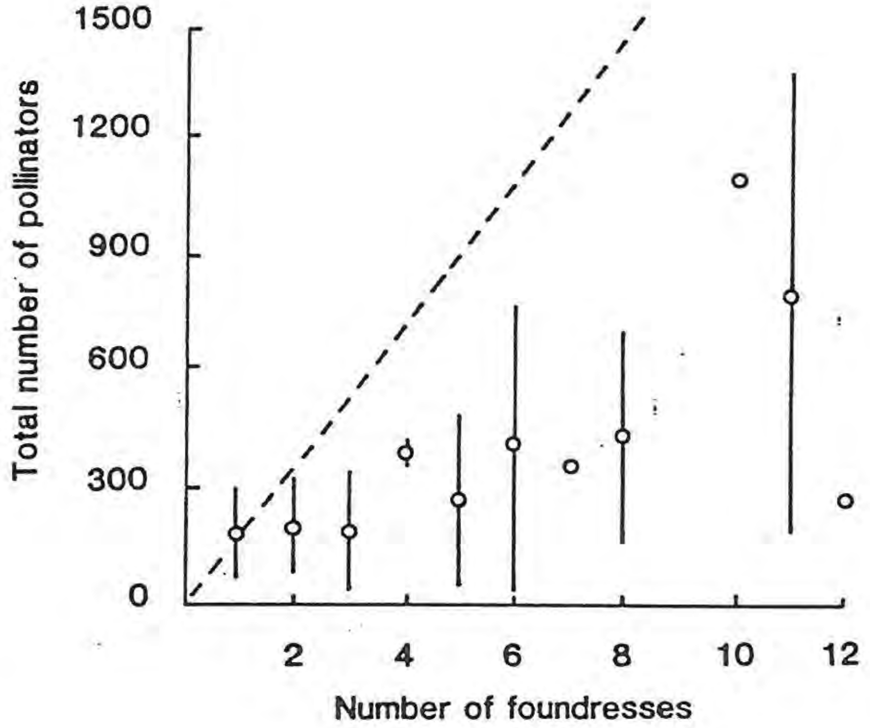

Figure 4.4 The effect of C. capensis foundress numbers on the total production of wasp progeny per fig (means + S.D.). The dotted line represents the cumulative number of offspring, assuming 187 eggs (the mean number of offspring produced by figs containing one foundress) are laid by all foundresses in each fig.

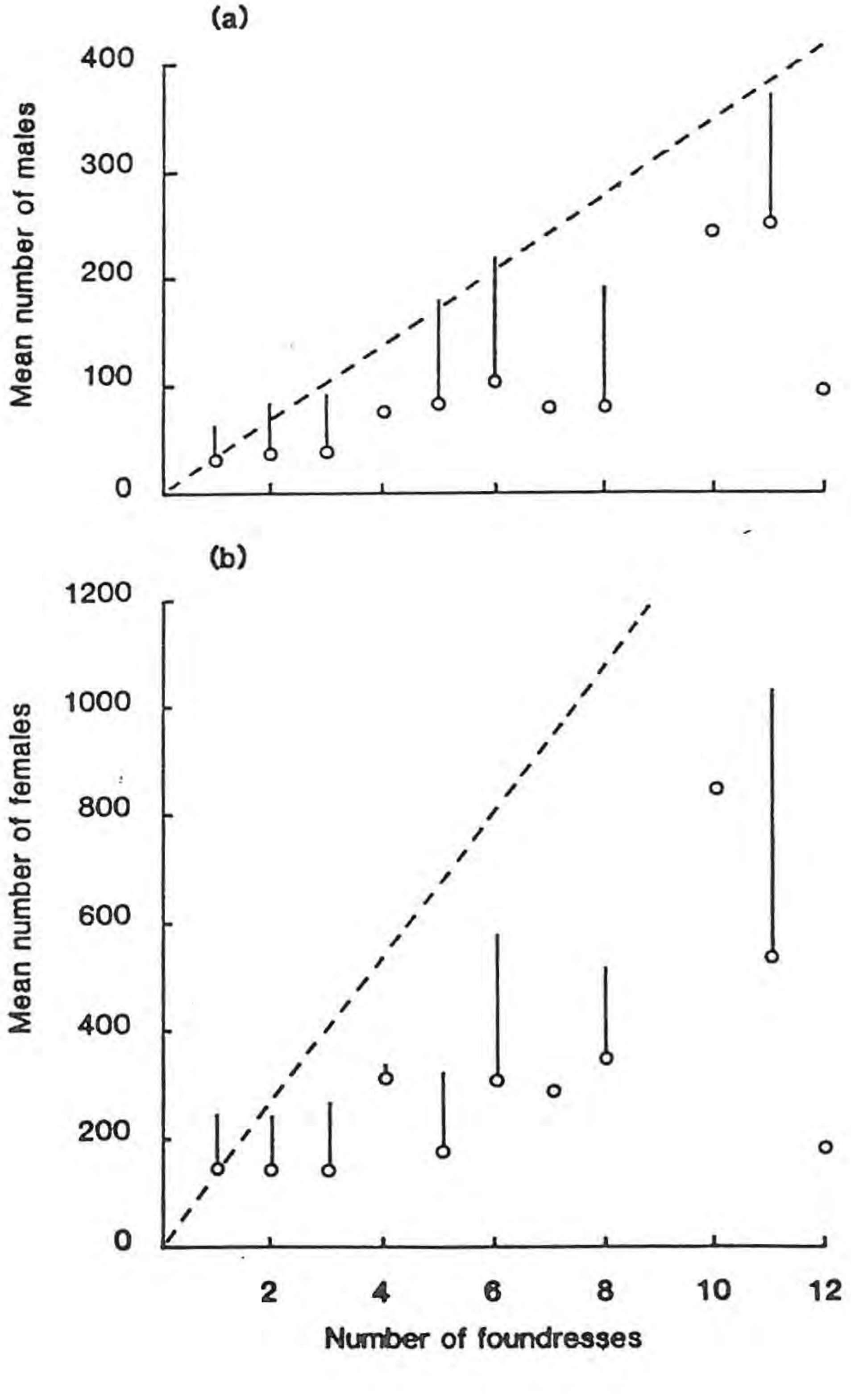

Figure 4.5 Male (a) and female (b) C. capensis progeny from figs which contained different numbers of foundresses (means + S.D.). The dotted lines represent the total number of males and females that would be produced assuming 17 male and 92 female eggs were laid.

four *C. capensis* foundresses, but despite this, the majority of flowers were not utilised. As in *F. burtt-davyi*, sex ratios became less female - biased at higher foundress numbers because fewer females were produced.

4.3.1.3 Is there sex ratio adjustment in *E. baijnathi* and *C. capensis* ?

i) *E. baijnathi*

The theoretical and the observed sex ratios of *E. baijnathi* are compared in Figure 4.6a. Since the sex ratio values often fall below 0.30 and consequently do not have a normal distribution, they were normalised by arcsine transformations for statistical analyses. Using the arcsine transformed data (all data points) the line of best fit was $y = 2.92 x^{0.24}$ ($r^2 = 40.2\%$, SE = 0.17, ANOVA, F = 11.427, DF = 1, $P < 0.004$). This confirms that wasps in figs with more foundresses produced less biased sex ratios.

Except at one foundress per fig, points from Herre's theoretical curve were not different from the observed values (Wilcoxon signed ranks tests, all $P > 0.05$), although at four foundresses the sex ratios appeared lower than the predicted curve, with a border-line significance of $P = 0.059$ (Figure 4.6a). Therefore, the data from this experiment supports the predictions of Herre's model. When one foundress is present inside a fig, theory predicts that there should be just enough sons to mate all their sisters. All figs which contained a single foundress produced some males, with a mean sex ratio of 0.11 (Table 4.2),

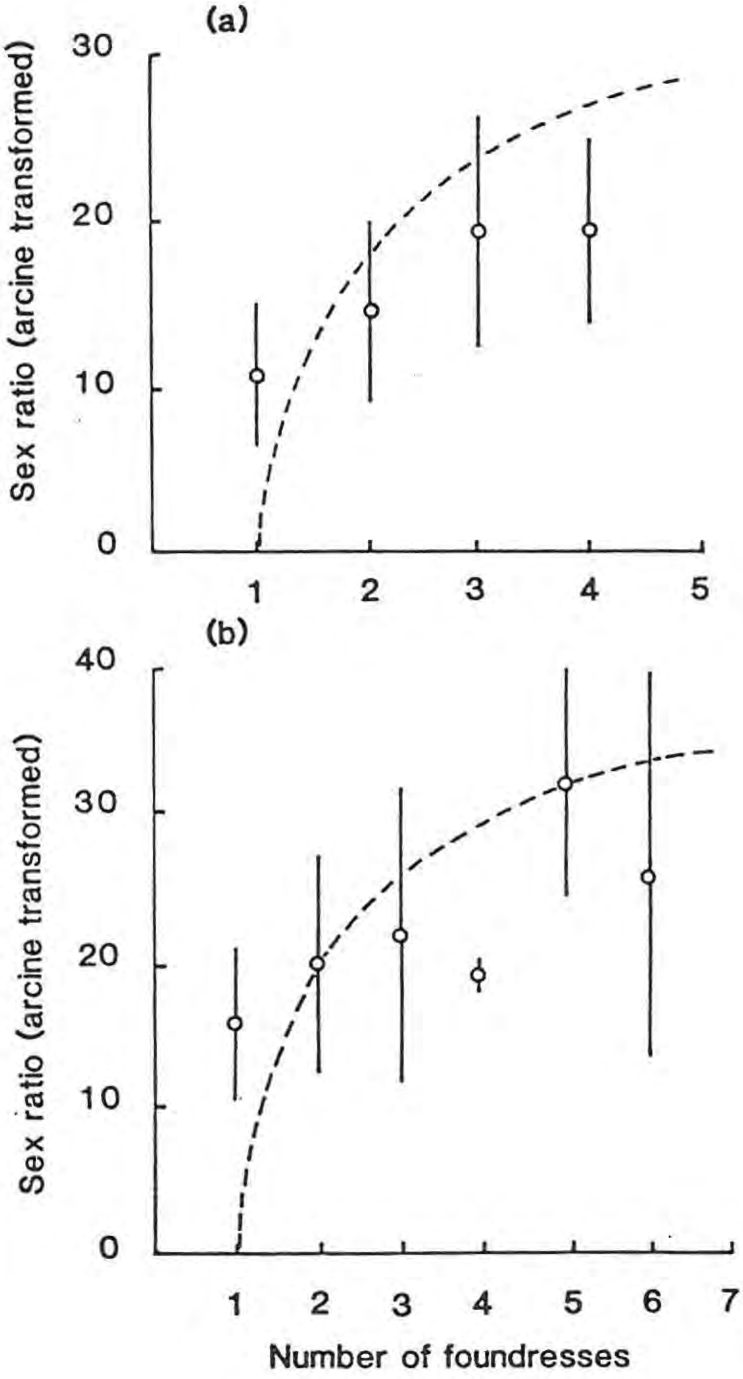

Figure 4.6 Sex ratio adjustment in a) E. baijnathi and b) C. capensis. Mean sex ratios (and standard deviations) from sets of figs which contained different foundress numbers. The dotted lines represent the theoretical sex ratio curves calculated from Herre's (1985) equation.

presumably enough to mate all the females.

ii) *C. capensis*

Herre's (1985) model predicted less biased sex ratios for *C. capensis* than *E. baijnathi*. The results of the experiment supported this prediction. The observed sex ratios generally conformed to the predictions of Herre's model, but like the offspring production numbers, the variances of these sex ratios were high (Table 4.6b). Arcsine transformed data gave a goodness of fit equation of $y = 3.15\ x^{0.13}$ (r^2 = 16%, SE = 0.23, ANOVA, F = 7.675, DF = 1, P = 0.008). The medians of the sex ratios were not significantly different from the theoretical curve (Wilcoxan - Sign Ranks - tests, all $P > 0.05$), again supporting the predictions of Herre's model. The sample size with four foundresses was too small for comparisons to be made.

4.3.1.4 Can sex-related style length utilisation by agaonid progeny provide an explanation for sex ratio adjustment ?

The relative numbers of male and female *E. baijnathi* progeny were found to differ according to the style lengths of the flowers they inhabited (Figure 4.7). A two way ANOVA showed that the mean style lengths differed both between the sexes (F = 76.7, DF = 1, $P < 0.001$) and between treatments (foundress numbers) (F = 20.4, DF = 3, $P < 0.001$). The interaction between mean style lengths of flowers inhabited by the sexes and those of the treatments was also significant (F = 5.46, DF = 3, P = 0.001). Overall, male *E. baijnathi* occupied shorter styled

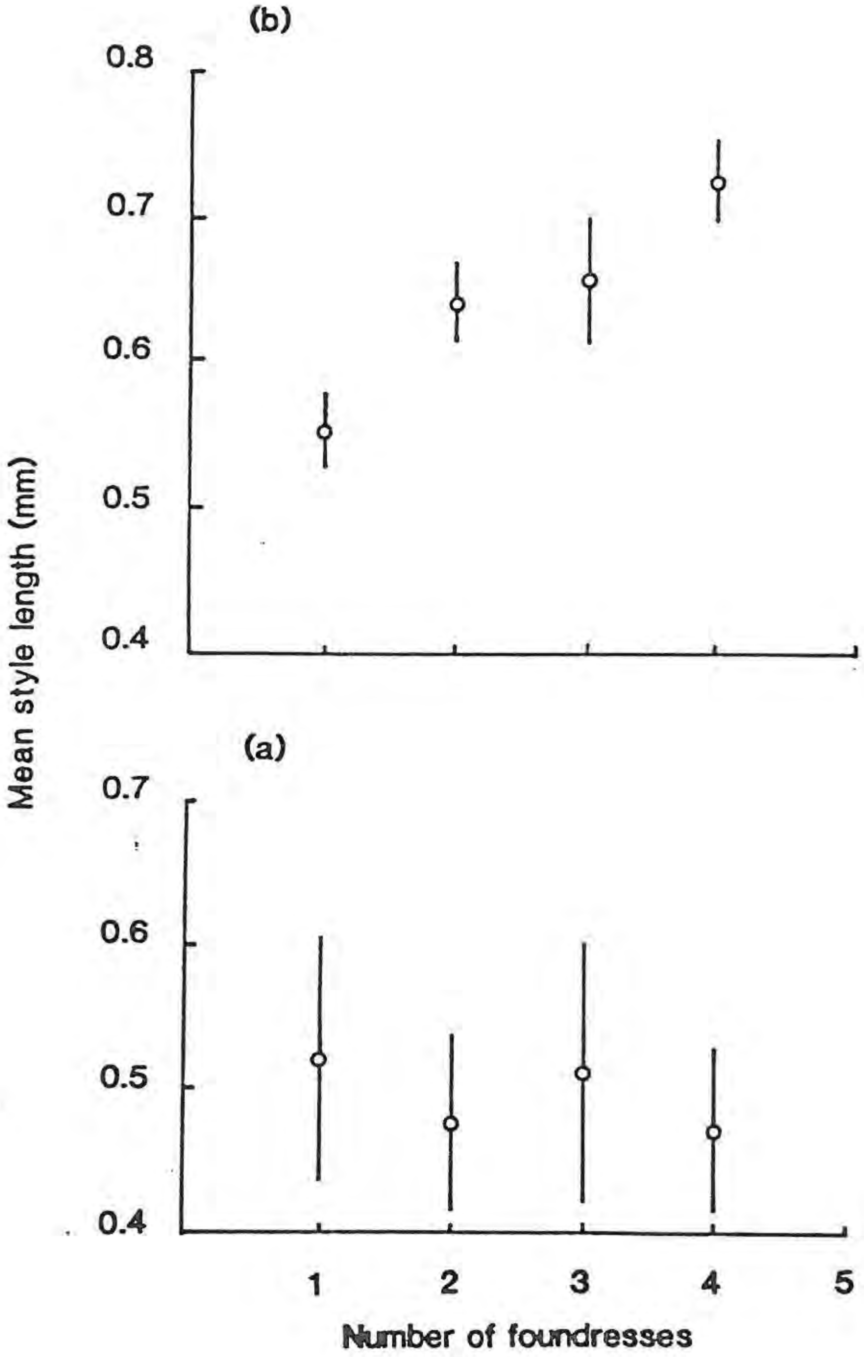

Figure 4.7 The style lengths of flowers containing a) male and b) female progeny in sets of figs containing different numbers of <u>E. baijnathi</u> foundresses (means + S.D.).

flowers (mean = 0.490mm, SD = 0.158, n = 130) than females (mean = 0.637mm, SD = 0.219, n = 718). This separation of sexes according to style lengths was only present in figs containing more than a single foundress (Figure 4.7). In figs containing one foundress the mean style length of flowers occupied by males did not differ from those occupied by female progeny (T - test, $P > 0.05$). Mean style lengths of flowers occupied by female progeny varied in relation to foundress number (one way ANOVA, F = 23.817, DF = 3, $P < 0.001$), while males occupied flowers with the same mean style lengths, regardless of the number of foundresses (one way ANOVA, F = 0.740, DF = 3, P = 0.53). With an increase in foundress number, more long styled flowers were utilised by female progeny, whereas male progeny remained limited to the shorter styled flowers.

Distributions of male and female *C. capensis* progeny inside figs of *F. sur* were found to follow almost exactly the same pattern as *E. baijnathi* in *F. burtt - davyi* (compare Figures 4.7 and 4.8). Overall, males occupied shorter styled flowers (mean = 1.423mm, SD = 0.302, n = 168) than females (mean = 1.635mm, SD = 0.322, n = 781) (two sampled T-test, t = -7.80, $P < 0.001$). Addition of extra foundresses caused a proportion of the female progeny to inhabit longer styled flowers. At least one pair of the means of style lengths occupied by female progeny were different between the treatments (one way ANOVA, F = 31.798, DF = 3, $P < 0.001$). This separation of sexes according to style lengths was only significantly different in figs with two and four foundresses, while the style lengths of male inhabited and female inhabited flowers from figs with one and three foundresses did not differ significantly (T - test, P

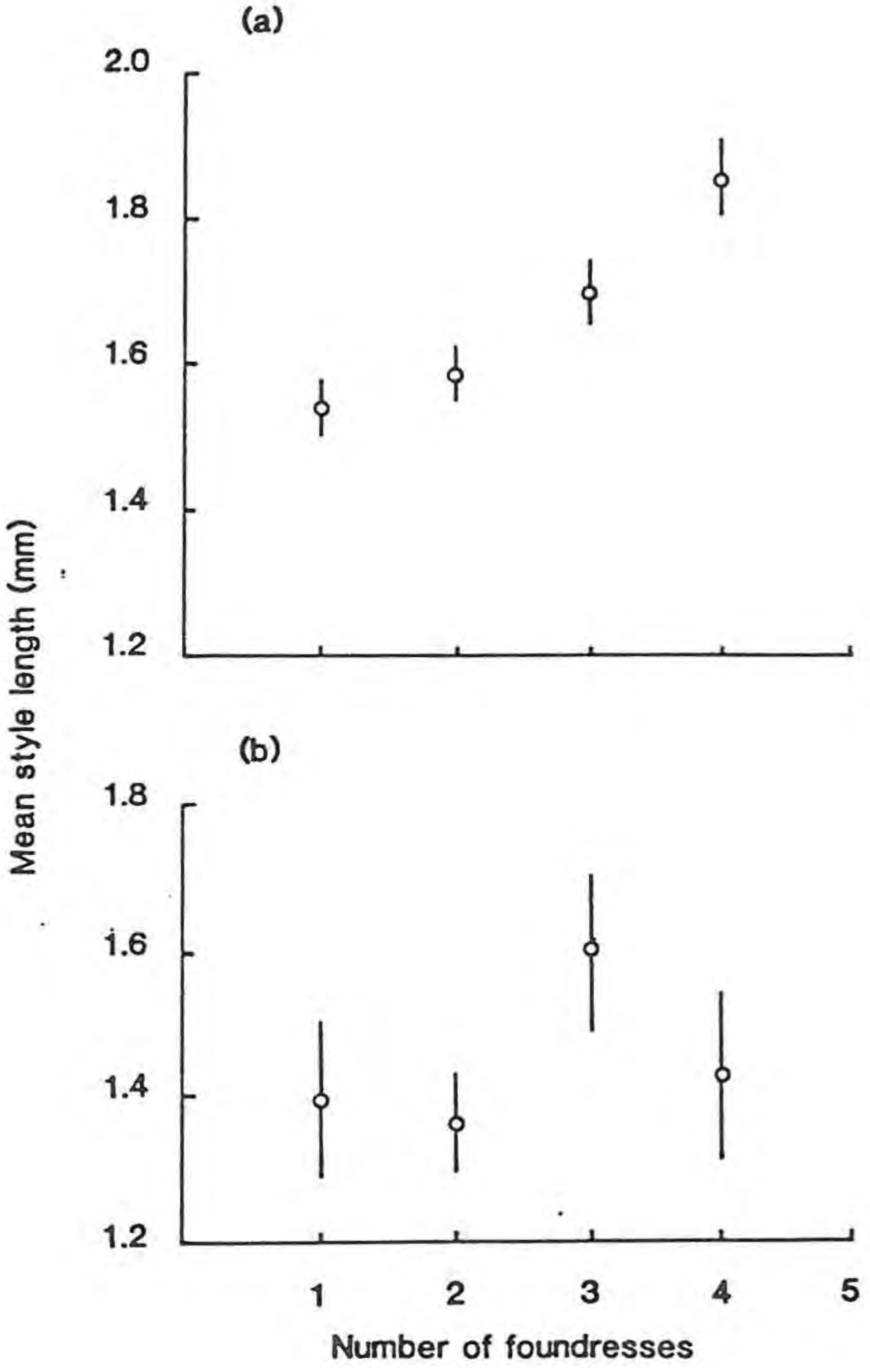

Figure 4.8 The style lengths of flowers containing a) male and b) female progeny in sets of figs containing different numbers of C. capensis foundresses (means + S.D.).

> 0.05).

4.3.2 Experiment 2 - Does an absence of pollination induce differential mortality of the sexes.

i) *F. burtt-davyi*

The effects of pollination of *F. burtt-davyi* on seed set, bladder and wasp production and sex ratios are given in Table 4.4. Unpollinated figs developed normally and did not abort. They were seedless, and contained mainly unpollinated flowers, but also some bladders and perfectly healthy *E. baijnathi* offspring. This confirms that the act of oviposition or the presence of *E. baijnathi* larvae *per se* prevents abortion, regardless of whether the flowers have been pollinated or not. Control figs produced a mean of 44.27 seeds, confirming that pollination had taken place. A few additional flowers were soft and infertile and presumably had not received any pollen.

There was a 30% reduction of the numbers of agaonid progeny from unpollinated figs (mean numbers of progeny = 43.0, range = 16 - 82, n = 12) compared with offspring numbers from pollinated figs (mean = 65.6, range = 62 - 73, n = 6) (T - test Statistic = -2.74, P = 0.015). Thus there were fewer progeny in figs with unpollinated flowers, although the single largest number recorded was from an unpollinated fig. The significantly larger numbers of bladders from unpollinated figs (T - test statistic = 4.01, P = 0.001) suggests that there was increased mortality of agaonids when pollination was prevented, rather than

Table 4.4 Experiment 2: The effects of pollen absence on seed, bladder and wasp production in *F. burtt-davyi*.

	Unpollinated figs					Pollinated figs				
Fig	Seeds	Infertile	Bladders	Wasps	Sex Ratio	Seeds	Infertile	Bladders	Wasps	Sex Ratio
1	0	0	16	53	0.075	55	12	15	73	0.082
2	0	145	52	28	0.107	20	15	15	66	0.106
3	0	91	17	82	0.110	40	22	15	68	0.132
4	0	159	38	30	0.067	25	30	22	38	0.079
5	0	136	37	59	0.051	65	25	6	86	0.070
6	0	115	50	37	0.081	60	40	10	62	0.177
7	0	108	42	46	0.087					
8	0	110	67	16	0.188					
9	0	117	46	38	0.053					
10	0	133	22	42	0.048					
11	0	144	45	42	0.095					
12	0	55	42	43	0.070					
Mean	0	119.36	39.50	43.00	0.086	44.27	24.00	13.67	65.60	0.108
SD	0	29.13	15.02	16.71	0.038	18.82	10.22	5.39	15.82	0.041

reduced oviposition.

The absence of pollen therefore prevents production of seeds, reduces the survival of wasp progeny inside the galled flowers and increases the frequency of bladders. However, there was no differential mortality of the sexes in unpollinated figs. In both the unpollinated and pollinated figs, the sex ratios were highly female biased and did not differ (Mann - Whitney Test, Z = 1.12, P = 0.26). The absence of pollination therefore did not influence the sex ratio of *E. baijnathi*, suggesting that male and female larvae are equally susceptible to death under conditions of reduced nutrient quality.

ii) *F. sur*

As expected, no seeds were produced in unpollinated figs of *F. sur* (Table 4.5). Between 50% and 67% of the flowers were infertile, depending on the number of foundresses which entered during oviposition. However, these differences between groups of figs containing different numbers of foundresses were not significant (Kruskal - Wallis one way ANOVA, test statistic = 2.73, P = 0.256).

There was no significant difference in bladder production between unpollinated figs and pollinated figs (Wilcoxon ranks paired test, Z = -1.35, P = 0.78), although this may be due to the small sample sizes. This test calculates the maximum distance between the cumulative distributions for the two samples, and tests whether the two samples come from the same distribution. Pollinated figs containing two

Table 4.5 Experiment 2: The effects of pollen absence on seed set, bladder and wasp production in F. sur figs with varying numbers of foundresses. In contrast to Table 4.1, proportions of flower types and wasp density are presented as a percentage of a subsample of 100 flowers.

	Number of foundress							
	1		2		3		4	
	mean	SD	mean	SD	mean	SD	mean	SD
Unpollinated figs	(n=4)		(n=3)		(n=3)		(n=3)	
Seeds (%)	0	-	0	-	0	-	0	-
Infertile (%)	60.75	9.40	51.33	15.31	67.00	8.00	57.67	7.51
Bladders (%)	24.50	9.68	29.00	8.89	18.00	6.24	22.00	7.81
Wasps (%)	14.75	10.05	19.20	3.02	15.00	2.65	20.00	10.82
Females	80.25	18.71	72.33	19.04	73.27	20.79	91.33	63.81
Males	17.25	11.70	30.00	8.54	16.00	6.08	20.33	5.69
Sex ratios	0.17	0.094	0.29	0.10	0.19	0.10	0.20	0.07
Pollinated figs	(n=1)		(n=4)		(n=2)			
Seeds (%)	61.00	-	43.50	21.81	4.50	2.12		
Infertile (%)	4.00	-	11.50	14.52	76.50	28.99		
Bladders (%)	9.00	-	17.00	15.58	13.50	19.09		
Wasps (%)	26.00	-	27.27	5.68	6.00	7.07		
Females	140.00	-	157.50	57.23	30.50	31.82		
Males	15.00	-	35.50	12.87	5.00	7.07		
Sex ratios	0.09	-	0.19	0.09	0.19	0.12		

foundresses (the only pairing where samples sizes were large enough for valid statistical analysis) produced marginally more progeny than unpollinated figs (Wilcoxon paired test, Z = 1.98, P = 0.04). Therefore it seems that in both *F. sur* and *F. burtt-davyi* pollination ensures improved survival of the pollinator larvae.

Sex ratios from unpollinated *F. sur* figs were not significantly different from sex ratios reared from pollinated figs (Wilcoxon Paired test, Z = -1.24, P = 0.22). This suggests that like *E. baijnathi*, both male and female *C. capensis* progeny are equally likely to suffer mortalities when developing in unpollinated flowers.

4.3.3 Experiment 3 - Does physical contact with other females cause sex ratio adjustment ?

Up to six ovipositor - amputated wasps were seen to enter a fig, but the exact number that reached the lumen could not be recorded because at the end of the experiment the remains of some of these wasps had decayed badly.

The sex ratios and total number of offspring from the figs with amputated wasps were compared with the sex ratios from 6 control figs, each with one foundress only (Table 4.6). The mean number of progeny produced from the control figs with one foundress (mean = 65.5, range = 38 - 86, n = 6) did not differ from the experimental figs (mean = 50.6, range = 14 - 95, n = 8) (T - test statistic = 1.36, P > 0.05). The mean

Table 4.6 Experiment 3: Offspring sex ratios of *E. baijnathi* from control figs containing one intact foundress and experimental figs containing one intact foundress and amputated foundresses.

	Fig									
	1	2	3	4	5	6	7	8	Mean	SD
Figs with one foundress (Control):										
Sex Ratios	0.082	0.106	0.132	0.079	0.079	0.177	-	-	0.109	0.039
Total numbers of offspring	73	66	68	38	86	62	-	-	65.5	15.82
One intact foundress plus amputees:										
Sex ratios	0.136	0.105	0.137	0.109	0.071	0.047	0.057	0.122	0.098	0.035
Total numbers of offspring	44	95	51	64	14	43	53	41	50.6	22.96

sex ratios of figs with a single foundress (0.109 $^{+}$ 0.039), also did not differ significantly from the 0.098 $^{+}$ 0.035 mean sex ratio from the experimental figs (Wilcoxon paired test, Z = -323, P = 0.747). The presence of additional foundresses therefore did not change the sex ratio, nor the number of offspring produced by a single foundress.

4.4 Discussion

The offspring sex ratios of *E. baijnathi* and *C. capensis* both conform to the predictions of Herre's (1985) model. Sex ratios in both these species were density dependent and became less female biased with an increase in foundress number. *E. baijnathi* was more inbred than *C. capensis* and, as predicted, this was reflected in a relatively more female-biased sex ratio for any given number of foundresses. Hence Herre's (1985) model which combines inbreeding and LMC, appears to generate more precise predictions than the simpler model of Hamilton (1967).

When using data of secondary sex ratios as an indication of the sex ratio laid by a female, it is usually assumed that mortality acts equally on both male and female progeny. At this stage, the determination of the primary sex ratio of fig wasps is problematic, although the potential exists to distinguish eggs or larvae by chromosome counts (Waage, 1986). Various techniques have been devised to permit the analysis of secondary sex ratios as primary sex ratios. For example, larval competition may be measured to allow for differential mortality (Werren, 1983). Alternatively, analysis of differential larval mortality can be used to estimate the primary sex ratio from the secondary sex ratio (Wellings *et al*, 1986).

The question arises as to whether larval mortality can effect the secondary sex ratio of agaonids. Death rates were found to be higher in unpollinated flowers, as shown previously in *C. arabicus* (Galil & Eisikowitch, 1971). That mortality rates are higher in unpollinated figs is to be expected, given that agaonid larvae are known to consume the endosperm, part of the seed that only develops if the flower has been pollinated (Verkerke, 1988a; 1988d). This constitutes a powerful selection pressure to maintain active pollination by ovipositing wasps. The experiments described in this chapter suggest however, in contrast to Galil & Eiskowitch's results, that differential mortalities did not occur. This therefore cannot explain the sex ratio adjustment seen in broods produced by different numbers of foundresses. The changes in secondary sex ratio in fig wasps should therefore reflect the primary sex ratio just after oviposition.

The third experiment on the proximal factors leading to sex ratio adjustment in *E. baijnathi*, showed that the physical presence of other females did not influence sex ratios. Direct contact therefore seems an unlikely explanation for sex ratio adjustment in this species. Pheromonal communication between the females also seems unlikely, although the possibility that foundresses 'cue' in to chemical markers left during oviposition cannot be ruled out.

The number of foundresses not only influences the sex ratios of agaonid progeny, but also seed set and the distribution of eggs in relation to flowers of differing style lengths. In terms of sex ratio adjustment, the most major influence of increasing foundress number seems to be in generating competition between the ovipositing females for oviposition sites. For every additional female entering a fig, the total number of eggs available for oviposition increases at a linear rate. Once the total complement of eggs among all the foundresses exceeds the number of accessible flowers, a proportion of eggs cannot be deposited. Fig wasps preferentially oviposit into shorter styled flowers, and as these become utilised, progressively longer styled flowers are inhabited (Chapter 3). It is clear that most male progeny of both *E. baijnathi* and *C. capensis* are found in shorter styled flowers, whereas females are found in both short as well as longer styled flowers. This indicates that fig wasps lay many or all of their male eggs first, followed by female eggs, as described in some other hymenopterous species (Waage, 1982a; 1982b; 1987; Waage & Ng, 1984; Strand, 1988). The first eggs are thus laid into shorter styled flowers and give rise to male progeny, while subsequent eggs, some of which are deposited into longer styled flowers, give rise to females.

The total egg complement of three *E. baijnathi* foundresses and four *C. capensis* foundresses exceeds the maximum number of accessible flowers of their respective fig species. In addition, even when there are enough oviposition sites (for example when only one foundress enters), oviposition becomes hindered as the styles wilt. This may be the reason that females prefer to oviposit in shorter styled flowers as it may allow them to maximise their rate of oviposition. Oviposition is necessarily rapid and competition between individual wasps for flowers is heightened by the short time period during which the flowers are flowers of differing style lengths. In terms of sex ratio adjustment, the most major influence of increasing foundress number seems to be in generating competition between the ovipositing females for oviposition sites. For every additional female entering a fig, the total number of eggs available for oviposition increases at a linear rate. Once the total complement of eggs among all the foundresses exceeds the number of accessible flowers, a proportion of eggs cannot be deposited. Fig wasps preferentially oviposit into shorter styled flowers, and as these become utilised, progressively longer styled flowers are inhabited (Chapter 3). It is clear that most male progeny of both *E. baijnathi* and *C. capensis* are found in shorter styled flowers, whereas females are found in both short as well as longer styled flowers. This indicates that fig wasps lay many or all of their male eggs first, followed by female eggs, as described in some other hymenopterous species (Waage, 1982a; 1982b; 1987; Waage & Ng, 1984; Strand, 1988). The first eggs are thus laid into shorter styled flowers and give rise to male progeny, while subsequent eggs, some of which are deposited into longer styled flowers, give rise to females.

The total egg complement of three *E. baijnathi* foundresses and four *C. capensis* foundresses exceeds the maximum number of accessible flowers of their respective fig species. In addition, even when there are enough oviposition sites (for example when only one foundress enters), oviposition becomes hindered as the styles wilt. This may be the reason that females prefer to oviposit in shorter styled flowers as it may allow them to maximise their rate of oviposition. Oviposition is necessarily rapid and competition between individual wasps for flowers is heightened by the short time period during which the flowers are experiments will help confirm whether agaonids produce male progeny first in an attempt to clarify how agaonid fig wasps regulate their sex ratios.

CHAPTER 5

Access to oviposition sites by non-pollinating fig wasps

5.1 Introduction

Numerous fig wasp species from several families of the superfamily Chalcidoidea may attack a single species of *Ficus* (Wiebes, 1977; Boucek *et al*, 1981). Normally a single agaonid wasp species is the pollinator, while the remainder are either phytophages feeding exclusively on the ovules / seeds (Ansari, 1966), or inquilines and parasitoids that develop inside flowers already containing the larvae of other fig wasps (Abdurahiman, 1980; Abdurahiman & Joseph, 1978a). Some studies indicate that certain fig wasps are parasitoids (Ulenberg, 1985; Waage, 1986), whereas the majority suggest that many fig wasps are cleptoparasitic, and feed on the galled tissue at the expense of the host larvae (Joseph, 1954; 1955; 1956; 1959a; 1984; Hill, 1967; Wiebes, 1977; Abdurahiman & Joseph, 1978a; 1978b; 1978c; Janzen, 1979b; Godfray, 1988). Godfray (1988) who studied fig wasps associated with *F. hispidioides* S. Moore found that *Philotrypesis* sp. indesc. and *Apocrypta mega* Ulenberg were parasitic on the agaonid, *Ceratesolen dentifer* Wiebes. and *Apocryptophagus* sp. indesc., a phytophage, respectively. Neves (1987) reported that an undescribed species of *Otitesella* galled the ovules of *F. microcarpa* L.f., despite the absence of the pollinator, confirming that it was a phytophage.

Most inquiline / parasitoid fig wasps are host tree specific, with each species attacking hosts in only one fig species, though a few exceptions exist (Boucek, 1988). Similarly, non - pollinating phytophage fig wasps are also generally host specific. Most fig wasps oviposit through the fig wall from the outside, while members of the Sycophaginae and Sycoecinae (Wiebes, 1979a) enter figs through the ostiole and insert their ovipositors down the styles, like their agaonid counterparts. An important difference in the biology of the sycophagines and sycoecines is that at least some species of the latter group are able to re-emerge from figs by forcing their way out back through the ostiole (Michaloud 1982; pers. observ.). This behaviour allows a single female sycoecine to infest a number of separate figs with her progeny. Thus, unlike species of the Agaonidae and Sycophaginae, they potentially have an unlimited supply of accessible flowers.

The fig wasps which oviposit through the walls of the figs all have relatively long ovipositors, although this is not always obvious externally. The ovipositors of *Otitesella* spp. for example, appear short because most of their length is coiled inside the gaster. Although the act of oviposition has been described for several groups of fig wasps (Abdurahiman & Joseph, 1978a; 1978b; 1979; Ulenberg, 1985; Wiebes, 1977), there have been no attempts to quantify oviposition site access by either the external phytophages or the inquilines / parasitoids.

Within each fig wasp community, each species of fig wasp lays its eggs into figs at characteristic stages of their development. Growth curves of *F. sur* (Baijnath & Ramcharun, 1983) and *F. burtt-davyi* (Baijnath & Ramcharun, 1988) figs show that the diameter increases in a non - linear manner as the fig cycle progresses. Fig growth means that at later stages the ovules are further from the outer surface of the fig (Nefdt,

1987) and therefore the fig wasps have to penetrate a greater distance to reach their oviposition sites. Fig wasps ovipositing at later stages of the fig's developmental cycle would therefore be expected to have evolved longer ovipositors.

Studies concerning other chalcid communities have shown that ovipositor lengths are adaptive (Brandl & Vidal, 1987) and may be important when considering competition between species (Heatwole & Davis, 1965) and the interactions between plants, their herbivores and parasitoids (Price & Clancy, 1986; Weis, 1982). This chapter examines the accessibilities of ovules (and the larvae they contain) to 17 non-pollinating fig wasps asssociated with six species of *Ficus*. Ovipositor lengths were compared with the distances required to reach their respective oviposition sites and the observed depths at which ovipositions took place were recorded. Furthermore, for each fig wasp species, the females' egg loads were examined in relation to the number of available oviposition sites.

5.2 Methods

5.2.1 Measurements of wasp ovipositors and the distances to ovules

Two species of sycophagines and five species of sycoecines were collected from the figs of six *Ficus* species at various localities in southern and central Africa (Table 5.1). The ovipositors and sheaths were measured using the same techniques as already described for the agaonids in chapter 3 and compared with the style lengths of their associated fig species. Style length data from the six fig species are the same as in chapter 3. Estimates of the proportion of flowers in

Table 5.1 Classification of African figs and the non - pollinating fig wasps that were investigated.

Fig species	Collected (figs)	Wasp species	Family / Subfamily	Oviposition site	Collected (wasps)	Collectors
F. burtt-davyi Hutch.	Grahamstown South Africa	Phagoblastus sp. 1 indesc.	Sycoecinae	Internal	Grahamstown South Africa	R.J.C. Nefdt
		Otitesella uluzi Compton	Pteromalidae	External	Grahamstown South Africa	R.J.C. Nefdt
		Otitesella sesquianellata van Noort	Pteromalidae	External	Grahamstown South Africa	R.J.C. Nefdt
		Sycoryctes sp. indesc.	Torymidae	External	Grahamstown South Africa	R.J.C. Nefdt
		Philotrypesis sp. indesc.	Torymidae	External	Grahamstown South Africa	R.J.C. Nefdt
F. lutea Vahl	Umhlanga Rocks South Africa	Phagoblastus sp. 2 indesc.	Sycoecinae	Internal	Umhlanga Rocks South Africa	S.G. Compton
F. thonningii Bl.	Grahamstown South Africa	Phagoblastus barbarus Grandi	Sycoecinae	Internal	Grahamstown South Africa	R.J.C. Nefdt
		Crossogaster odorans Wiebes	Sycoecinae	Internal	Lusaka Zambia	R.J.C. Nefdt
		Otitesella tsamvi Wiebes	Pteromalidae	External	Grahamstown South Africa	R.J.C. Nefdt
		Philotrypesis parca	Torymidae	External	Grahamstown South Africa	R.J.C. Nefdt

Table 5.1 continued

Fig species	Collected	Wasp species	Family / Subfamily	Oviposition site	Collected	Collectors
F. sycomorus L.	Gobabeb Namibia	Sycophaga sycomori L.	Sycophaginae	Internal	Lusaka Zambia	S.G. Compton R.J. Nefdt
F. sur Forsk.	Grahamstown South Africa	Sycophaga cyclostigma Grandi	Sycophaginae	Internal	Grahamstown South Africa	R.J.C. Nefdt
		Apocryptophagus sp. 1 Indesc.	Torymidae	External	Grahamstown South Africa	R.J.C. Nefdt
		Apocryptophagus sp. 2 Indesc.	Torymidae	External	Grahamstown South Africa	R.J.C. Nefdt
		Apocryptophagus sp. 2 Indesc.	Torymidae	External	Grahamstown South Africa	R.J.C. Nefdt
		Apocrypta longitarsus	Torymidae	External	Grahamstown South Africa	R.J.C.Nefdt
F. sansibarica Warb.	Kruger Park South Africa	Seres sp. indesc.	Sycoecinae	Internal	Kabompo Zambia	R.J.C. Nefdt S.G. Compton

which these wasps could reach the ovules was based on the number of flowers with styles less than or equal to their mean ovipositor lengths.

The accessibilities of flowers to fig wasps ovipositing from the outside were also examined. The ovipositors and sheaths of 20 individuals of ten species (Table 5.1) were measured under a dissecting microscope. Their mean ovipositor lengths were then related to the distances required to reach the ovules if they oviposited at stage D in the fig cycle (stage C in *F. sur*). 25 *F. burtt-davyi* phase D figs were collected from 11 trees and were cut in half. The distances from the fig's exterior to the equator of the ovules of 50 flowers in each fig were measured under a dissecting microscope. This was repeated with *F. thonningii* where 10 phase D figs were collected from four trees and with *F. sur* where four phase C figs from one *F. sur* tree were collected. The proportion of accessible flowers was estimated by calculating the number of flowers with distances to the outer fig surface that were either equal or shorter than the mean ovipositor length of each wasp species.

5.2.2 Flower utilisation

Estimated flower accessibility was related to ovary utilisation. Figs of *F. burtt - davyi*, *F. thonningii* and *F. sur* were collected just prior to the emergence of male wasps, a time when pupae are still inside their galls and are identifiable. The figs were preserved in 70% ethanol or were frozen prior to dissection. 53 figs from 11 *F. burtt-davyi* trees, 11 figs from four *F. sur* trees and ten figs from four *F. thonningii* trees were collected. Flowers from each fig were removed by scraping the inside surface of the peel with a scalpel. 50 flowers were then selected randomly from the flowers collected from each fig. The style of each flower was measured to the nearest 0.01 mm under a dissecting microscope. The 50 flowers were then divided into the following categories; 1) intact, healthy seeds distinguished by their white cotyledons and hard testas. 2) galls that were dissected open to reveal the species of wasp inside. 3) 'bladders' (Galil & Eisikowitch, 1971) that had the same external appearance as galls, but were empty and dry inside. 4) Infertile / unpollinated flowers that were smaller than other flower categories and lacked a hard testa. Overall the styles of 494 *F. sur*, 500 *F. thonningii* and 1730 *F. burtt-davyi* flowers were measured.

5.2.3 Egg production by non - pollinating fig wasps

The reproductive systems of 12 wasp species were examined by gently squashing up to 20 females individually in a drop of water between a slide and a glass coverslip. Under the compound microscope this revealed the reproductive systems (Copland & King, 1972; 1973). Just prior to squashing the insect, an estimate of body size was obtained by measuring the distance between the inner margins of the compound eyes. Once squashed, the total number of eggs was counted from each female.

To investigate the sites of oviposition by fig wasps ovipositing through the fig wall from the outside, wasps that were in the act of oviposition were killed by simply squashing them against the fig's surface. The paths followed by their ovipositors were then followed by carefully dissecting the fig wall, until the gall that had been penetrated could be identified.

5.3 Results

5.3.1 Wasps ovipositing down the styles of flowers

5.3.1.1 Sycophaginae

The ovipositor lengths of two species of Sycophaginae were measured. The mean ovipositor length of *S. sycomori*, from *F. sycomorus* was significantly longer than that of *C. arabicus*, the tree's pollinator (Figure 5.1) (T - test statistic = -7.72, $P < 0.001$). The same pattern was present in the wasps from *F. sur*, where the ovipositor of *S. cyclostigma* was significantly longer than that of the associated pollinator *C. capensis* (Figure 5.1). The sheath : ovipositor length ratios of the sycophagines were larger than those of the pollinators, with that of *S. sycomori* being 0.76 and *S. cyclostigma* being 0.69 (Table 5.2). Based on the proportion of styles which were shorter or equal in length to the mean ovipositor lengths of the sycophagines, it was estimated that 100% and 77% of the flowers were accessible to *S.sycomori* and *S. cyclostigma* respectively.

The mean number of eggs from 20 individuals of *S. cyclostigma* was 124 (Table 5.3). Since an estimated 1253 flowers are accessible in each fig, there are enough oviposition sites to accommodate up to 10 foundresses. In contrast, only four individuals of the associated pollinator, *C. capensis*, which has a relatively shorter ovipositor, can be accommodated in a single fig (Chapter 4).

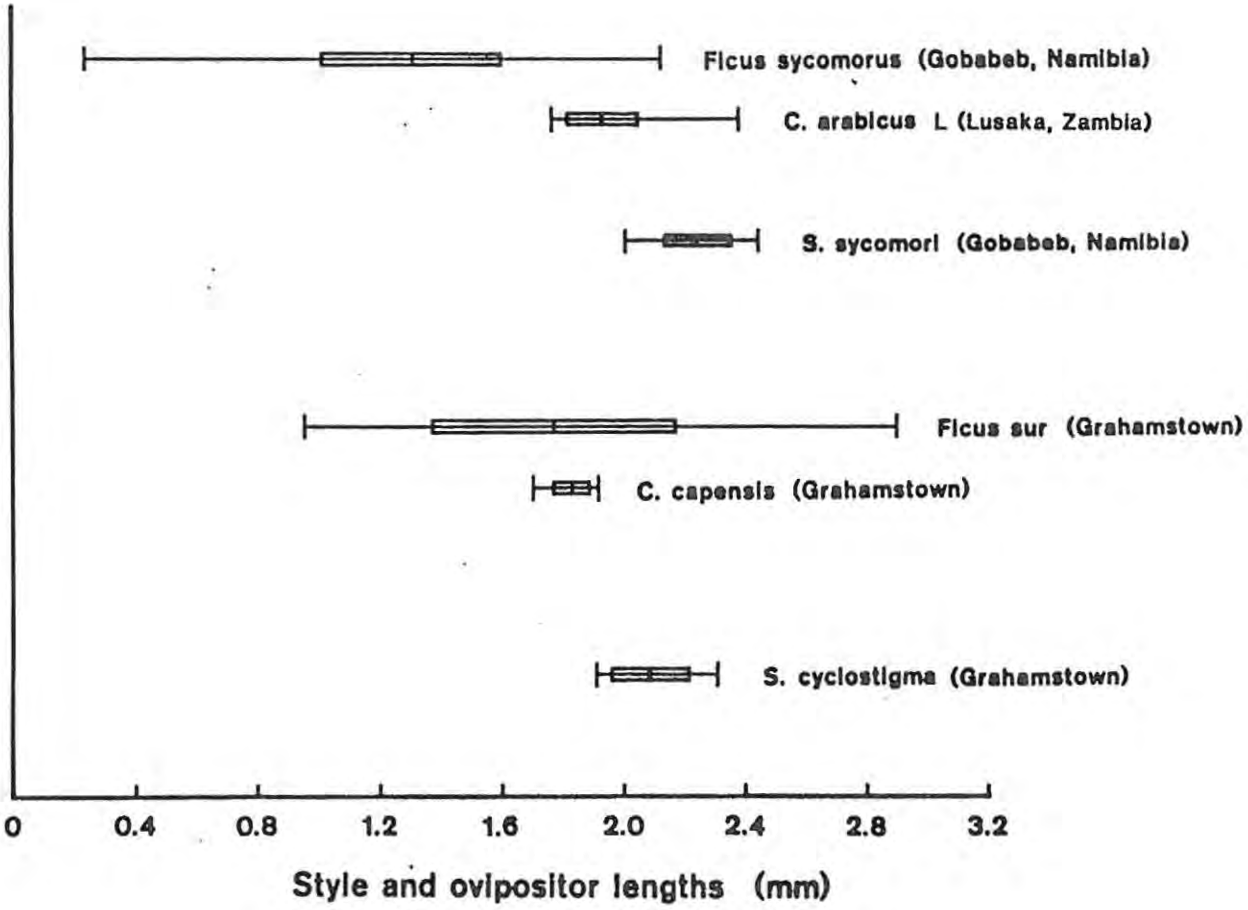

Figure 5.1 Comparisons between style lengths of F. sycomorus and F. sur and ovipositor lengths of the associated pollinators and sycophagines. Means standard deviations and ranges are indicated.

Table 5.2 Ovipositor, sheath and style lengths, and the estimated flowers accessible to two sycophagines and five sycoecines from southern Africa.

Sycophagine or sycoecine	Ovipositor length (mean + SD) (mm)		Sheath length (mean + SD) (mm)		Ratio (sheath/ovipositor)	Style length (mean + SD) (mm)		Estimated accessible flowers (%)
Sycophaga sycomori	2.26	0.13	1.72	0.10	0.76	1.33	0.30	100
Sycophaga cyclostigma	2.10	0.13	1.45	0.07	0.69	1.79	0.40	77
Phagoblastus sp. 1 indesc.	0.84	0.04	0.27	0.02	0.32	0.80	0.23	58
Phagoblastus sp. 2 indesc.	1.10	0.03	0.23	0.02	0.21	1.09	0.30	55
Phagoblastus barbarus	0.94	0.04	0.25	0.02	0.27	1.10	0.36	37
Crossogaster odorans	1.02	0.08	0.29	0.01	0.28	1.10	0.36	45
Seres sp. indesc.	1.84	0.10	-	-	-	2.13	0.44	30

Table 5.3 Body sizes (measured as the inter-ocular distance), clutch sizes and the number of flowers accessible to three internally - ovipositing fig wasps. n = 20 individuals for each species.

Fig wasp	Inter-ocular distance (mm)		Number of eggs		Number of accessible flowers
	mean	range	mean	range	mean
Sycophaga cyclostigma	0.36	0.35-0.38	124	96-158	1253
Phagoblastus barbarus	0.32	0.27-0.34	58	42-72	70
Phagoblastus sp. 1	0.32	0.30-0.33	88	59-121	166

5.3.1.2 Sycoecinae

The five species of sycoecines had ovipositor lengths that were between 30% to 45% shorter than the ovipositor lengths of the pollinators sharing the same fig species (Figure 5.2). The sheaths of this subfamily are relatively short since most of the ovipositor remains retracted within the gaster. The sheath:ovipositor ratios ranged from 0.21 to 0.32, depending on the species (Table 5.2).

It was estimated that between 30% and 58% of the flowers were accessible. Mean ovipositor lengths of the 5 species of sycoecines were positively correlated with the mean style lengths of their respective fig species (Figures 5.2 & 5.3, $r^2 = 69\%$, $P < 0.001$). A comparison of this regression with that of the agaonids shows that they have similar slopes, but the intercept (corresponding to the mean ovipositor length) was 60% less than that of the agaonid regression.

Figure 5.4 shows the frequency distributions of the ovipositor and style lengths of *Phagoblastus barbarus* and its associated fig, *F. thonningii*. These are compared with the actual utilisation of flowers of varying style lengths. Some flowers with styles greater than the maximum recorded ovipositor length were found to contain *P. barbarus* pupae, indicating that some wasps have longer ovipositors than those measured in this study. Nevertheless, flowers with styles longer than 1.3mm never contained any *P. barbarus* confirming that the longer styled flowers are out of reach. Like the pollinators, *P. barbarus* preferentially oviposits into shorter styled flowers.

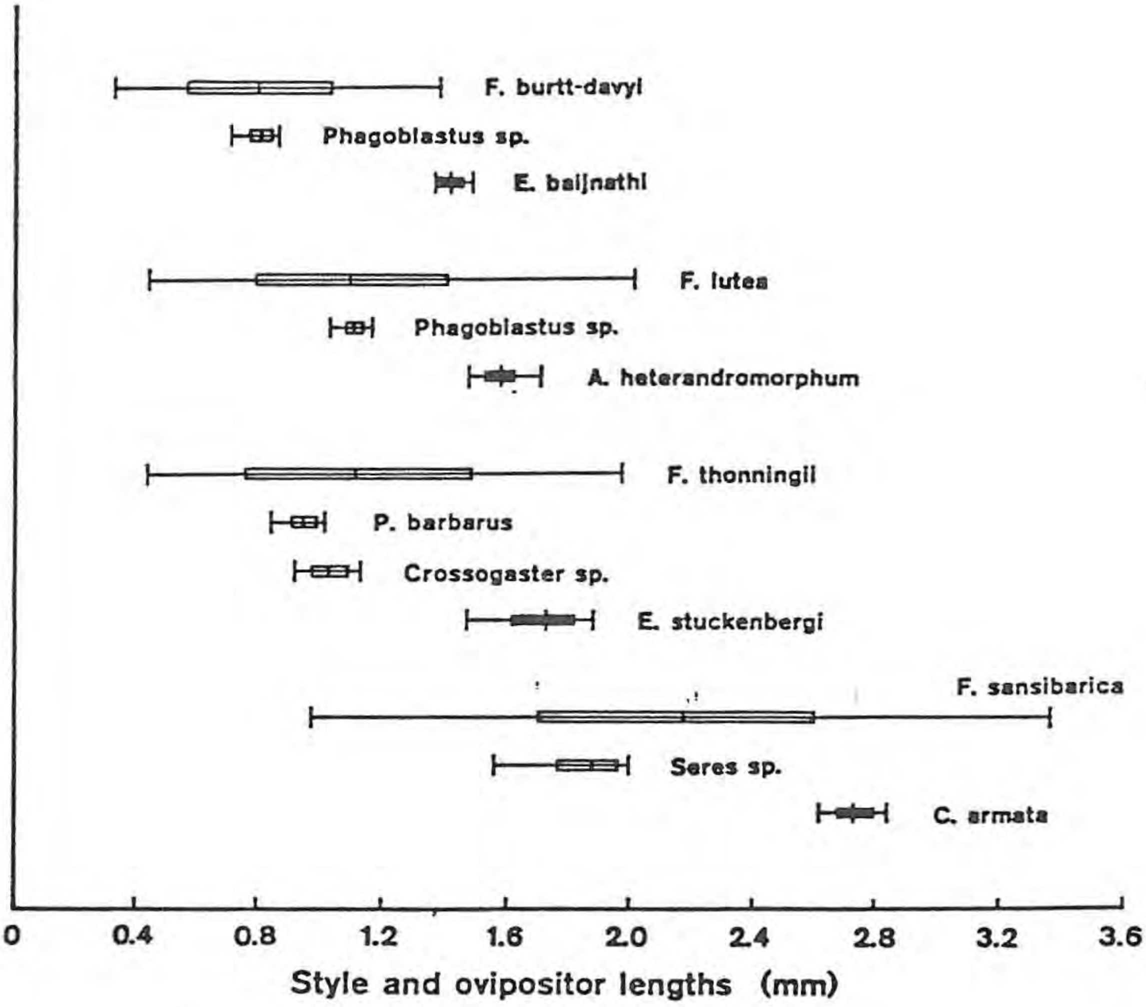

Figure 5.2 Comparisons between style lengths from four fig species and the ovipositors of the associated pollinators and sycoecines. Means, standard deviations and ranges are indicated.

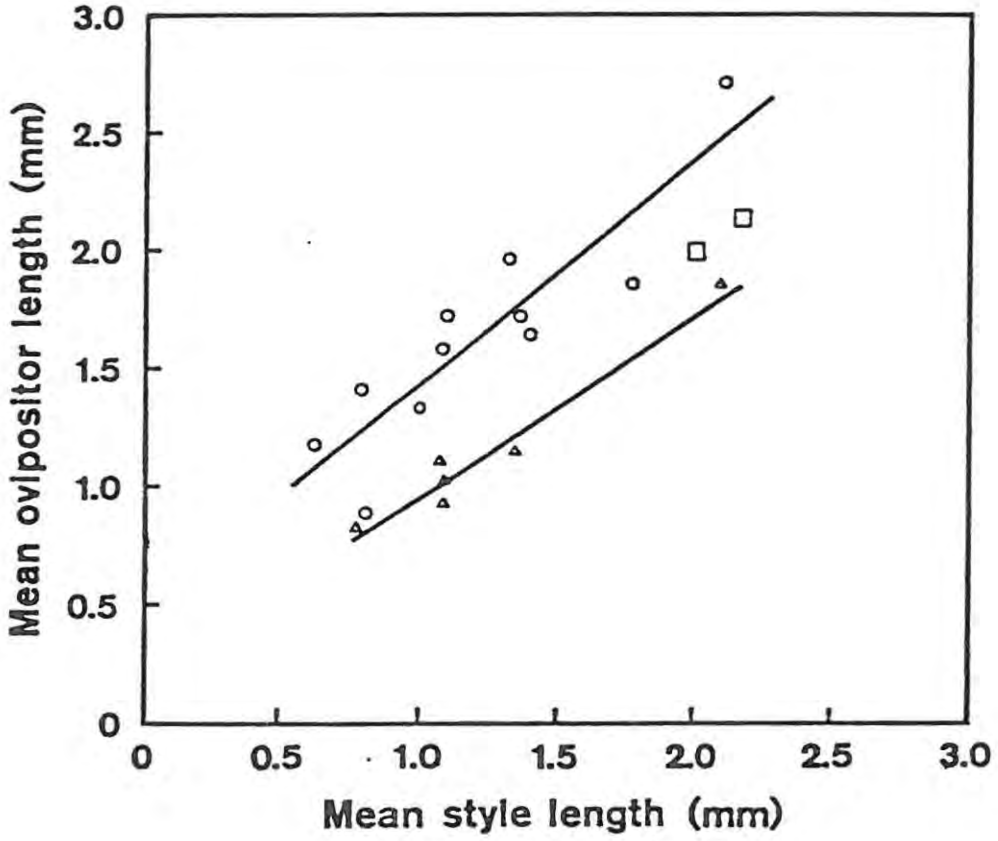

Figure 5.3 The relationships between mean style lengths of various *Ficus* species and the ovipositor lengths of their associated agaonids (circles), sycoecines (triangles) and sycophagines (squares).

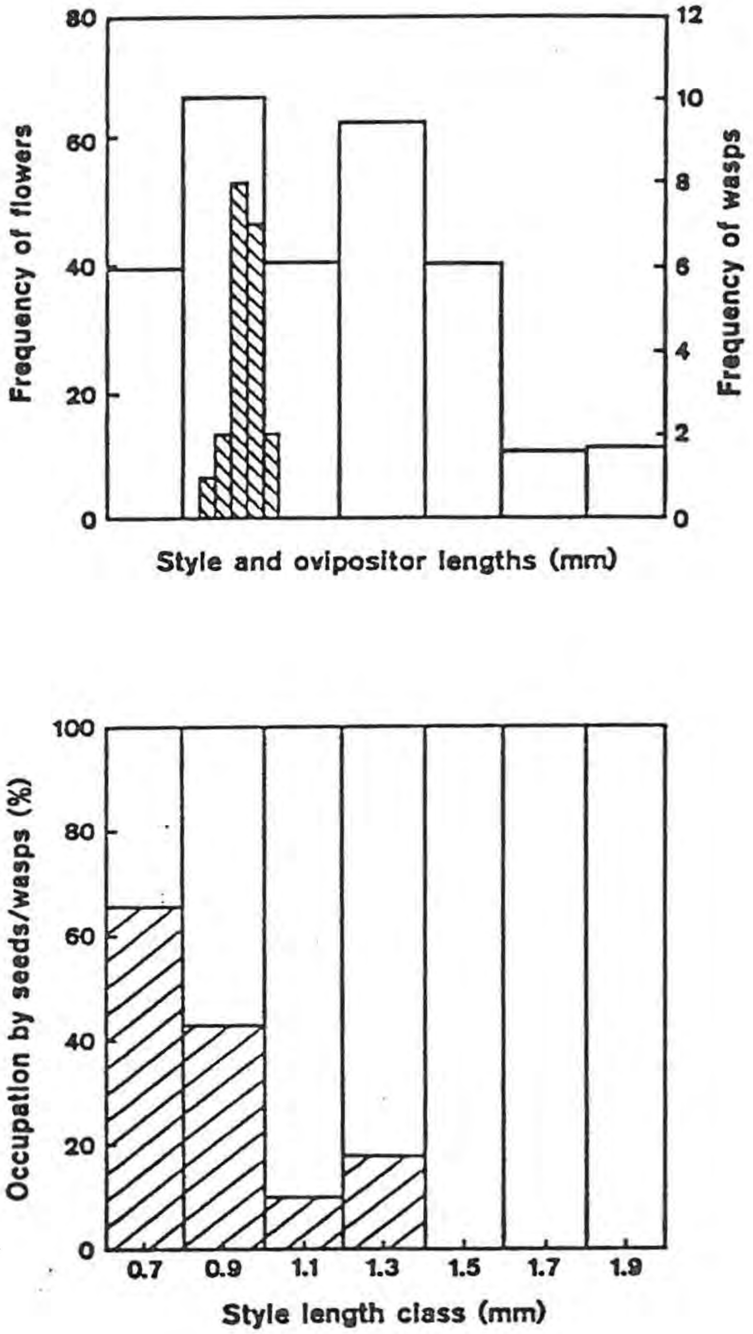

Figure 5.4 The relationship between style and ovipositor lengths as a predictor of actual utilisation of *F. thonningii* flowers by *P. barbarus* progeny. The histogram (top) compares style (open bars) and ovipositor (lined bars) length frequency distributions. The lower histogram shows the proportion of flowers that were found to contain *P. barbarus* progeny from phase D figs.

The mean number of eggs recorded from 20 individuals of *P. barbarus* was 58, and 37% of the flowers in figs from *F. thonningii* were estimated to be accessible, which would allow two females to lay all their eggs in one fig. *Phagoblastus* sp. from *F. burtt-davyi* had a mean clutch size of 88 eggs (Table 5.3). The number of accessible flowers was estimated to be 166. Thus, on average, two foundresses of this species would be unable to lay all their eggs into the same fig.

5.3.2 Wasps which oviposit through the fig wall from the outside

5.3.2.1 Wasps associated with F. burtt-davyi

The two species of *Otitesella* utilising the ovules of *F. burtt-davyi* had shorter ovipositors than the other wasps ovipositing from the outside (Figure 5.5 & Table 5.4). The mean ovipositor length of *O. uluzi*, which oviposits earlier in the fig's developmental cycle (Dallas, pers. comm.; pers. observ.), was shorter than that of *O. sesquianellata* (T - test statistic = -2.49, n = 20, $P < 0.05$). It was estimated that *O. uluzi* would have access to only 10% of the flowers inside a fig at phase D, representing 29 flowers and *O. sesquianellata* 15%, representing 43 flowers per fig out of a mean total of 286 flowers per fig (n = 37, range = 193 - 363) (Compton & Nefdt, 1989). However, these wasps oviposit much earlier in the fig's cycle, when the ovules are closer to the exterior. This explains why progeny of *Otitesella* spp. were recorded in all style length classes (Figure 5.6a). Members of this genus therefore actually have the ability to oviposit into all the flowers in *F. burtt-davyi* and are not limited to any particular style length

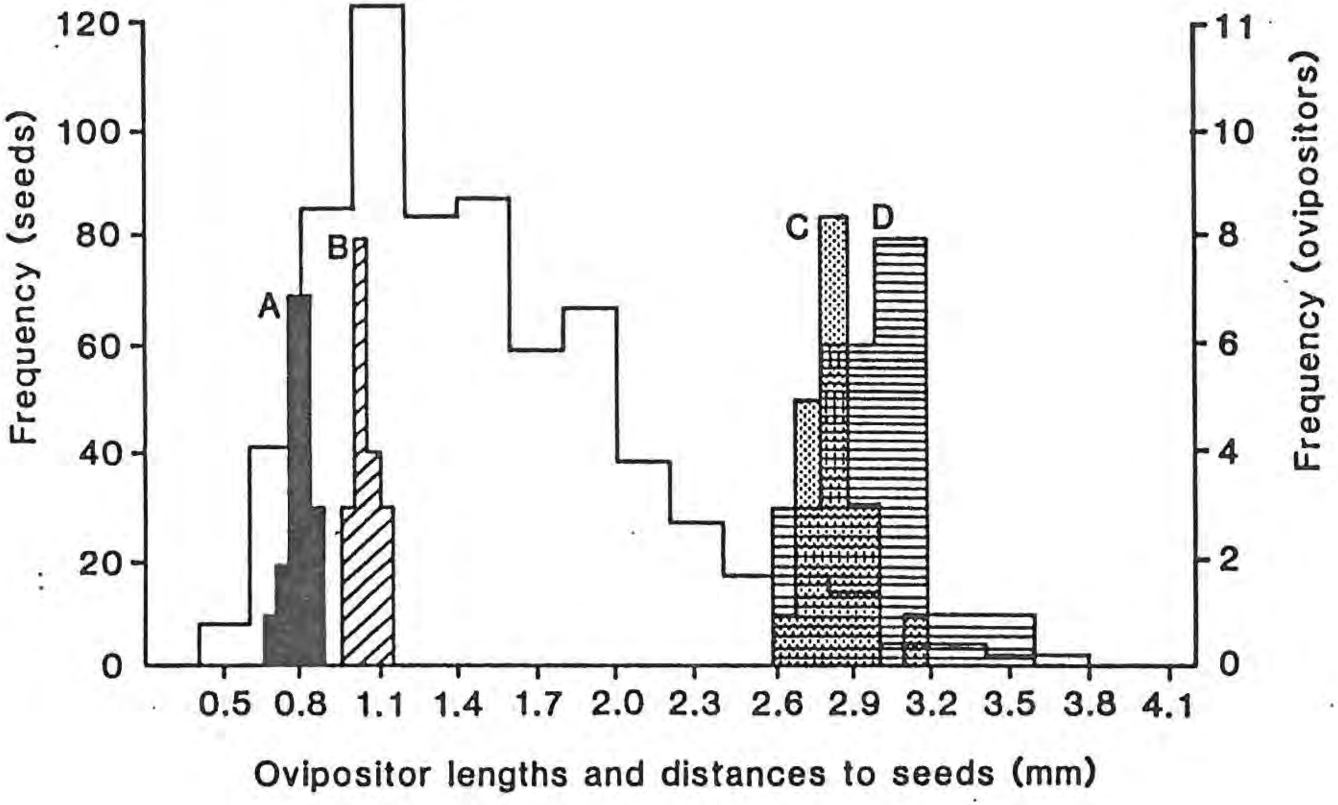

Figure 5.5 Frequency distributions of the ovipositor lengths of four wasps ovipositing through the walls of *F. burtt-davyi* figs from the exterior. The open histogram is the frequency distribution of the distances between the outer surfaces of phase C figs to the equators of the flowers. A: *Otitesella uluzi* B: *O. sesquianellata* C: *Sycoryctes* sp. D: *Philotrypesis* sp.

Table 5.4 Body sizes (measured as the inter-ocular distance), ovipositor lengths, numbers of eggs and the estimated accessibilities of 11 non - pollinating fig wasps. Estimated numbers accessible are those which would be accessible if the wasps oviposited late in the fig's cycle (phase C or D).

Host trees and wasp	Sample size	Inter-ocular distance (mm)		Ovipositor length (mm)		Number of eggs		Estimated accessible flowers
	n	mean	range	mean	range	mean	range	(%)
F. burtt-davyi								
Otitesella uluzi	9	0.32	0.30-0.35	0.79	0.69-0.87	91	65-119	10
Otitesella sesquianellata	4	0.21	0.19-0.23	0.86	0.79-0.93	15	8-23	15
Philotrypesis sp. indesc.	10	0.21	0.18-0.25	3.28	2.69-4.62	25	15-36	100
Sycoryctes sp. indesc.	10	0.22	0.20-0.25	2.92	2.62-3.47	41	34-51	100
F. thonningii								
Otitesella tsamvi	12	0.32	0.28-0.36	1.30	1.12-1.43	65	40-108	8
Sycoryctes sp. indesc.	10	0.26	0.24-0.30	3.19	3.03-3.33	50	45-58	100
F. sur								
Apocryptophagus sp. 1 indesc.	20	0.47	0.43-0.50	3.97	3.11-4.39	300	210-360	100
Apocryptophagus sp. 2 indesc.	10	0.40	0.33-0.49	6.98	5.82-8.30	131	37-420	100
Apocryptophagus sp. 3 indesc.	2	0.37	0.34-0.40	2.19	2.15-2.23	52	25-80	?
Apocrypta longitarsus	10	0.28	0.25-0.32	5.99	4.64-7.21	20	8-40	100

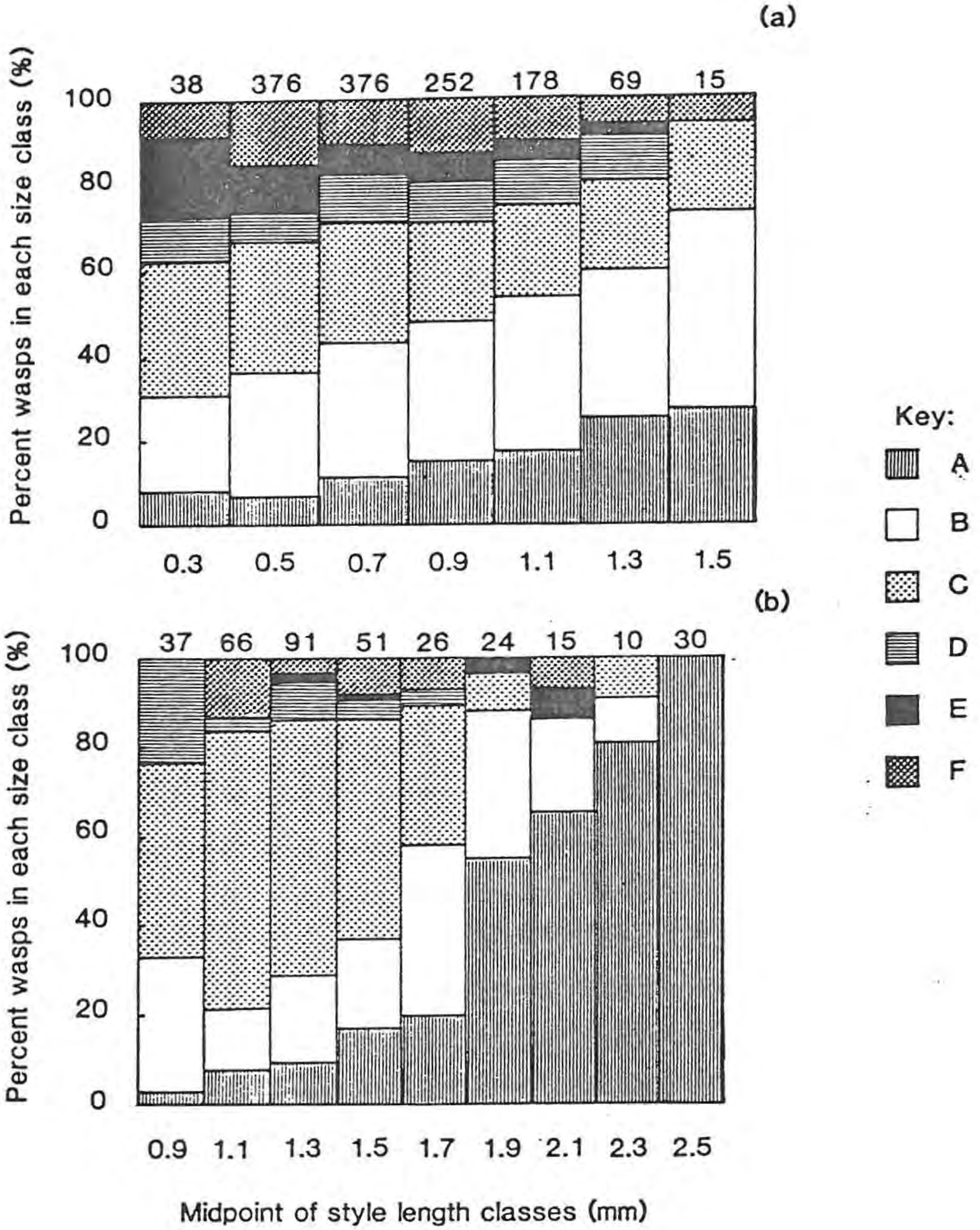

Figure 5.6 The observed utilisation of flowers in a) <u>F. burtt-davyi</u> and b) <u>F. sur</u> in relation to style lengths. For <u>F. burtt-davyi</u>: A - seeds, B = bladders, C = <u>E. baijnathi</u>, D = <u>Otitesella</u> spp., E = <u>Sycoryctes</u> sp., F = <u>Philotrypesis</u> sp. For <u>F. sur</u>: a = seeds, b = bladders, c = <u>C. capensis</u>, d = <u>S. cyclostigma</u>, f = <u>A. longitarsus</u>, e = infertile flowers. Numbers in brackets refer to sample size in each size class.

category. Individuals of *O. uluzi* on average contained 91 eggs and nine trophamaria (immature eggs), while its congener had a mean of 15 eggs and five trophamaria.

The remaining wasps associated with *F. burtt-davyi* had much longer ovipositors (Figure 5.5). There was no significant difference between the mean ovipositor lengths of *Philotrypesis* sp. and *Sycoryctes* sp. (Table 5.4 & Figure 5.5) (n = 20, t = -1.39, P > 0.05). Their ovipositors were longer than the maximum distance required to reach any of the flowers and therefore it was estimated that they potentially have access to all the ovules. The progeny of both species were found in almost all the style length categories (E & F in Figure 5.6a). There were proportionately more agaonid progeny in the shorter styled flowers, leaving the longer styled flowers to produce seeds (A & C in Figure 5.6a). The style length classes of flowers utilised by *E. baijnathi* did not differ with those used by *Philotrypesis* sp. (Chi square = 2.69, DF = 5, P = 0.80) and *Sycoryctes* sp. (Chi square = 5.63, DF = 5, P = 0.34). The bladders (B in Figure 5.6a), which in part may result from probings by parasitoids killing hosts, were found in a wide range of style length classes and their distribution was not significantly different to that of *E. baijnathi* (Chi square = 9.19, DF = 5, P = 0.10). Thus there was no refuge from parasitoids for agaonids developing in ovules furthest from the outside of the figs.

Dissections of figs with wasps that were killed while ovipositing, also revealed that the two inquilines can deposit eggs in flowers at any depths (Figure 5.7) they choose. Seven individuals of *Philotrypesis* sp.

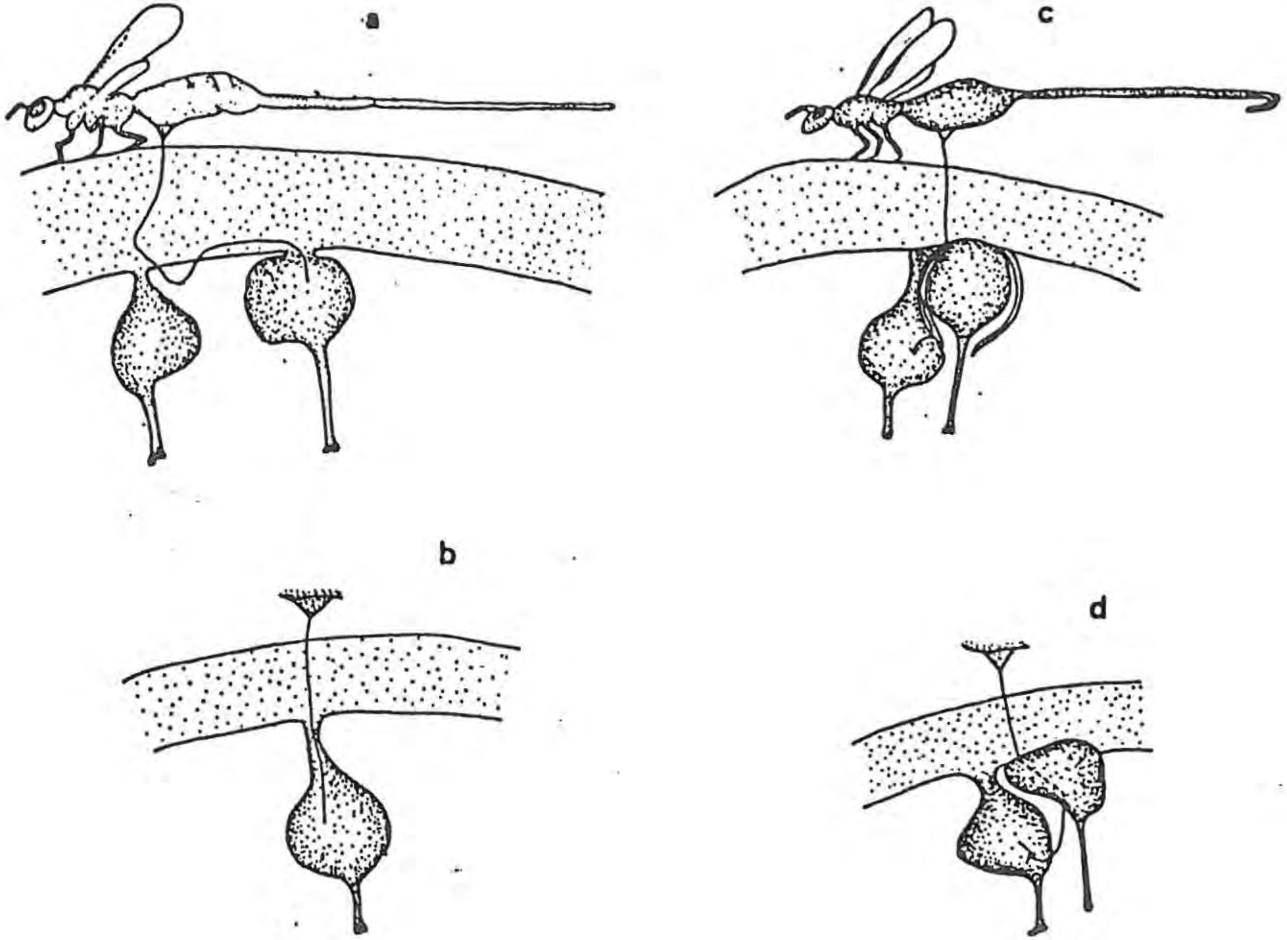

Figure 5.7 Examples of the paths taken by ovipositors of <u>Philotrypesis</u> sp. (a & b) and Sycoryctes sp. (c & d) while probing into figs of <u>F. burtt-davyi</u>.

were observed. The ovipositors of two penetrated the fig wall obliquely, and then travelled parallel to the outer fig surface before entering a seed (Figure 5.7). Other routes included looping back into the fig wall and then entering the flower via the pedicel (Figure 5.7a) and straight forward direct entry (Figure 5.7b).

On five out of ten occasions, the ovipositor of *Sycoryctes* sp. travelled within the lamina of a floral bract before bending ninety degrees and penetrating an adjacent seed (Figure 5.7c). One individual was found to insert its ovipositor right through a seed before entering another (Figure 5.7d).

5.3.2.2 Wasps associated with F. thonningii

I recorded the body sizes, ovipositor lengths and numbers of eggs per wasp in two fig wasps which oviposit from the exterior of *F. thonningii* figs (Table 5.4). The ovipositors of *O. tsamvi* were at least 34% longer than the congenerics from *F. burtt-davyi*. Egg counts from 12 individuals gave a mean of 65 eggs and 5 trophamaria. *Sycoryctes* sp. also had a longer ovipositor than its congener from *F. burtt-davyi* and was estimated to be able reach 100% of the *F. thonningii* ovules. On average individuals of this species carried 50 eggs. The utilisation of ovules by these species was not examined.

5.3.2.3 Wasps associated with F. sur

A. longitarsus had a mean ovipositor length of 5.99mm, while the maximum distance between the fig surface and the ovules it attacks was 3.80 mm (Table 5.4). Therefore the ovipositor of *A. largitarsus* was estimated to be long enough to reach all the ovules in *F. sur* (Figure 5.8). This prediction was tested by dissecting figs at phase D containing *A. longitarsus* progeny. Figure 5.6b shows that this parasitoid can lay eggs in nearly all the style length classes, though no progeny were recorded in the shortest and longest style length classes. However, their absence from short and long styled flowers may be due to the low sample size. 188 flowers contained either *S. cyclostigma* or *C. capensis*, while 20 were attacked by *A. longitarsus*, a parasitism rate of 11%. Individuals of this species had relatively few eggs, with a mean of 20 per female. On average there were 10 trophamaria at different stages of development, confirming that new eggs are continuously produced even after oviposition has been initiated. Other externally ovipositing species were not present in the sample.

Two of the species of *Apocryptophagus* had mean ovipositor lengths longer than the maximum distance between the fig's exterior and the ovules (Table 5.4), while the third, with the shortest ovipositor, was estimated to reach a smaller proportion of the flowers. Within the three species of *Apocryptophagus* the number of eggs was variable, with *Apocryptophagus* sp. 3 containing a mean of 52 eggs and *Apocryptophagus* sp. 1 containing a mean of 300 eggs. The mean ovipositor lengths of all four species were significantly different between one another (T -tests, all $P < 0.001$).

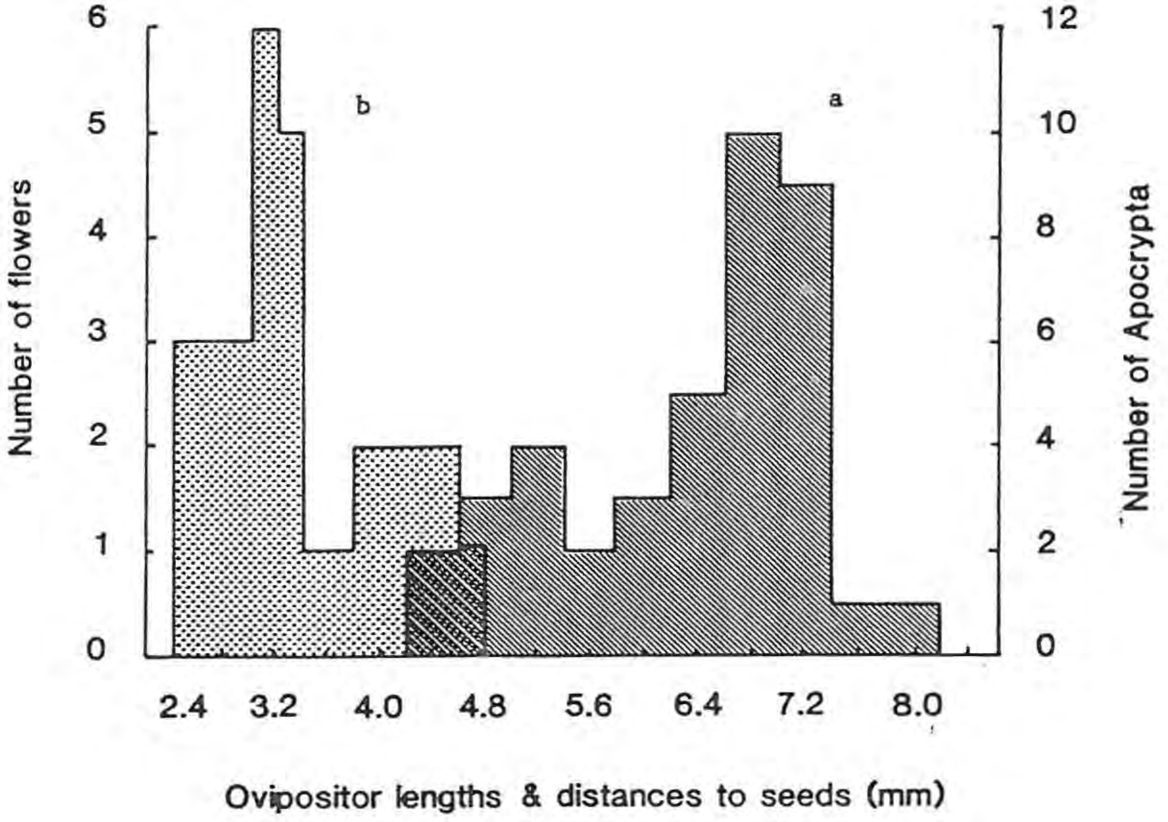

Figure 5.8 Frequency distributions of the ovipositor lengths (a) of Apocrypta longitarsus, which oviposits through the wall of F. sur figs from the exterior. Histogram (b) is the frequency distribution of the distances between the outer surfaces of phase C figs to the equators of the flowers.

5.3.3 Body size and egg numbers

There were positive correlations between body sizes and egg numbers per female from five of the nine pro - ovigenic fig wasps species that were examined (Table 5.5). In contrast, there were no significant relationships among the five syn - ovigenic species.

5.3.4 Oviposition in relation to the host fig's developmental cycle

Figure 5.9 shows the times at which different species of wasps were seen to probe into figs of *F. burtt - davyi* and *F. sur*. The thickness of the line gives a subjective estimate of the density of wasps that were observed probing into the figs. *O. uluzi* probed during phase A, before pollination, while *O. sesquianellata* was most active in the early parts of phase C, after pollination. *E. baijnathi* and *Phagoblastus* sp. were only seen during phase B, when the ostioles were open. The largest concentrations of *Philotrypesis* sp. were usually present between one and seven days pollination, but a few individuals were also seen probing towards the end of pase C. Roughly equal numbers of *Sycoryctes* sp. were observed probing throughout most of phase C.

Apocryptophagus spp. (the different species were not separated) oviposited into phase A figs of *F. sur*. This is clearly evident in figs collected at phase B, where *Apocryptophagus* spp. progeny are found in large galls that sometimes even occlude the lumen. *C. capensis* and *S. cyclostigma* both appear during phase B and enter the ostioles. *A.*

Table 5.5 Correlations between female body size (estimated from inter - ocular distances), and number of eggs inside 14 different species of fig wasps.

Species	Ovigenesis	Degrees of freedom	r^2 (%)	Significance
Pollinators				
E. baijnathi	Pro - ovigenic	18	64	$P<0.001$
E. stuckenbergi	Pro - ovigenic	18	-	NS
C. capensis	Pro - ovigenic	18	31	$P<0.01$
P. soraria	Pro - ovigenic	5	-	NS
Internal Phytophages				
Phagoblastus sp.1	Pro - ovigenic	5	-	NS
P. barbarus	Pro - ovigenic	18	53	$P<0.01$
S. cyclostigma	Pro - ovigenic	18	-	NS
External Phytophages				
O. uluzi	syn - ovigenic	5	-	NS
Apocryptophagus sp.1	syn - ovigenic	18	52	$P<0.01$
Apocryptophagus sp.2	syn - ovigenic	7	73	$P<0.01$
Inquilines				
Sycoryctes sp.				
(F. burtt-davyi)	syn - ovigenic	14	-	NS
(F. thonningii)	syn - ovigenic	8	-	NS
Philotrypesis sp.				
(F. burtt-davyi)	syn - ovigenic	6	-	NS
A. longitarsus	syn - ovigenic	7	-	NS

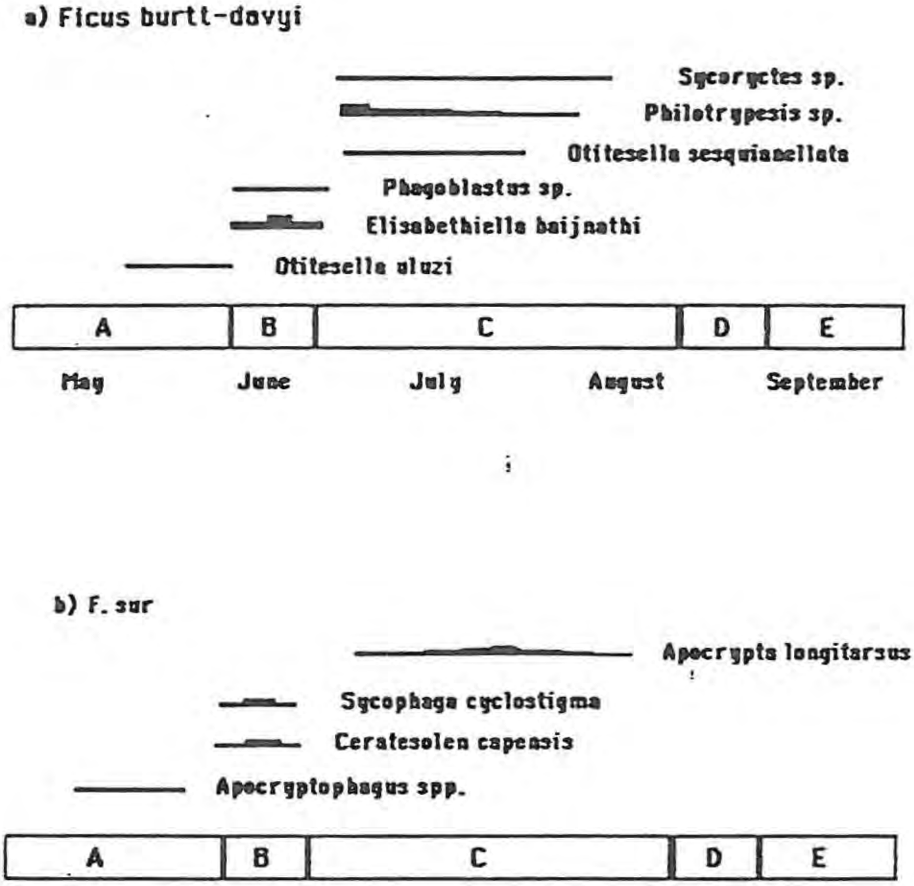

Figure 5.9 The times at which fig wasps probe into the figs in relation to the fig's cycle of a) F. burtt-davyi and b) F. sur trees. The thickness of the lines is a subjective estimate of the density of wasps probing.

longitarsus, the only parasitoid studied here, was observed to oviposit throughout phase C, when its hosts were available. On one tree noticeably higher concentrations of *A. longitarsus* were seen during the middle of phase C, but this seems to vary between trees and is influenced by changes in ambient temperatures.

Table 5.6 compares the ovipositor lengths of externally ovipositing wasps with the diameter of their associated figs at different stages of their development (Baijnath & Ramcharun, 1983; 1988). It was found that the species of wasps from *F. burtt-davyi* possessing longer ovipositors probed at later stages of the fig's development. *O. uluzi* which had the shortest ovipositor, probed at the earliest stage, before pollination. Its congener, *O. sesquianellata* had a slightly longer ovipositor and oviposited later during phase C. The two inquilines, *Philotrypesis* sp. and *Sycoryctes* sp. are obliged to oviposit during phase C when hosts are available. They had even longer ovipositors.

The ovipositor lengths of the four wasp species ovipositing into *F. sur*, were not related to the stage at which they probe (Table 5.6). All three species of *Apocryptophagus* probe at phase A, but each have ovipositors of different lengths. *A. longitarsus* was the only species found ovipositing at phase C. It had an ovipositor longer than two species of *Apocryptophagus*, but was shorter than *Apocryptophagus* sp. 2.

Table 5.6 The ovipositor lengths of fig wasps in relation to when they oviposit. Fig diameters at each of the developmental stages are from Baijnath & Ramcharun (1983; 1988).

Species	Developmental stage of fig	Fig diameter (mm)	Mean ovipositor length (mm)
F. burtt-davyi			
O. uluzi	A	4-7	0.79
O. sesquianellata	Early C	7-9	0.86
Philotrypesis sp.	Early C	7-9	3.28
Sycoryctes sp.	Mid C	9-10	2.92
F. sur			
Apocryptophagus sp. 1	A	5-12	3.91
Apocryptophagus sp. 2	A	5-12	6.98
Apocryptophagus sp. 3	A	5-12	2.19
A. longitarsus	C	14-23	5.99

5.4 Discussion

Various studies have shown that the length and structure of insect ovipositors are adapted to the particular habitat in which they lay their eggs (Heatwole & Davis, 1965; Price, 1972; Achterberg, 1986; Fergusson, 1988). In chapter 3 it was shown that agaonid species have ovipositors which are adapted to the style lengths of their respective fig species. Species of figs with flowers having long styles have agaonids with long ovipositors, enabling the wasps to reach enough ovules to lay their eggs. It is likely that the ovipositor lengths of *Sycophaginae* follow the same patterns, but insufficient species were available to confirm this. Both *S. sycomori* and *S. cyclostigma* have high sheath : ovipositor ratios, with the sheath enveloping almost the entire ovipositor. Thus, sheath measurements of sycophagines more accurately reflect the true ovipositor length than they do in the Agaonidae or Sycoecinae.

Once inside a fig, both agaonids and sycophagines are unable to leave, and so are obliged to lay their total clutch into the flowers of a single fig. One would expect then that in both groups natural selection would favour individuals with ovipositors that are at least long enough to reach enough flowers to offload all their eggs. Unlike their respective agaonids, the two sycophagines had ovipositors which were long enough to reach almost all of the flowers of their associated figs. The method of oviposition between agaonids and sycophagines differs (Galil *et al*, 1970; pers. observ.). Sycophagines oviposit by inserting the tip of their ovipositor into the style and then move backwards slowly, forcing the ovipositor towards the ovule. Agaonids on the other hand, bring their ovipositor under their gaster and insert it down the style in a vertical plane. This method used by agaonids may not require as long an ovipositor as the sycophagines would need to penetrate the same distance.

The sycoecines had ovipositors shorter even than those of their respective pollinators. This may be related to the ability of females to infest more than one fig. *Phagoblastus* from *F. natalensis* have been reported to force their way back out of a fig after depositing some eggs down some of the styles (Michaloud, 1987). This behaviour was also observed in *Phagoblastus* sp. from *F. burtt-davyi* figs and *P. barbarus* from *F. thonningii* (van Noort, pers. comm.). Because sycoecines can spread their eggs among many figs, only a few ovules in each fig need be accessible, but the actual number of potential oviposition sites in a single fig exceeds the number of eggs produced by a single wasp. Hence lone foundress sycoecines do not need longer ovipositors to offload all their eggs. Shorter ovipositors may be beneficial to sycoecines because longer ovipositors may hinder their re -emergence from figs and entry into other figs. Also by ovipositing into a number of different figs, sycoecines may avoid competing with agaonids for oviposition sites.

Observed utilisation patterns showed that female *P. barbarus*, like the agaonids studied, oviposited preferentially into shorter styled flowers. As the styles of flowers they use approach the length of the ovipositor, fewer flowers contained *P. barbarus* pupae. This again suggests that fig wasps probing down styles preferentially oviposit into shorter styled flowers, probably because the resistance when probing down shorter styled flowers is less.

By spreading their clutches among several figs, there may not be any competition between conspecific sycoecine foundresses for oviposition sites. However, they may still compete for oviposition sites with other genera of fig wasps, including the pollinators and the secondary sycophiles that oviposit from the outside of the figs. Some figs of *F thonningii* were found to contain the remains of many pollinators, as well as *P. barbarus*. The majority of short styled flowers were soft and contained neither seeds nor wasp progeny, while longer styled flowers were inhabited mainly by agaonid pupae. This suggests that *P. barbarus* does not recognise the flowers containing agaonid eggs or larvae, and many short styled flowers received an egg from each species. Under these circumstances the larvae of both species appear to die.

Price (1970) concluded that differences in ovipositor lengths may be a factor allowing coexistence among ichneumonid parasitoids of *Neodiprion swainei* Midd., a sawfly. Heatwole & Davis (1965) earlier showed that species of *Megarhyssa* (Hymenoptera: Ichneumonidae) are largely segregated ecologically in that they parasitise different segments of the host population, *Tremex columba* (Hymenoptera: Siricidae) as a result of possessing ovipositors of different lengths. Although some of the externally ovipositing fig wasps sharing a fig species had ovipositors that were different in lengths, it seems unlikely that this has evolved to reduce competition for oviposition sites because actual utilisation showed that the progeny were not segregated spatially. The ovipositors lengths of *Sycoryctes* sp. and *Philotrypesis* sp. were similar and allowed females to attack pollinator larvae in flowers of all style lengths.

Ovipositor lengths in any case were a poor indicator of potential utilisation because in both *Philotrypesis* sp. and *Sycoryctes* sp. the paths of the ovipositors were twisted as they penetrated the figs. Progeny of these two species were also found in the same style length classes as those of *O. uluzi* and *O. sesquianellata* and these species are occasionally parasitised (Compton, pers. comm.). Hence there was no apparent spatial segregation of oviposition sites by the four fig wasps ovipositing into figs of *F. burtt-davyi* from the outside.

The ovipositor lengths of the four externally ovipositing wasp species from *F. sur* were all different and potentially could result in segregation of oviposition sites if those with the largest ovipositors avoided the more peripheral ovules. Data on utilisation by *Apocryptophagus* spp. was not collected, but the progeny of *A. longitarsus* were located in ovules regardless of the distance required to reach them from the outside. Hence, oviposition sites of wasps ovipositing into *F. sur* are also unlikely to be segregated spatially.

In *F. burtt-davyi* the two species of *Otitesella* which had short ovipositors oviposited at an earlier stage than *Philotrypesis* sp. and *Sycoryctes* sp. which have much longer ovipositors. However, there appeared to be no such pattern with the wasps from *F. sur*, where ovipositor length was independent of the time of oviposition. Despite only a slightly longer ovipositor than *O. uluzi*, *O. sesquianellata* probed into figs at phase C, a much later stage of the fig's cycle. However, because the progeny from all the wasps ovipositing into *F. burrt-davyi* were found in all style lengths classes, it is unlikely that there is ecological isolation due to ovipositing at different times of the fig's developmental cycle.

Most *Philotrypesis* sp. and *Sycoryctes* sp. progeny were found in the shorter styled flowers. These results confirm that both *Philotrypesis* sp and *Sycoryctes* sp. can oviposit into any of the ovules inside a fig, independent of style length. The same applied to *A. longitarsus* in *F. sur*. As the majority of agaonid hosts are also in the shorter styled flowers, this suggests that the inquilines are ovipositing into areas of greater host density. Agaonid larvae are therefore equally susceptible to attack in flowers of all style lengths and there is no selective advantage for them to avoid flowers that are closer to the fig's exterior. It can thus be concluded that in the fig wasp communities investigated here, inquilines and parasitoids do not constitute a selection pressure acting on ovipositor lengths of the agaonids and the results are not compatible with the predictions of the 'enemy free space' hypothesis (Kjellberg *et al*, 1984).

The adaptive significance of ovipositors are closely related to other reproductive characteristics, such as longevity and egg production (Price, 1970; 1971; 1973a; 1973b; 1974; Charnov & Skinner, 1984; 1985). Sycophagines and sycoecines are pro - ovigenic, with mature eggs when they emerge from their galls. Hence, egg numbers counted from females represent their lifetime reproductive success, in contrast to syn - ovigenic species where eggs continue to develop after oviposition has begun (Iwata, 1962). In the laboratory, adult agaonids live for a maximum of two days and sycophagines a few days more (Dallas, 1987). Like the agaonids, sycophagines have only a short period in which to lay their eggs, because they cannot re - emerge from figs they have entered and are therefore obliged to lay all their eggs inside one fig. Once inside a fig, they can lay eggs quickly because oviposition sites are readily accessible. Because of their high rate of egg production and short lifespans, the agaonids and sycophagines may be seen as r-strategists (Pianka, 1970).

Most of the wasps ovipositing through the fig wall from the exterior are syn - ovigenic and are long lived (Dallas, 1987). They are therefore more closely aligned with the K-strategists and are characterised by slow production of eggs and greater investment during each oviposition event (Force, 1972). They lay their eggs into many different figs, perhaps on different trees and have ovipositors that are long enough to allow access to most of the flowers. The inquiline fig wasps expend much more time for each oviposition event in comparison to the internally ovipositing fig wasps. On average, *C. capensis* oviposited 26 times per hour and each oviposition event lasted between 5 and 10 seconds (n = 5), whereas *Sycoryctes* sp. from *F. burtt-davyi* oviposited between 1.8 to 3.1 times per hour and each oviposition event lasted between 6 to 23 minutes (n = 12) (Nefdt, unpubl. data). Inquilines have to penetrate through the fig wall and locate galled flowers containing host larvae. This may require greater time and energy investments than fig wasps which have more accessible oviposition sites by ovipositing down styles and into hundreds of available flowers. Hence externally ovipositing fig wasps are characterised by longer lifespans and continuous production of eggs after oviposition has begun.

In summary, fig wasps living on single fig species can be divided on the basis of their oviposition behaviour. Those that oviposit inside figs have large numbers of eggs that can be laid quickly in many readily available ovules. These species have characteristics of r - strategists. Fig wasps which oviposit from the outside seem to be aligned with K - strategists and are characterised by being syn - ovigenic, live much longer and invest much more time and energy during each oviposition event. There appears to be no segregation of oviposition sites between species and parasitism by inquilines / parasitoids does not constitute any selection pressure for shorter agaonid ovipositors.

CHAPTER 6

Discussion

6.1 Factors maintaining the stability of the fig - fig wasp mutualism

A major objective of this study was to investigate the factors involved in maintaining a stable coexistence between agaonids and their host *Ficus* species. Various studies had accepted that agaonid ovipositors were long enough to allow oviposition into about half the ovules, leaving the remainder to produce seeds. From a telelogical perspective, this may seem to have evolved to allow the fig tree to produce seeds, which in later generations would benefit the wasps' descendents by providing oviposition sites. However, Kjellberg *et al* (1988) noted that the generation times of fig wasps and fig trees are quite different, with the latter measured in centuries and the former in months. Therefore short term selection could act on fig wasps so that they would evolve longer ovipositors, as this would allow them to produce more progeny. In support of the original hypothesis are data from Galil & Eisikowitch (1968a) showing that the ovipositor of *C. arabicus* was long enough to reach less than half the ovules of *F. sycomorus*. However, they measured the lengths of sheaths, which in this study was shown to often dramatically underestimate the length of the true ovipositor. Recent work by Baijnath & Ramcharun (1988) and Bronstein (1988a) who used the true ovipositor lengths, showed that in two species of figs a much larger proportion of ovules are available to oviposition than originally thought. However, Bronstein (1988a) still estimated that in *F. pertusa* figs, 20% of the ovules were inaccessible.

I investigated the accessibilities of fig ovules for 10 African agaonid species and found that in six species more than 80% of the flowers were available for oviposition, with the extreme example being *P. awekei* which was estimated to reach 99% of the ovules. The ovipositor lengths of each of these species were found to be closely related to to the style lengths of their associated fig species, suggesting that the ovipositors are adapted to the ovipositional requirements. In fig wasps which are able to reach all the ovules or sufficient to normally lay their full egg load, there would be no selective advantage in possessing even longer ovipositors, which would be disadvantageous during flight and penetration through the ostiole. In other insect groups, ovipositor lengths are often related with the depth required to penetrate in order to reach the oviposition sites. Most wasps have ovipositors long enough to reach all the potential oviposition sites (Achterberg, 1986; Brandl & Vidal, 1987), while a few studies have suggested that ovipositor lengths may be governed by inter-specific competition (Heatwole & Davis, 1965; Price, 1970; 1971; 1972). Natural enemies may also attack individuals with ovipositors falling into particular length categories, resulting in the hosts evolving ways of reducing their vulnerability to attack. However, because inquilines and parasitoids appear to reach all the agaonids, independent of the distances between the galls and the fig's exterior, the enemy free space hypothesis can be rejected. Agaonid fig wasps appear to have ovipositors related to the length of the styles irrespective of these other influences.

With regard to availability of ovules to agaonids, *F. sur* was an exception, with more than half the flowers having styles longer than the ovipositor of *C. capensis*. It seems then that even though many mutualisms have agaonids with ovipositors long enough to reach nearly all the flowers, this is not inevitably the case. That some agaonids can potentially reach 100% of the ovules dispels the style length hypothesis (Murray, 1985a) as a universal explanation of maintaining a stable coexistance between figs and their fig wasps.

In cases where agaonid species are potentially able to destroy 100% of the ovules, the question arises as to what factors are involved in ensuring that fig trees still produce enough seeds to avoid extinction? Possibly factors regulating seed and wasp production may act by maintaining control of the number of wasps able to enter through the ostiole (Janzen, 1979a) and the number of eggs they are able to lay (Bronstein, 1988a). Data in this thesis showed that production of offspring increased with agaonid entries into figs of *F. burtt-davyi* and *F. sur* and then plateaued below the number of accessible ovules (Chapter 4). A proportion of these progeny however, may have died, resulting in an underestimate of the numbers of eggs that were laid. Furthermore interference among ovipositing agaonids may reduce the total number of eggs laid in multiply entered figs. Keeping these biases in mind, these data suggest that offspring numbers may be constrained by the number of eggs that each wasp is able to lay.

In most species of agaonids it seems likely that the collective number of eggs among foundresses entering a single fig may occasionally exceed the number of accessible oviposition sites. As a result a certain proportion of eggs cannot be deposited and will remain inside the ovaries after death. It could therefore be hypothesised that high densities of foundresses per fig may represent a significant selective disadvantage due to intra-specific competition, which would lead to the evolution of smaller clutch sizes. However, this is a group selectionist view that is unlikely to operate with the fig wasp system because individuals with larger numbers of eggs would be at a competitive advantage.

A more likely scenario is that the number of eggs per wasp may be determined by body size. Larger species had more eggs and in three of the five agaonid species examined, larger individuals had more eggs in their ovaries. However, the upper limit to body size is likely to be set by the size of the fig ostiole. *Ficus* with smaller figs are likely to have narrower ostioles through which the female foundresses have to penetrate. It is not uncommon to find dead foundresses stuck inside ostioles, particularly when there are high concentrations of wasps surrounding the tree (Nefdt, pers. observ.). Natural selection would therefore favour smaller body sizes which would allow for easier access into the lumen. Quantification of the mortality rates resulting from agaonids dying in the ostiole may show that there are selection pressures for smaller body sizes. In addition, selection differentials could be calculated by comparing body sizes of wasps which successfully enter figs with those that fail to reproduce because of their inability to pass through the ostioles. Selection will also be acting on the trees, however. Individuals with small ostioles will have a higher mortality rates among the wasps trying to enter, and will consequently set less seed and produce fewer wasps carrying their pollen.

Larger figs such as *F. sur* have many more flowers than smaller figs such as *F. burtt-davyi*, and their agaonids tend to have relatively more eggs. It is possible that the larger ostioles and greater numbers of oviposition sites in bigger fig species have, together, favoured larger agaonid species which contain many eggs. A further factor affecting the number of eggs that can be carried by wasps is egg size. Parker & Begon (1986) have tested models concerning the relationship between egg size and egg number, and suggest that production of smaller eggs could lead to larger clutch sizes. The lower limit of the size of agaonid eggs may be set by the minimum amount of reserves inside each egg required for the survival of the progeny. Assuming a trade - off between egg sizes and egg numbers, this could control the maximum number of eggs that an agaonid produces. There is some data to support the idea that species producing smaller eggs contain larger numbers of eggs in their ovaries (Nefdt, unpubl.). Non - pollinating fig wasps such as *A. longitarsus* ovipositing from the outside often have fewer and larger eggs than the agaonids associated with the same fig species. However, there are other large species, such as *Apocryptophagus* spp. which contain both larger and more numerous eggs. Clearly the factors which control the number of eggs produced by individuals of different wasp species are complex and form the, perhaps unique, reproductive strategy of each species.

Overall, it seems that the number of foundresses entering a fig may be the most important factor regulating seed and wasp production. Consequently, the population dynamics of agaonids will play an important role in influencing the 'stability' of the mutualism. Fig trees tend to be widely dispersed, resulting in wasps often having to travel considerable distances to find 'receptive' trees. Where wasp densities are low, trees receive fewer wasps, and many figs may remain unpollinated and consequently abort (Bronstein, 1988a). Therefore, the evolutionary history of the mutualism may have been one where there have been too few wasps for trees, not too many. Even when wasp densities are high, the number of wasps able to enter a fig may be limited by the short period during phase B of the fig's cycle when the wasps can enter and oviposition can take place. This is because the ostiole closes soon after pollination, blocking the entrance of additional wasps. This short period allowed for wasp entry may be an adaptive feature that could improve the fig tree's fitness by increasing seed production. It also has the consequence of leading to more 'sharing' of wasps between the figs on the same tree.

6.2 Sex Ratio Adjustment

Sex ratio studies of agaonid 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 help provide explanations of the proximate mechanisms of sex ratio adjustment. The problem of identifying such mechanisms is exasperated by difficulties of disentangling the primary sex ratios, controlled intrinsically by sperm release or retention, and secondary sex ratios controlled by a multitude of extrinsic factors (Werren *et al*, 1986; King, 1987).

Adult sex ratios may not reflect the primary sex ratios at the time eggs are laid, owing to differential mortalities of the sexes. Sex-biased mortalities have been reported in several groups of Parasitica, such as in *Torymus chilonis* (Suzuki *et al*, 1984), *Torymus evanescens* Westwood (Dijken & Waage, 1987) and in the eulophids *Dahlbominus fuliginosus* Nees (Wilkes, 1963) and *Pediobius foveolatus* (Crawford) (Kauffman & Flanders, 1985). However, Hooker & Barrows (1989) detected no differential sex mortalities in the offspring of *P. foveolatus* and many other studies have shown that primary sex ratios are manipulated in accordance with sex ratio theory (Trivers & Willard, 1973; Wellings *et al*, 1986; Welzen & Waage, 1987; Strand, 1988). The experiments in this thesis suggest that in fig wasps, secondary sex ratios may accurately reflect primary sex ratios, as there was no differential mortality of male or female progeny when nutrient quality was reduced. This is in contrast to Galil & Eisikowitch (1971) who by introducing pollenless female *Blastophaga quadraticeps* into figs of *F. religiosa*, found that the offspring sex ratios were unusually male-biased, with a sex ratio of 3.9 as compared to 0.4 in normal figs. They concluded that this was a result of proportionately higher mortalities of female progeny due to the absence of pollination. Female *B. quadraticeps* were collected from galls that had been perforated by males, as a way of ensuring that the females were inseminated. However, it is also possible that in some cases the females had made these holes and were not nescessarily inseminated, as can happen in *C. capensis* (pers. observ.). Since an uninseminated female will only produce male progeny, her presence inside a fig containing other foundresses would result in a highly male-biased sex ratio. In the experiments carried out using *E. baijnathi* and *C. capensis*, subsamples of foundresses were checked for charged spermathecae, ensuring that they had been inseminated. In contrast to Galil & Eisikowitches' results, the sex ratios from these experimental figs did not differ with sex ratios from normal figs, even though there was a significant mortality rate of both *E. baijnathi* and *C. capensis*. Therefore, differential mortality of the sexes seems an unlikely explanation of how sex ratios change with different foundress numbers.

The sex ratio adjustment curves from this study were similar to those for American agaonids (Herre, 1985; 1987). However, the variances within the different experimental sets of figs (each set with a constant number of foundresses) were much greater than those of Herre (1985). This discrepancy could be attributable to the variable foundress relatedness of the wasps used in the experiment. When there is competition among relatives of the same sex for limited resources, the theoretical sex ratio tends to be biased towards the sex with the lower average relatedness among competitors (Hamilton, 1967; Clark, 1978; Taylor & Bulmer, 1980; Fisher & Harper , 1986). Herre recorded the progeny sex ratios from naturally collected figs and related them to the number of dead foundresses found inside the lumen. In this study agaonids were first collected from a small number of figs from *one* tree, and introduced experimentally into sets of figs on another tree. Hence, unnaturely biased relatedness between foundresses cannot be ruled out and may have confounded the offspring sex ratios in some figs. However, in a study involving *Pegoscapus assuetus* Grandi, Frank (1985a) found no differences between sex ratios of broods produced from sib-mated foundresses and broods from non-related foundresses. The importance, if any, of foundress relatedness has therefore not yet been established. Since fig wasps offer opportunities for rearing isolated broods from parents of known relatednesses, this is a question which can be answered by future experiments.

LMC theory predicts that more sons are produced when many foundresses contribute to an isolated brood (Hamilton, 1967; Werren, 1980; 1983; 1984; Green *et al*, 1982; Frank, 1985a; 1986a; 1986b; Herre, 1985; 1987; Ramirez, 1987; Griffiths & Godfray, 1988). If some agaonid mothers are unable to lay their total egg complement, what would be the advantage of only producing sons when many foundresses enter a fig? A mother which produces a son inside a fig with many female progeny is likely to have greater fitness than a mother which produces a daughter. This is because a son has the potential to inseminate all the female progeny and propagate his mother's genes via many clutches. 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.

There are various ways of refining the LMC model (Frank, 1986a), such as incorporating inbreeding (Green *et al*, 1982; Frank, 1985a; 1985b; Herre, 1985; 1987), sib-mating, foundress relatedness (Clark, 1978; Taylor & Bulmer, 1980), and group selection (Colwell, 1981; Harvey *et al*, 1985) that may better predict sex ratios. Fig wasps offer a unique system where the selection of sex ratios can be tested experimentally. For example, as mentioned above, the influences of foundress relatedness on sex ratios could be tested by introducing wasps of different relatednesses into figs and then comparing their offspring sex ratios.

Daughter - biased sex ratios are commonly found in Hymenoptera, Parasitica. Examples include the scelionid *Gryon atriscapis* Gahan (Waage, 1982a; 1982b), the eulophids *Nesolynx albiclavis* (Kerrich) (Putters & Assem, 1985), *P. foveolatus* (Hooker & Barrows, 1989) and

Colpoclypeus florus (Walker) (Dijkstra, 1986), and the trichogrammatid *T. evanescens* Westwood (Waage & Ng, 1984). It appears that the mechanisms involved in regulating sex ratios vary depending on the species. Gregarious parasites often oviposit many eggs over a short time period, resulting in variable sex ratios (Ikawa & Suzuki, 1982). The pteromalid *Anisopteromalus calandrae* (Howard) oviposits a fixed number of sons during its lifetime, but then decreases or increases daughter numbers in response to host quality (Assem *et al*, 1984). *Bracon hebetor* Say even produces more female-biased sex ratios with increases in the numbers of foundresses contributing to an isolated brood (Galloway & Grant, 1989). Recently the sequence of laying male and female eggs in relation to clutch sizes have been shown to be important when considering sex ratio adjustments. Hooker & Burrows (1989) found that within clutches, *P. foveolatus* initially laid female eggs followed by male eggs. The eulophid, *Colpoclypeus florus* also produces female progeny in the early stages of the oviposition sequence followed by a few male eggs (Dijkstra, 1986). However, the strategy of laying male eggs early, but not necessarily first, and female eggs later is much more common. Examples include *Gryon atriscapus* (Waage, 1982a; 1982b), the pteromalid *Spalangia endius* Walker (Donaldson & Walter, 1984), *Torymus chilonis* (Suzuki *et al*, 1984) and *T. evanescens* (Waage & Ng, 1984).

Data presented in this thesis support the hypothesis that agaonid sex ratios change as a result of sequential laying of male eggs first followed by female eggs under conditions of variable oviposition site limitation. It was shown that when several foundresses enter a fig there is competition for ovules between them. This is clearly evident from the total production of offspring, which levels off despite increases in foundress numbers once the total number of accessible ovules is approached. If foundresses fail to lay the last few eggs, which would have produced mainly females, then the sex ratio would become more male-biased, as predicted by LMC. However, the 'male eggs first' hypothesis depends on two assumptions. Firstly, it must be confirmed that when all the flowers are utilised, excess eggs are not deposited into ovules already containing eggs. This could be tested experimentally by introducing different numbers of foundresses into different sets of figs. As soon as oviposition has ceased, the number of eggs still inside their ovaries could be counted and compared between the different sets of figs. If they do fail to lay all their eggs when oviposition sites are all utilised, one would predict that the number of eggs remaining in their ovaries would increase with foundress number.

The second assumption is that female fig wasps initially lay eggs which produce males, and then lay eggs which give rise to female progeny. There is circumstantial evidence to support this assumption. In three of the agaonid species studied here (including a non - pollinating fig wasp, *P. barbarus*) and in others studied elsewhere (Galil & Eisikowitch, 1968a; 1968b; Verkerke, 1988a), oviposition is more concentrated in the shorter styled flowers. As the number of females inside a fig increases, more of the longer styled flowers are utilised. This suggests that the first eggs are laid into short styled flowers and these flowers have been shown to give rise mainly to male progeny. Eggs laid later were often deposited into longer styled flowers and gave rise mainly to female progeny.

One way to confirm that agaonids lay male producing eggs first is to experimentally kill ovipositing foundresses at different stages of their oviposition sequence and compare the resulting offspring sex ratios. Galil & Eisikowitch (1971) used a systemic insecticide to kill the larvae inside ovules of *F. religiosa*. It may be possible to inject a contact insecticide into the fig lumen via the ostiole to kill the foundress, but not their eggs or larvae. If agaonids sequentially segregate male and female eggs, the figs which contained females killed shortly after oviposition had begun should produce only male progeny.

6.3 The reproductive strategies of fig wasps

The continued survival of the different species of fig wasps living on one species of *Ficus* depends on their ability to reach their oviposition sites. However, different fig wasps have evolved different reproductive strategies depending on their particular life style. Other studies have shown that access to oviposition sites, competition between individuals for oviposition sites (Heatwole & Davis, 1965; Price, 1970; 1973a; 1973b) and natural enemies (Price *et al*, 1980; Price, 1980; 1984; Waage *et al*, 1985; 1986; Weis & Abrahamson, 1985; Price & Clancy, 1986; Price & Pschorn - Walcher, 1988) may be important forces which affect the reproductive characteristics of different insect species. Price (1972) found that six parasitoids utilising one sawfly host, *Neodiprion swainei* Middleton, destroy different segments of the host population by having different ovipositor lengths. Each species attacked hosts buried at different depths in the forest litter. In contrast to this, oviposition by fig wasps ovipositing into galls containing their hosts was not segregated spatially, despite some species possessing ovipositors of different lengths. Therefore, the ovipositors of externally ovipositing fig wasps are unlikely to have evolved as a consequence of competitive interactions between different species. It is more likely that ovipositor lengths are adapted so that the wasp can reach as many oviposition sites as is normally required. Since ovipositions are not spatially segregated, interactions between internally ovipositing fig wasps and their inquilines / parasitoids are also unlikely to have been important selection pressures on ovipositor length.

The fig wasps studied in this thesis employed two main reproductive strategies, distinguished by their method of oviposition. The first group includes the sycophagines and agaonids that oviposit down the styles from inside the fig's lumen. Since they cannot escape from the lumen of the fig they have entered, they must lay all their eggs in a short time in one place. Hence, they are pro - ovigenic, with all their eggs being mature when they emerge from their galls and the number of eggs represents the total lifetime reproductive success. They have also evolved ovipositors that are long enough to reach most of the ovules. As already described for the agaonids, figs may have ostioles which regulate the number and sizes of sycophagine females that enter them.

Many sycophagine foundresses entering a single fig could lead to competition between females for oviposition sites. Sycophagine sex ratios are highly female - biased, like their agaonid counterparts (pers. observ.), and they may also adjust their sex ratios in relation to foundress numbers. If they also lay male and female eggs sequentially under oviposition site limitation, sex ratios will also become more female-biased with increases in foundress numbers, as hypothesised for the agaonidae in chapter 4. The effects of the presence of sycophagines on agaonid sex ratios and vice versa, are also unknown. The presence of sycophagines or sycoecines could cause agaonid offspring sex ratios to become more male - biased if agaonids failed to oviposit in ovules containing eggs of other species; or when the agaonid foundresses were unable to lay their last few eggs which would have given rise to females. This could be tested by experimentally introducing agaonid foundresses into figs already containing sycophagine progeny and comparing the resulting offspring sex ratios with those from control figs containing only agaonid foundresses. Competition between agaonids and sycoecines might also produce more male - biased sex ratios. Sycoecines lay mainly in short styled flowers, where male agaonids are concentrated. Competition leading to death agaonids would therefore differentially reduce male numbers.

The presence of several individuals of different fig wasp species sharing a fig should lead to inter - specific competition, made more intense by the biology of the wasps, which mean that each female can lay her eggs nowhere else. By 'spreading' their eggs among several figs, sycoecines may avoid severe competition with agaonids for oviposition sites. For this strategy to have evolved, its advantages must have outweighed the disadvantages of having to travel in a 'hostile' environment outside, between each fig and the costs of penetrating many ostioles. A consequence of this strategy is that shorter ovipositors are adequate, since they need only reach a few shorter styled flowers in each fig. Shorter ovipositors may even be advantageous, by making it easier for individuals to re - emerge through the ostiole. Oviposition in shorter styled flowers may be much more rapid than in long styled flowers, another advantage for sycoecines.

Nothing is known about competition between agaonids and sycoecines, and fig species having members from these two groups provide interesting opportunities for further research. Data are needed about how many different figs sycoecines can infest. On average single *F. burtt-davyi* figs produce ten or so *Phagoblastus* sp. offspring (Vincent, pers. comm.), and each foundress contains an average of 50 eggs. As sycoecines are pro - ovigenic, it can be inferred from these data that *Phagoblastus* sp. may oviposit into five different *F. burtt-davyi* figs (Low densities of the species make two foundresses in a fig unlikely). In order to investigate competition between sycoecine and agaonid foundresses, the proportion of ovules still available after agaonids have already oviposited could be examined. As sycoecines can only reach shorter styled flowers, they should be at a competitive disadvantage. My observations also indicate that there is sometimes multiparasitism (Hubbard *et al*, 1987), with agaonids and sycoecines ovipositing onto the same flowers. This raises the question of whether sycoecines have the ability to avoid flowers already utilised by agaonids and vice versa. In addition it would be interesting to test whether the presence of many agaonid foundresses inside a fig induces a sycoecine to re - emerge and find new oviposition sites in other figs. Agaonid wings left at the entrance would provide a useful cue, allowing sycoecines to assess a fig without even entering it.

The second major strategy employed by fig wasps is to oviposit from the outside. The important difference between these externally ovipositing species and the agaonids, sycoecines and sycophagines is that oviposition from the outside requires greater reproductive effort per oviposition event because the ovules are less readily available. This especially applies to the inquilines and parasitoids that are obliged to locate their hosts, which may be at low densities. Searching is clearly apparent from the twisting of the ovipositor before it reaches an inhabited ovule. As a result of greater energy and time expenditures for each oviposition event, externally ovipositing wasps are long - lived (Dallas, 1987) and have the ability to continuously develop new eggs. They are also at greater risk of predation (Compton & Robertson, 1988) and exposed to the elements of a hostile environment.

Although externally ovipositing fig wasps that are phytophagous do not have to locate hosts inside galls, it seems they too are constrained by less readily available fig ovules. Species of *Otitesella* and *Apocryptophagus* all expend large amounts of time and energy probing through the fig wall (Nefdt, unpubl. data). At least three species of *Otitesella* were found to contain trophamaria, confirming they are syn - ovigenic, although it is not yet known whether adult *Apocryptophagus* spp. develop new eggs. In terms of short - term egg output, two species of externally ovipositing fig wasps were found to contain numerous eggs per female. *Apocryptophagus* sp. 2 from *F. sur* was found to contain on average 300 eggs, while *Camarothorax equicollis* (Pteromalidae, Epichrysomalidae) from *F. thonningii* contained more than 1000 eggs (Nefdt, unpubl. data). In contrast *Apocryptophagus* sp. 3 had only 50 eggs. This shows that egg production varies greatly and independently of the accessibilities of oviposition sites.

How do fig wasp communities, with their two broad reproductive strategies, compare with other chalcid communities? Askew (1975) studied the communities centred upon oak galls made by species of Cynipidae. The major contrast between oak gall and fig wasp communities is that in oak gall communities, the phytophagous species are mainly cynipids, while the rest of the chalcids are inquilines or parasitoids (Askew & Shaw, 1986). Another contrast was in the degree of polyphagy. Fig wasps are characterised by monophagy, and only in the case of some eurytomids has oligophagy been confirmed. Askew (1975) found that the oak gall parasitoids adopted two broad strategies. The first group were characterised mainly by being monophagous, but contained large numbers of eggs per wasp and often suffered from high mortalities from attack by polyphagous parasitoids. These were described as r - strategists, after Pianka's (1970) r - k strategy model, though they were closely dependent on one host, unlike typical r - strategists parasitoids as defined by Price (1973a). Agaonids and sycophagines both have high rates of egg deposition and in many cases are subject to mortalities by parasitoids or inquilines. They too can be considered as r - strategists.

Polyphagous parasitoids attacking cynipids in oak galls employed a strategy which resembles that of the externally ovipositing fig wasps, particularly the inquilines / parasitoids. Like inquiline fig wasps, these species spent more time and energy depositing an egg than monophagous parasitoids. They also carried fewer eggs on average, but this indication of lower reproductive output was counter - balanced by their multivoltinism. Inquilines and parasitoid fig wasps had fewer eggs per female than the internally ovipositing phytophages, but the impression of reduced fecundity was counter - balanced by being syn - ovigenic and having longer lifespans than agaonids and sycophagines. By ovipositing later their survival should also be higher. The polyphagous parasitoids in the oak gall communities, and the majority of the externally ovipositing fig wasps have attributes of K - strategists, where few eggs are produced, but more time and energy is invested for the survival of each individual offspring.

Despite ovipositing from the exterior, *Apocryptophagus* spp. and *C. equicollis* appear to be aligned more closely with r - strategists. However, they must necessarily invest more time and effort into each oviposition event, despite their sometimes very high egg loads. Hence, the r - K strategy model cannot accomodate all species and is only useful within certain limits. As the reproductive characteristics of more species are examined, it is clear that many factors are involved in moulding the reproductive strategies of the insects. In Africa alone there are 105 *Ficus* species (Berg, in press), and each *Ficus* on average supports about ten fig wasp species (Compton, pers. comm.). This provides numerous opportunities for testing the generality of different evolutionary and ecological hypotheses. The 'replication' of 105 fig wasp communities in Africa offer a fascinating challenge for future fig wasp researchers.

REFERENCES

Abdurahiman U.C., 1980. Observations on the oviposition behaviour in Philotrypesis pilosa Mayr (Torymidae: Hymenoptera), *Proc. Symp. Environ. Biol. Trivandrum* 146-150.

Abdurahiman U.C. & K.J. Joseph, 1978a. Biology and behaviour of *Apocrypta bakeri* Joseph. (Torymidae), cleptoparasite of *Ceratosolen marchali* Mayr. (Agaonidae). *Entomon. 3(1)*, 31-36.

Abdurahiman U.C. & K.J. Joseph, 1978b. Cleptoparasitism of the fig wasps (Torymidae: Chalcidoidea) in *Ficus hispida* L. *Entomon 3(2)*, 181-186.

Abdurahiman U.C. & K.J. Joseph, 1978c. Bionomics of the fig chalcidoidea (Agaonidae: Hymenoptera). *J. Anim. Morph. Physiol: Silver jubilee Volume*, 18-26.

Abdurahiman U.C. & K.J. Joseph, 1979. Observations on the oviposition behaviour in *Apocrypta bakeri* Joseph (Torymidae : Hymenoptera). *J. Bombay Nat. Hist. Soc. 76*, 219-223.

Achterberg C. van., 1986. The oviposition behaviour of parasitic Hymenoptera with very long ovipositors (Ichneumonoidea: Braconidae). *Entomol. Ber. 46(1)*, 113-115.

Addicott J.F., 1986. Variation in the costs and benefits of mutualism: the interaction between yuccas and yucca moths. *Oecologia 70*, 486-494.

Ansari M.H., 1966. On a new species of fig insect (Chalcidoidea: Hymenoptera) from India. *Indian J. Ent., 28*, 74-83.

Ansari M.H., 1967. The process of egg laying in Idarninae (Chalcidoidea : Hymenoptera). *Indian J. Entomol. 29, 380-384*

Askew R.R., 1965. The biology of the British species of the genus *Torymus* Dalman (Hymenoptera: Torymidae) associated with the galls of Cynipidae (Hymenoptera) on oak, with special reference to alternation of forms. *Trans. Soc. Brit. Entomol., 16*, 217-232.

Askew R.R., 1975. The organisation of chalcid-dominated parasitoid communities centred upon endophytic hosts. In: Price, P.W., (ed), *Evolutionary Strategies of Parasitic Insects and Mites*. pp. 130-153., Plenum, London.

Askew R.R. & M.R. Shaw, 1986. Parasitoid Communities: their size, structure and development. In: Waage J. & D. Greathead., *Insect Parasitoids*. 13th Symposium of the Royal Entomological Society of London. London.

Assem van den J., F.A. Putters & T.C. Prins, 1984. Host quality effect on sex ratio of the parasitoid wasp, *Anisopteromalis calandrae* (Chalcidoidea: Pteromalidae). *Netherlands Journal of Zoology, 34*, 33-62.

Atsatt P.R., 1981. Lycaenid butterflies and ants: selection for enemy-free space. *Am. Nat. 118*, 638-654.

Baijnath H., 1988. Figs, wasps, birds, bats. *Aetfat., Twelfth Plenary meeting Douziene Reunion Pleniere*. Hambburg. (Abstract)

Baijnath H. & S. Ramcharun, 1983. Aspects of pollination and floral development in *Ficus capensis* Thunb (Moracaea). *Bothalia 14(3 & 4)*, 883-888.

Baijnath H. & S. Ramcharun, 1988. Reproductive biology and chalcid symbiosis in *Ficus burtt-davyi* (Moraceae). *Monogr. Syst. Bot Missouri Bot. Gard. 25*, 227-235.

Berg C.C., 1983. Floral differentiation and dioecism in *Ficus* (Moraceae). *Acta Bot. Neerl. 32*, 344-355.

Berg C.C., 1986. Subdivision of *Ficus* sibg. *Urostigma* sect. Galoglychia (Moraceae). *Botany Proceedings C 89(2)*, 121-127.

Berg C.C., 1989. Annotated chack-list of the *Ficus* species of the African floristic region, with special reference and key to the taxa of southern Africa. In press.

Boucek Z., 1988. *Australasian Chalcidoidea (Hymenoptera). A Biosystematic Revision of Genera of Fourteen Families, with Reclassification of Species*. C.A.B. International, Oxon.

Boucek Z., A. Watsham & J.T. Wiebes, 1981. The fig wasp fauna of the receptacles of *Ficus thonningii* (Hymenoptera : Chalcidoidea). *Tijdschr. Ent. 124*, 149-233.

Brandl R. & S.Vidal, 1987. Ovipositor length in parasitoids and tentiform leaf mines: adaptations in Eulophids (Hymenoptera : Chalcidoidea). *Biol. J. Linn. Soc. 32*, 351-355.

Breitenbach von F., 1985. *Southern Cape Tree Guide*. Department of Environmental Affairs, Pretoria, South Africa.

Bronstein J.C., 1987. Maintenance of species-specificity in a Neotropical fig pollinator mutualism. *Oikos 48(1)*, 39-46.

Bronstein J.C., 1988a. Mutualism, antagonism, and the fig-pollinator interaction. *Ecology 69(4)*, 1298-1302.

Bronstein J.C., 1988b. Predators of fig wasps. *Biotropica 20*, 215-219.

Cees R.L., van Welzen & J.K. Waage, 1987. Adaptive responses to local mate competition by the parasitoid, *Telenomus remus*. *Behav. Ecol. Sociobiol. 21*, 359-365.

Charnov E.L., 1982. *The evolution of sex allocation*. Princeton University Press., Princeton.

Charnov E.L., R.L. Los-Den harbugh, T. Jones & J van den Assem, 1981. Sex ratio evolution in a variable environment. *Nature 289*, 27-33.

Charnov E.L. & S.W. Skinner, 1984. Evolution of host selection and clutch size in parasitoid wasps. *Florida Entomol. 67(1)*, 5-21.

Charnov E.L. & S.W. Skinner, 1985. Complementary approaches to the understanding of parasitoid oviposition decisions. *Environ. Entom. 14(4)*, 383-391.

Clark, 1978. Sex ratio and local resource competition in a Prosimian Primate. *Science 201*, 163-165.

Clausen C.P., 1939. The effect of host size upon the sex ratio of Hymenopterous parasites and its relation to methods of rearing and colonization. *J. New York Entomol. Soc. XLVIII(1)*, 1-9.

Colwell R.K., 1981. Group selection is implicated in the evolution of female-biased sex ratios. *Nature 290*, 401-404.

Compton S.G.A. & R.G.E. Baker, 1986. Records of figs (*Ficus* spp.) and Fig - wasps (Hymenoptera: Chalcidoidea) in Cameroun, pp. 46-51. In: Baker R.G.E., K. Richards & C.A. Rimes, The Hull University Cameroun Expedition: 1981-1982. Final Report. University of Hull, Department of Geography. Miscellaneous Series No. 30.

Compton S.G. & F.A.C. McLaren, 1989. Respiratory adaptations in some male fig wasps. *Konink. Ned. Akad. Wetenschappen C, 92(1)*, 57-71.

Compton S.G. & R.J.C. Nefdt, 1989. The figs and fig wasps of *F. burtt-davyi* Hutch. *Mitt. Inst. Allgemeine Bot. Hamburg.* 1-9.

Compton S.G. & S. Robertson, 1988. Complex interactions between mutualisms: ants tending homopterans protecting fig seeds and pollinators. *Ecology, 69(4)*, 1302-1305.

Copland M.J.W. & P.E. King, 1972. The structure of the female reproductive system in the Torymidae. *Trans. R. Ent. Soc. london 124*, 191-212.

Copland M.J.W. & P.E. King, 1973. The structure of the female reproductive system in the agaonidae (Chalcidoidea, Hymenoptera). *J. Entom. (A) 48(1)*, 25-35.

Corner E.J.H., 1985. *Ficus* (Moraceae) and Hymenoptera (Chalcidoidea): figs and their pollinators. *Biol. J. Linn. Soc. 25*, 187-195.

Dallas H., 1987. Longevity and timing of oviposition in some South African fig wasps. Unpubl. B.Sc. (Honours) Project, Rhodes University.

Darwin C.R., 1899. *The origin of species by means of natural selection, or the preservation of favoured races in the struggle for life.* London, John Murray, 432pp.

Dijken M.J. van, J.K. Waage, 1987. Self and conspecific superparasitism by the egg parasitoid *Trichogramma evanescens*. *Entomol. Exp. Appl., 43,* 183-192.

Dijkstra L.J., 1986. Optimal selection and exploitation of the hosts in the parasitic wasp *Colpoclypeus florus* (Hymenoptera: Eulophidae). Ph.D. dissertation, Agriculture University, Wageningen, The Netherlands.

Donaldson J.S. & G.H. Walter, 1984. Sex ratios of *Spalangia endius* (Hymenoptera: Pteromalidae), in relation to current theory. *Ecol. Entom. 9*, 395-402.

Donaldson J.S. & G.H. Walter, 1988. Effects of egg availability and egg maturity on the ovipositional activity of the parasitic wasp, *Coccophaga atratus. Physiol. Entomol., 13(4)*, 407-417.

Faegri K. & L. van der Pijl, 1979. *The Principles of Pollination Ecology.* Pergamon Press, Oxford.

Fergusson N.D.M., 1988. A comparative study of the structures of phylogenetic importance of the female genetalia of the Cynipoidea (Hymenoptera). *System. Entom. 13*, 13-30.

Fischer E.A. & A. Harper, 1986. Local mate competition in finite groups. *Evolution 40(4)*, 862-863.

Fisher R.A., 1930. *The Genetical Theory of Natural Selection.* Oxford University Press.

Flanders S.E., 1939. Environmental control of sex in hymenopterous insects. *Ann. Entomol. Soc. America 32*, 11-26.

Force D.C., 1972. r- and K-stategists in endemic host-parasitoid communities. *Bull. Entomol. Soc. Am. 18*, 135-137.

Frank S.A., 1984. The behaviour and morphology of the fig wasps *Pegoscapus assuetus* and *P. jimenezi* : descriptions and suggested behavioural characters for phylogenetic studies. *Psyche 91*, 289-308.

Frank S.A., 1985a. Hierarchical selection theory and sex ratios II. On applying the theory, and a test with fig wasps. *Evolution 39(5)*, 949-964

Frank S.A., 1985b. Are mating and mate competition by the fig wasp *Pegoscapus assuetus* (Agaonidae) random within a fig. *Biotropica 17(2)*, 170-172.

Frank S.A., 1986a. The genetic value of sons and daughters. *Heredity 56*, 351-354.

Frank S.A., 1986b. Hierarchical delection theory and sex ratios I. General solutions for structured populations. *Theor. Pop. Biol. 30*, 215-231.

Galil J., 1977. Fig biology. *Endeavour 1*, 52-56.

Galil J., R. Dulberger & D. Rosen, 1970. The effects of *Sycophaga sycomori* L. on the structure and development of the syconia in *Ficus sycomorus* L. *New Phytol. 69*, 103-111.

Galil J. & D. Eisikowitch, 1968a. On the pollination ecology of *Ficus sycomorus* II. Pocket filling and emptying in *Ceratosolen arabicus* Mayr. *New Phytol. 73*, 515-528.

Galil J. & D. Eisikowitch, 1968b. Flowering cycles and fruit types of *Ficus sycomorus* in Israel. *New Phytol. 67*, 745-758.

Galil J. & D. Eisikowitch, 1968c. On the pollination ecology of *Ficus sycomorus* in East Africa. *Ecology 49(2)*, 259-269.

Galil J. & D. Eisikowitch, 1971. Studies on the mutualistic symbiosis between syconia and sycophilous wasps in monoecious figs. *New Phytol. 70*, 773-787.

Galil J. & D. Eisikowitch, 1973. Topocentric and ethodynamic pollination. In: Pollination and Dispersal (eds, N.B.M. Branjes & H.F. Linskens), pp 85-100. Department of Botany, University Nijmegen, Nijmegen, Netherlands.

Galil J. & D. Eisikowitch, 1974. Further studies on the pollination ecology in *Ficus sycomorus*. II Pocket filling and emptying by *Ceratosolen arabicus* Mayr. *New Phytol. 73*, 515-528.

Galil J. & Neeman 1977. Pollen transfer and pollination in the common fig (*Ficus carica*). *New Phytol. 79*, 163-171.

Galil J.W., B. Ramirez & D. Eisikowitch, 1973a. Pollination of *Ficus costaricana* and *F. hemsleyana* by *Blastophaga estherae* and *B. tonduzi* in Costa Rica (Hymenoptera : Chalcidoidea, Agaonidae). *Tijdschr. Entom. 16*, 175-183.

Galil J.W., M. Zeroni & D. Bar-Shalom, 1973b. Carbon dioxide and ethelene effects in coordination between the pollinator *Blastophaga quadraticeps* in the syconium in *Ficus religiosa*. *New Phytol. 72*, 113-127.

Galloway K.S. & B. Grant, 1989. Reverse sex-ratio adjustment in an apparently outbreeding wasp, *Bracon hebetor*. *Evolution, 43(2)*, 465-468.

Gardiner A., 1986. Sex ratios in fig wasps and why so many daughters. Unpubl. B.Sc. (Honours) Project, Rhodes University.

Gilbert F.S., E.F. Harding, J.M. Line & I. Perry, 1987. Morphological approaches to community structure in hoverflies (Diptera:Syrphidae). *Proc. R. Soc. London B 224*, 115-130.

Godfray H.C.J., 1988. Virginity in haplodiploid populations : a study on fig wasps. *Ecol. Entomol. 13*, 283-291.

Green R.F., G. Gordh & B.A. Hawkins 1982. Precise sex ratios in highly inbred parasitic wasps. *Am. Nat. 120*, 653-665.

Griffiths N.T. & H.C.J. Godfray, 1988. Local mate competition, sex ratio and clutch size in bethylid wasps. *Behav. Ecol. Sociobiol. 22*, 211-217.

Grosch D.S., 1948. Dwarfism and differential mortalilty in *Habrobracon. J.Exp. Zool. 107*, 289-313.

Hamilton W.D., 1967. Extraordinary sex ratios. *Science 156*, 477-488.

Harvey P., L. Partridge & L. Nunney 1985. Group selection and the sex ratio. *Nature 313*, 10-11.

Heatwole H., and D.M. Davis 1965. Ecology of three sympatric species of parasitic insects of the genus *Megarhyssa* (Hymenoptera : Ichneumonidae). *Ecology 46(1&2)*, 140-150.

Herre E.A., 1985. Sex ratio adjustment in Fig Wasps. *Science 228*, 896-898.

Herre E.A., 1987. Optimality, plasticity and selective regime in fig wasp sex ratios. *Nature 329*, 627-629.

Hespenheide H.A. 1973. Ecological inferences from morphological data. *Ann. Rev. Ecol. Syst., 4*, 213-229.

Hill D.S. 1967. Figs (*Ficus* spp) and fig wasps (Chalcidoidea). *J. Nat. Hist. 1 : 413-134.*

Hooker M.E. & E.M. Barrows, 1989. Clutch sizes and sex ratios in *Pediobus foveolatus* (Hymenoptera: Eulophidae), primary Parasites of *Epilachna varivestis* (Coleoptera: Coccinellinidae). *Entomol. Soc. America, 82(4)*, 460-465.

Hubbard S.F., G. Marris, A. Reynilds & G.W. Rowe 1987. Adaptive patterns in the avoidance of superparasitism by solitary parasitic wasps. *J. Anim. Ecol. 56*, 387-401.

Ikawa, T. & Y. Suzuki, 1982. Ovipositional experience of the gregarious parasitoid *Apanteles glomeratus* (Hymenoptera: Braconidae), influencing her discrimination of host larvae, *Pieris rapae crucivora. Applied Entomology and Zoology, 20*, 331-339.

Iwata K., 1962. The comparative anatomy of the ovary in Hymenoptera. Part VI. Chalcidoidea with descriptions of ovarian eggs. *Acta Hymenopterologica 1(4)*, 383-391.

Janzen D.H., 1979a. How to be a fig. *Annual Rev. Ecol. Sys. 10*, 13-51.

Janzen D.H., 1979b. How many babies do figs pay for babies. *Biotropica 11*, 48-50.

Jeffries M.J. & J.H. Lawton, 1984. Enemy free space and the structure of ecological communities. *Biol. J. Linn. Soc. 23*, 269-286.

Joseph K.J., 1954. Contributions to our knowledge of fig insects (Chalcidoidea: Parasitic Hymenoptera) from India. V-On seven species of the genus *Philotrypesis* Frsst., with a note on unisexual variations and polymorphism: *Agra. Univ. J. Res. III*, 43-94.

Joseph K.J., 1955. Observations sur la biologie de *Philotrypesis caricae* L. (Hym., Chalcidiens, Callomomidae). *C.R. Acad. Aci. Paris 241*, 1626-1635.

Joseph K.J., 1956. De la presence de chimiorecepteurs sur la tariere de *Pilotrypesis caricae* L. *C.r. Acad. Sci. Paris 143*, 1163-1164.

Joseph K.J., 1958. Recherches sur les chalcidiens *Blastophaga psenes* L. et *Philotrypesis caricae* L. du figuier *Ficus carica* L. *Ann. Sci. nat. Zoologie 20*, 197-260.

Joseph K.J., 1959. The biology of *Philotrypesis caricae* L., parasite of *Blastophaga psenes* L. (Chalcidoidea : parasitic Hymenoptera). *Proc. XVth Intern. Cong. Zool., London 1958*, 662-664.

Joseph K.J., 1964. A proposed revision of the classification of the fig insects of the families Agaonidae and Torymidae (Hymenoptera). *Proc. R. Ent. Soc. London B,33*, 63-66.

Joseph K.J., 1966. Taxonomy, biology and adaptations in fig insects (Chalcidoidea). In *second* All-India Cong. Zool. Proc., Varanasi (1962), *2*, 400-403.

Joseph K.J., 1984. The reproductive strategies in fig wasps (Chalcidoidea : Hymenoptera) - a review. *Proc. Indian nat. Sci. Acad. B50 (5)*, 449-460.

Joseph M. & V.C. Abdurahiman, 1981. Oviposition behaviour of *Ceratosolen fusciceps* Mayr (Agaonidae: Hymenoptera) and the mechanism of pollination in *Ficus Racemosa* L. *J. Bombay Nat. Hist. Soc. 78*, 287-291.

Kauffman W.C. & R.V. Flanders, 1985. Effects of variably resistant soybean and lima bean cultivars on *Pediobus foveolatus* (Hymenoptera: a parasitoid of the Mexican bean beetle, *Epilachna varivestis* (Coleoptera: Coccinellidae). *Environ. Entomol., 14*, 678-682.

King B.H., 1987. Offspring sex ratios in parasitoid wasps. *Quart. Rev. Biol. 62(4)*, 367-392.

Kjellberg F. & D. Damgin, M. Ibrahim & G, Valdeyron, 1984. Sex ratio in the progeny of related females breeding under local mate competition conditions. pp45-52. In: *Mini Symposium Fig and Fig insects*, Centre Louis Emberger, France.

Kjellberg F., G. Michaloud & G. Valderyon 1987a. The *Ficus-Ficus* pollinator mutualism : how can it be evolutionary stable? Pages 335-340, In: Labergie V., V. Fabres & D. Lachaise, (eds). *Insects - plants. Dr W. Junk, Dordrecht, The Netherlands.*

Kjellberg F., P.H. Gouyon, M. Ibrahim, M. Raymond & G. Valderyon 1987b. The stability of the symbiosis between dioecious figs and their pollinators: a study of *Ficus carica* L. and *Blastophaga psenes* L. *Evolution 41*, 693-704.

Kjellberg F., B. Doumesche & J.L. Bronstein, 1988. Longevity of a fig wasp (*Blastophaga psenes*). *Proc. Konink. Ned. Akad. Wetenschappen C 91(2)*, 177-122.

Lee P.E. & A. Wilkes, 1965. Polymorphic spermatozoa in the hymenopterous wasp *Dahlbominas. Science 147*, 1445-1446.

MacArther R.H. & E.O. Wilson, 1967. *The theory of island biogeography.* Princeton, Princeton University Press, 202pp.

May R.M. & J. Seger, 1985. Sex ratios in wasps and aphids. *Nature 318*, 408-409.

Maynard Smith J., 1978. *The evolution of sex.* Cambridge University Press, Cambridge.

Maynard Smith J., 1984. The Ecology of Sex. In: Krebs & Davies (eds), *Behavioural Ecology. An Evolutionary Approach.* Blackwell Scientific Publications, Oxford.

Michaloud, G., 1982. *Figuiers tropicaux et pollinisation.* (Motion picture film: available from French Consulates). Service du film de Recherche Scientifique, Paris, France.

Michaloud G., S. Michaloud-Pelletier, J.T. Weibes, & C.C. Berg, 1985. The co-occurence of two pollinating species of fig wasp and one species of fig. *Proc. Konink. Ned. Akad. Wetenschappen C 88*, 93-119.

Murray M.G., 1985a. Figs (*Ficus* spp) and fig wasps (Chalcidoidea : Agaonidae): hypotheses for an ancient symbiosis. *Biol. J. Linn. Soc. 26*, 69-81.

Murray M.G., 1985b. Putting the challenge into resource exploitation: a model of contest competition. *J. Theor. Biol. 115*, 367-389.

Murray M.G., 1987. The closed environment of the fig receptacle and its influence on male conflict in the Old World fig wasp, *Philotrypesis pilosa. Anim behav. 35*, 488-506.

Nefdt R.J.C., 1987. *Interactions between Figs and Fig wasps.* Unpubl. B.Sc. (Honours) project. Rhodes University.

Neves L. de Jesus, 1987. Ocorrencia de agente galhador em Flores de *Ficus microcarpa* L.f. *Boletim do Herbarium Bradeanum, iv,*, 327-330.

Newton L.E. & A Lomo 1979. The pollination of *Ficus vogeli* in Ghana. *Bot. J. Linn. Soc. 78*, 21-30.

Noort S. van & S.G. Compton, 1988. Two new species of *Otitesella* (Hymenoptera: Chalcidoidea: Pteromalidae) from *F. burtt-davyi. Proc. Konink. Ned. Akad. van Wetenschappen C91*, 419-427.

Noort S. van, A.B. Ware & S.G. Compton, 1989. Pollinator-specific volatile attractants released from figs of *F. burtt-davyi. South African Journal of Science, 85*, 323-324.

Nunney L., 1985. Female-biased sex ratios: individual or group selection? *Evolution 39*, 349-361.

Owen R.E., 1983. Sex ratio adjustment in *Asobara persimilis* (Hymenoptera: Braconidae), a parasitoid of *Drosophila. Oecologia 59*, 402-404.

Palgrave K.C., 1983. *Trees of southern Africa 2nd ed.* C. Struik Publishers, Cape town.

Parker G.A. & M. Begon, 1986. Optimal egg size and clutch size: effects of environment and maternal phenotype. *Am. Nat. 128*, 573-592.

Pianka E.R., 1970. On r- and K-selection. *Am. Nat. 104*, 529-597.

Price P.W., 1970. Characteristics permitting coexistence among parasitoids of a sawfly in Quebec. *Ecology 51(3)*, 445-454.

Price P.W., 1971. Niche Breadth and dominance of parasitic insects sharing the same host species. *Ecology 52(4)*, 587-596.

Price P.W., 1972. Parasitoids utilising the same host : adaptive nature of differences in size and form. *Ecology 53(1)*, 190-195.

Price P.W., 1973a. Parasitoid Strategies and community Organisation. *Environ. Entomol. 2(4)*, 623-626.

Price P.W., 1973b. Reproductive strategies of parasitoid wasps *Am. Nat. 107*, 684-693.

Price P.W., 1974. Strategies for egg production. *Evolution 28*, 76-84.

Price P.W., 1984. 2nd edition. *Insect Ecology*. John Wiley, New York.

Price P.W., 1980. *Evolutionary biology of parasites*. Princeton University Press, Princeton

Price P.W., C.E. Bouton, P. Gross, B.A. McPheron, J.N. Thompson & A.E. Weis, 1980. Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Ann. Rev. Ecol. Syst. 11*,41-65.

Price P.W. & K.M. Clancy 1986. Interactions among three trophic levels : Gall size and parasitoid attack. *Ecology 67(6)*, 1593-1600.

Price P.W. & H. Pschorn-Walcher, 1988. Are galling insects better protected against parasitoids than exposed feeders? : a test using tenthredinid sawflies. *Ecological Entomol., 13*, 195-205.

Putters F.A. & J. van den Assem, 1985. Precise sex ratio in a parasitic wasp: the results of counting eggs. *Behav. Ecol. Sociobiol., 17*, 265-270.

Putters F.A. & J. van den Assem, 1988. The analysis of Partial Preferences in a Parasitic Wasp. *Anim. Behav. 36*, 933-948.

Ramirez W., 1969. Fig wasps : mechanism of pollen transfer. *Science 163*, 580-581.

Ramirez W., 1970. Host specificity of fig wasps (Agaonidae). *Evolution 24. 680-691.*

Ramirez W., 1974. Coevolution of *Ficus* and Agaonidae. *Ann. Missouri. Bot. Gdn. 64*: 296 - 310.

Ramirez W., 1976. Evolution of Blastophagy. *Brenasia 9*, 1-13.

Ramirez W., 1978. Evolution of mechanisms to carry pollen in Agaonidae (Hymenoptera: Chalcidoidea). *Tijdschr. Ent. 121*: 279 - 293.

Ramirez W., 1981. Evolution of the monoecious and dioecious habit in *Ficus* (Moraceae). *Brenasia 18*: 207 - 216.

Ramirez W., 1987. The influence of the microenvironment - The interior of the syconium-in the coevolution between fig wasps (Agaonidae) and the fig (*Ficus*). In: Labeyrie V., G. Fabres & D. Lachaise (eds) *Insects-Plants*. Dr W. junk Publishers. Dordrecht.

Roitberg B.D. & R.J. Prokopy, 1987. Insects that Mark Host Plants. An ecological, evolutionary perspective on host - marking chemicals. *BioScience 37(6)*: 400 - 406.

Storey W.B., 1975. Figs. In; *Advances in Fruit Breeding*. J. Janide & J.N. Moore eds. Purdue University Press. pp. 568-589.

Strand M.R., 1988. Variable sex ratio strategy of *Telenomus heliothidis* (Hymenoptera: Scelionidae): adaptation to host and conspecific density. *Oecologia 77*, 219-224.

Strong D.R., J.H. Lawton, & T.R.E. Southwood, 1984. *Insects on Plants. Community Patterns and Mechanisms*. Blackwell, Oxford.

Suzuki Y., H. Tsuji & M Sasakawa, 1984.Sex allocation and effects of superparasitism on secondary sex ratios in the gregarious parasitoid, *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) *Anim. Behav. 32*, 478-484.

Taylor P.D. & M.G. Bulmer, 1980. Local mate competition and the sex ratio. *J. Theor. Biol. 86*, 409-419.

Trivers R.L. & D.E. Willard 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science 179*, 90-92.

Ulenberg S.A., 1985. The systematics of the fig wasp parasites of the genus *Apocrypta* Coquerel. *Proc. Ned. Akad. Wetenschappen c 83*, 1 -7.

Valderyon G. & D.G. Lloyd 1979. Sex differences and flowering phenology in the common fig, *Ficus carica* L. *Evolution 33*, 673-685.

Verkerke W., 1986. Anatomy of *Ficus ottoniifolia* (Moracaea) syconia and its role in the fig-fig wasp symbiosis. *Proc. Kon. Ned. Akad. van Wetenschapen C89*, 443-469.

Verkerke W., 1987. Ovule dimorphism in *Ficus asperifolia* Miquel. *Acta Bot. Neerl. 36*, 121-124.

Verkerke W., 1988a. Syconial anatomy of *Ficus asperifolia* (Moracaea), a gynodioecious tropical fig. *Proc. Kon. Ned. Akad. van Wetenschapen C 90(4)*, 461-492.

Verkerke W., 1988b. Flower development in *F. sur* Forsskal (Moraceae). *Proc. Kon. Ned. Akad. van Wetenschapen C91*, 175-195.

Verkerke W., 1988c. Sycone morphology and its influence on the flower structure of *F. sur* (Moraceae). *Proc. Kon. Ned. Akad. van Wetenschapen C91*, 319-344.

Verkerke W., 1989. Structure and function of the fig. *Experientia, 45*, 612-622.

Waage J.K., 1982a. Sib-mating and sex ratio strategies in scelionid wasps. *Ecol. Entomol. 7*, 103-112.

Waage J.K., 1982b. Sex ratio and population dynamics in natural enemies-some possible interactions. *Annals of Applied Biology 101*, 159-164.

Waage J.K., 1986. Family planning in parasitoids: adaptive patterns of Progeny an Sex Allocation. In: Waage J.K. & D.J. Greathead (eds) *Insect Parasitoids*. Academic Press, London.

Waage J.K. & J.A. Lane, 1984. The reproductive strategy of a parasitic wasp. II. Sex allocation and local mate competition in *Trichogramma* Waage J.K., *evanescens*. *J. Anim. Ecology 53*, 417-426.

Waage J.K. & Ng Sook Ming, 1984. The reproductive strategy of a parasitic wasp. I. Optimal progeny and sex allocation in *Trichogramma evanescens*. *J. Anim. Ecology 53*, 401-416.

Waage J.K., Weis A.E. & W.G. Abrahamson, 1985. Potential selection pressures by parasitoids on a plant-herbivore interaction. *Ecology 66*, 1261-1269.

Waage J.K., Weis A.E. & W.G. Abrahamson, 1986. Evolution of host plant manipulation by gall-makers : ecological and genetic factors in the *Solidago-Eurosta* system. *Am. Nat. 127*, 781-694.

Waring L.W. & P.W. Price, 1989. Parasitoid pressure and the radiation of a gall-forming group (Cecidiomyiidae: *Asphondylia* spp.) on creosote bush (*Larrea tridentata*). *Oecologia, 79*, 293-299.

Weis A.E., 1982. Resource utilisation patterns in a community of gall-attacking parasitoids. *Proc. Entomol. Soc. America, 11*, 809-815.

Weis A.E. & W.G. Abrahamson, 1986. Evolution of host-plant manipulation by gall makers: ecological and genetic factors in the *Solidago-Eurosta* system. *Am. Nat., 127*, 681-695.

Wellings P.W., R. Morton & P.J. Hart, 1986. Primary sex-ratio and differential progeny survivorship in solitary haplo-diploid parasitoids. *Ecol. Entomol. 11* 341-348.

Welzen C.R.V. & J.K. Waage, 1987. Adaptive responses to local mate competition by the parasitoids, *Telonomus reames*. *Behav. Ecol. Sociobiol. 21*, 359-365.

Werren J.H., 1980. Sex ratio adaptations to local mate competition in a parasitc wasp. *Science 208*, 1157-1159.

Werren J.H., 1983. Sex ratio evolution under local mate competition in a parasitic wasp. *Evolution 37*, 116-124.

Werren J.H., 1984. A model for sex ratio selection in parasitic wasps : local mate competition and host quality effects. *Netherlands J. Zool. 34(1)*, 81-86.

Werren J.H., S.W. Skinner & A.M., Huger, 1986. Male-killing Bacteria in Parasitic wasps. *Science 231*, 990-992.

Wharton R.A., J.W. Tilson & R.L. Tilson, 1980. Asynchrony in a wild population of *Ficus sycomorus*. *South African Journal Science 76*, 478-480.

Wiebes J.T., 1973. Phylogenetic specificity of fig and fig wasps. In: Branjes N.B.M. (ed), *Pollination and dispersal*, pp. 21-25.

Wiebes J.T., 1977. A short history of fig wasp research. *Gardens Bulletin Singapore 29*, 207-232.

Wiebes J.T., 1979a. Fig wasps from Gabon: new species of *Agaon* (Agaonidae) and *Phagoblastus* (Torymidae) (Hymenoptera Chalcidoidea). *Konink. Ned. Akad. Wetenschappen. (C) 82*: 391 - 400.

Wiebes J.T., 1979b. Co-evolution of figs and their insect pollinators. *Ann. Rev. Ecol. Syst 10*, 1-12.

Wiebes J.T., 1982. The phylogeny of the Agaonidae (Hymenoptera: Chalcidoidea). *Netherlands Journal of Zoology 32*, 295-411.

Wiebes J.T., 1984. Fig wasp-fig Co-evolution. *Antenna 8*, 122-127.

Wiebes J.T., 1986. The association of figs and fig insects. *Revue Zool. Afr. 100*, 63-71.

Wiebes J.T., 1987. Coevolution as a test of the phylogenetic tree. In: P. Horenkamp *et al* (eds), *Systematics and Evolution: a matter of diversity*. Utrecht University. 309-314.

Wiebes J.T., 1989. Agaonidae (Hymenoptera Chalcidoidea) and *Ficus* (Moraceae): fig wasps and their figs, III (*Elisabethiella*). *Proc. Kon. Ned. Akad. Wetenschappen C92*, 117-136.

Wilkes A., 1963. Environmental causes of variation in the sex ratio of an arrhenotokous insect, *Dahlbominus fulininosus* (Nees)(Hymenoptera: Eulophidae). *Can. Entomol., 95*, 183-202.

Wylie H.G., 1966. Some mechanisms that affect the sex ratio of *Nasonia vitripennis* (Walk.) (Hymenoptera: Pteromalidae) reared from superparasitised housefly pupae. *Can. Entomol.*, *98*, 645-653.