

UC Irvine

UC Irvine Previously Published Works

Title

Theoretical and Empirical Studies of Sex Ratios, Mainly in Fig Wasps

Permalink

<https://escholarship.org/uc/item/31g2p9pc>

Author

Frank, Steven A

Publication Date

1983-08-31

Copyright Information

This work is made available under the terms of a Creative Commons Attribution-NonCommercial-ShareAlike License, available at <https://creativecommons.org/licenses/by-nc-sa/3.0/>

THEORETICAL AND EMPIRICAL STUDIES OF
SEX RATIOS, MAINLY IN FIG WASPS

By

STEVEN ALAN FRANK

A THESIS PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

1983

ACKNOWLEDGMENTS

Many people have selflessly offered their ideas, patience, and friendship. First my teachers. Jane Brockmann and her students provided much inspiration with their dedication to the difficult union of theory with empiricism. Jane invested countless hours in reading and commenting on my work over the past three years. Her prodding when I fatigued and striving for clarity of ideas and expression are responsible for any coherency achieved in this thesis. Jane has stood steadfastly by me through difficult times of struggle and growth, for which I am grateful.

Jim Lloyd has turned a gentle and knowing eye toward me. A young man could not ask for a better role model both as a person, and as a truly dedicated scientist and artist. His friendship is a rare gift. I was never able to drop by his office without staying for two hours and discussing fireflies, fig wasps, science, or people and the world in general. The paper on sex ratios Jim allowed me to publish in the symposium he edits yearly for the Florida Entomologist is the foundation for many of the ideas in this thesis, and some of the work that I hope to pursue in the coming years. Without his editorial help, encouragement, and faith, these ideas would never have crystallized. Pauline Lawrence always had time to listen to my ideas and ask probing and creative questions about my research. The roots of my interest in biology are at the University of Michigan, where I was deeply influenced by R. D. Alexander, W. D. Hamilton, and B. S. Low. W. D.

Hamilton introduced me to fig wasps, and has generously shared his ideas during the past few years.

The intellectual milieu of a graduate student is mainly other graduate students. One could not imagine a better office-mate than Jeff Lucas. We spent countless hours in the corner of our cubby-hole waving our arms while discussing science, always striving for the marriage of ideas with facts. More than once Jeff suggested the next step for my ideas when I could not see it. Marty Obin and I worked together on our overhand curves during late-afternoon wiffleball sessions, and wagged our fingers on all subjects through many nights. Pat Murphy always had a patient ear, and Susan Hadley listened often and advised wisely. Kevin Brown and Steve DiMattia have provided steadfast and invaluable friendships over the years. Many others read my work and discussed ideas with me. These include A. Bolten, J. Cohen, T. Forrest, A. Grafen, C. Johnson, J. A. Pounds, S. Skinner, J. Sivinski, and T. J. Walker. J. T. Wiebes made specific identifications of the fig wasps, and provided helpful suggestions.

Financial support for my research included grants from the Alexander Bache Fund of the National Academy of Sciences, the Theodore Roosevelt Fund of the American Museum of Natural History, and two grants from Sigma Xi. The Department of Zoology at the University of Florida provided supplies and a research assistantship. The Everglades National Park granted permission to work within their grounds, and the South Florida Research Center headed by Gary Hendrix allowed me to use their facilities, and offered a friendly atmosphere. The Agricultural Research and Education Center of the University of Florida, in Homestead, provided housing in the summer of 1982, and Van Waddill generously gave lab space, supplies, and kindness.

My parents Irwin and Marilyn Frank have continued to provide stability and an unquestioning faith in me, as well as financial support. This thesis is dedicated to my parents, teachers, and friends who have given me so much.

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGMENTS	ii
ABSTRACT	vii
CHAPTER I: OVERVIEW	1
Introduction	1
Sex-Ratio Theory	2
The Natural History of Fig Wasps	6
CHAPTER II: ASYMMETRIC RELATEDNESS AND LOCAL MATE COMPETITION--THEORY	8
Introduction	8
Intuitive Development of Concepts	9
Formal Arguments and Major Results	10
Definitions and Previous Results	13
Relaxing the Assumptions	17
Further Extensions of the Theory	23
Discussion	24
Asymmetric Relatedness	25
Within-Sex Competition for Limited Resources	27
Some Comments on Genetic Models	31
Relating this Study to Previous Work	33
Summary	38
CHAPTER III: SEX RATIOS OF THE FLORIDA FIG WASPS	39
Introduction	39
Previous Reports on Local Mate Competition	39
Previous Reports on Fig Wasp Sex Ratios	40
Goals and Structure of the Empirical Research	41
Organization of this Chapter	43
Natural History	44
Life Cycles of Florida Figs and Wasps	45
General Methods	51
Locations and Dates	51
Rearing Pollinator Wasps for Experiments	52
Preparing Trees for Study	53
Collecting Figs for Sex-Ratio Counts	56
Handling the Wasps	57

	PAGE
Species Recognition of Fig-Tree Hosts by Pollinators ..	58
Breeding Structure	60
Subpopulation Structure	62
Settling of Foundresses from Local Subpopulations ..	65
Male Pollinator Wasp Mating Preference	78
Summary of Breeding Structure	82
Sex Ratios in Florida Fig Wasps	85
Data from Unmanipulated Figs-- <u>F. citrifolia</u> and	
<u>aurea</u>	86
Sex Ratio versus Foundress Number-- <u>F. citrifolia</u> ...	88
Mechanism of Sex-Ratio Adjustment	96
Genetic Recognition and the Sex ratio--	
<u>F. citrifolia</u>	101
Sex Ratio versus Foundress Number-- <u>F. aurea</u>	106
Discussion of Data and Theory	111
Quantitative Analysis of <u>P. assuetus</u> Sex Ratios	111
Qualitative Discussion of <u>P. jimenezi</u> Sex Ratios ...	122
Thesis Summary	123
Large-Scale Population Structure	123
Population Bottlenecks	124
Patterns of Mating and Mate Competition	124
Conditional Sex Ratios	125
Sex Ratios Conditional on Number of Foundresses	
Expected versus Number Present	127
Control over the Sex Ratio	128
Sex Ratio or Investment Ratio?	129
Efficiency of Theoretical Modeling Exceeds	
Efficiency of Adaptation?	130
Future Directions	130
APPENDIX A: MATHEMATICS FOR LOCAL MATE COMPETITION ...	131
Index of Symbols Used	131
Derivation of Results	133
Variance Terms	137
APPENDIX B: STATISTICAL NOTE	140
LITERATURE CITED	142
BIOGRAPHICAL SKETCH	148

Abstract of Thesis Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Master of Science

THEORETICAL AND EMPIRICAL STUDIES OF
SEX RATIOS, MAINLY IN FIG WASPS

By

Steven Alan Frank

August, 1983

Chairperson: H. Jane Brockmann
Cochairperson: James E. Lloyd
Major Department: Zoology

The area of sex-ratio theory known as 'local mate competition' is analyzed and extended. New models are presented by adding several realistic assumptions to previous work, including (i) different clutch sizes among females, and (ii) different mating-group sizes within a population. Also, following Hamilton's general formulation for local mate competition, insights into several related sex-ratio phenomena are discussed. These include conflict situations over the sex ratio, and the effect of asymmetric relatedness of parents to sons versus parents to daughters on the sex ratio.

The new theory generated in this thesis is applied to data I collected on the sex ratios of Florida fig wasps, Pegoscapus assuetus Grandi and P. jimenezi Grandi, which pollinate the fig species Ficus citrifolia P. Miller and F. aurea Nuttall, respectively. These empirical studies were conducted in the Everglades National Park and on

Key Largo, Florida. Due to their unique natural history, fig wasps are an ideal system for the study of local mate competition in natural populations. The number of female fig wasps colonizing an isolated, locally mating patch (= a fig) was experimentally controlled, and the sex ratio as a function of foundress number was measured. The qualitative agreement between predictions and observations is excellent; however, the observed sex ratios are consistently more female biased than predicted. A theoretical exploration of some factors that may explain this quantitative discrepancy is presented. Two a priori candidates are the most compelling at this time: (i) different clutch sizes among females within an isolated, locally mating group, and (ii) genetic differentiation among subpopulations of fig wasps. Empirical methods for the further study of fig wasp sex ratios are discussed extensively. Additionally, information and discussion is presented on the natural history of Florida fig wasps, their nematode and wasp parasites, the mechanism of sex-ratio adjustment, and breeding structure of the wasps, including subpopulation structure, the number of foundresses per fig and the cues assessed by the wasps when choosing a fig in which to oviposit.

Fig wasps provide an excellent opportunity for evaluating sex-ratio theory and the success of population-genetic models in predicting behavior patterns accurately. The wasps are easily observed, important parameters can be readily quantified in natural populations, and they are amenable to experimentation in the field. Also, since there are 900 fig wasp species, this group provides a rich source of comparative information.



H. Jane Brockmann, Chairperson
Associate Professor of Zoology

CHAPTER I OVERVIEW

Introduction

The subject of sex ratios has received much attention over the past few years. The reasons are compelling: sex ratios are relatively easily measured traits that are highly correlated with fitness. Further, it seems likely that the relative fitness of males and females must vary with a wide range of ecological factors, and variability in the sex ratio both within and among species has been widely documented. Hence, in many ways sex ratios provide an ideal forum for testing the explanatory and predictive power of theories about adaptation.

The goals of this thesis are as follows.

(i) To extend a particular subset of sex-ratio theory, known as 'local mate competition,' in order to generate a set of biologically meaningful and testable hypotheses (Chapter II).

(ii) To apply this extended theory to a relevant data set I obtained while studying the biology of two fig wasp species in south Florida, Pegoscapus assuetus Grandi and P. jimenezi Grandi that pollinate Ficus citrifolia P. Miller and F. aurea Nuttall, respectively (Chapter III).

The remainder of this introductory chapter is devoted (a) to defining the sex-ratio phenomenon discussed in this thesis, local mate competition and asymmetric relatedness of parents to sons versus parents to daughters, within the broader class of sex-ratio phenomena in general, and (b) to introducing the natural history of fig wasps, in particular those features that make fig wasps an excellent model system for the study of local mate competition and asymmetric relatedness.

Sex-Ratio Theory

Darwin (1871) was the first to identify sex ratios as an intriguing puzzle within the framework of natural selection and adaptation. He focused on the problem of why most species have approximately equal numbers of males and females at birth, and possible reasons for the occasional observations of excess males or females. Although there are hints of some of the great debates of twentieth-century evolutionary biology in Darwin's (1871) writing on sex ratios (e.g., population regulation versus individual selection), he arrives at no general conclusions, and states of sex ratios ". . . but I now see that the whole problem is so intricate that it is safer to leave its solution for the future" (Darwin 1871).

The first major development in sex-ratio theory was by Fisher (1930), who argued that parental investment in the sexes over the entire population should be equal. The essence of his idea is that a rarer sex (in terms of investment of resources by parents) would always be favored in a frequency-dependent manner. However Fisher

did not specify some latent assumptions necessary for his equal investment theory. One necessary assumption was given by Shaw and Mohler (1953). They noted that any explanation of natural selection of the sex ratio must assume that the sex ratio has a component that is variable and heritable. They cited the correlation between highly skewed sex ratios and sex-linked genes observed in Drosophila (Novitski 1947) as an example of genetic variability in the sex ratio. Having realized that the mechanism underlying sex-ratio variability is critical, Shaw and Mohler constructed a model assuming that control of the sex ratio is at an autosomal locus. Based on this assumption and a further assumption that males and females are equally costly, they offered a proof that the overall population sex ratio will stabilize at 1:1.

Kolman (1960) extended this result by proving, as Fisher had asserted, that the total expenditure on male offspring should equal the total expenditure on female offspring, when summed over the entire population. His model is an individual-selection model, which is essentially equivalent to an autosomal-allele model (Hamilton 1967). Foreshadowing further developments in sex-ratio theory, Kolman (1960, p. 377) stated, "Moreover, since the selection is only for the total expenditure [over the entire population], only the mean sex ratio is fixed and there is no effect on the variance, that is, a population can have any degree of heterogeneity so long as the totals expended on the production of each of the sexes are equal." Trivers and Willard (1973) recognized that since the variance of the sex ratio among broods was under no direct selective pressure, that local sex ratios would be favored according to ecological correlates of the relative fitness of males and females, under the constraint that the population

expenditure remains 1:1. Charnov (1982) reviews and discusses this pattern in detail.

Two necessary assumptions of the equal investment theory will be discussed. The first is that ". . . the total number of the gene-bearer's grandchildren is a true measure of the propagation of the gene [that controls the sex ratio]" (Hamilton 1967, p. 477). When this assumption is violated, parents are asymmetrically related to sons versus daughters, and asymmetric population investment ratios are predicted. An early example of locally asymmetric investment ratios due to a violation of this assumption was noted by Rhoades (1933), who reported cytoplasmic inheritance of male sterility in Zea mays. In this case, overall population investment ratios were not measured (see Frank 1983 for a discussion of the interaction between locally asymmetric investment ratios due to cytoplasmic elements and the population investment ratio). The several well-documented cases of meiotic drive of sex chromosomes and biased investment ratios in Drosophila (Stalker 1961, White 1973) are analogous to cytoplasmic elements affecting the sex ratio, since in both cases there is a conflict, in terms of fitness, between subsets of the genome and the individual over the investment ratio in offspring (Alexander and Borgia 1978, Hamilton 1979, Frank 1983). The second assumption is that competition for mates among males is random with respect to genotype. The pattern that results when there is both genotypic correlation among competing males and asymmetric relatedness of parents to sons versus parents to daughters (Hamilton 1979) is the topic of this thesis.

Hamilton (1967) was the first to analyze sex ratios when the assumption of population-wide competition among males for mates is violated. The essence of the resulting sex-ratio pattern when competition for mates is non-random is captured in the following scenario. Assume exactly two previously mated diploid females (= foundresses) arrive in a patch and lay four eggs each. The offspring of these foundresses grow to adulthood within the patch, mate randomly among themselves, the males die after mating, and each female flies off to a new patch and lays four eggs. Further, assume the cost of producing a male equals the cost of producing a female, so that the sex ratio and the investment ratio are equivalent. What sex ratio produced by a foundress maximizes her number of grandchildren? Each daughter yields four grandchildren, and each son mates on average with F/M females, where F and M are the total number of females and males in the patch, respectively; so each son yields $4(F/M)$ grandchildren. When each foundress produces two sons and two daughters, each will have eight grandchildren through sons and eight through daughters, for a total of 16. When each foundress produces one son and three daughters, each will have 12 grandchildren through sons and 12 through daughters, for a total of 24. By examining all possible combinations of sex ratios for the two foundresses, it can be seen that a sex ratio of $1/4$ (males/total) is the stable sex ratio in the sense that no other can do better in terms of fitness. The important point here is that competition among brothers for mates can lead to female-biased sex ratios, and that the sex ratio becomes more female biased as the genotypic correlation among males competing for mates increases (hence the name 'local mate competition'). Formal arguments and proofs will be developed in detail in Chapter II.

The Natural History of Fig Wasps

The empirical goal of this research was to collect sex-ratio data from a population with local competition for mates and asymmetric relatedness of parents to sons versus parents to daughters. These data are relevant to quantitative models derived from population genetics theory (in particular, Hamilton 1979). Fig wasps are in many respects ideal for testing the quantitative predictions of these models due to their unique natural history. In bare outline, the life of a fig wasp (Agaonidae, Pegoscapus) that pollinates a monoecious group of New World fig trees (Moraceae, Ficus, subgenus Urostigma) can be summarized as follows.

Fig trees bear inflorescences (= figs) that are hollow receptacles with hundreds of pistillate and staminate flowers opening into a sealed, central cavity. A few female fig wasps (= foundresses) enter the fig through a narrow passage, and once inside can never leave. While laying eggs in the ovaries of approximately one-half of the pistillate flowers they dust the stigma of the flowers with pollen carried from the fig from which they emerged. After egg laying, the females die within the fig, and each larva develops by eating the tissue of a single pistillate flower. Pistillate flowers that do not harbor a developing fig wasp produce one seed each. About one month later the next generation of wingless males emerges and mates with the winged females within the still-sealed fig. After mating, the male wasps chew an exit tunnel through the wall of the fig and die soon afterwards. The females then collect pollen and escape the fig through the exit tunnel to find and enter a receptive fig and begin a new generation (Galil 1977, Chapter III).

Since there are only a few foundresses within each fig, and since all matings occur within the fig before the females disperse, there is competition among brothers for mates in each generation. Also, fig wasps are haplodiploid (females arise from biparental diploid zygotes and males arise from unfertilized eggs) and sib mating leads to asymmetric relatedness of mothers to sons versus mothers to daughters. Since the foundresses die within the fig, the distribution of the number of foundresses per fig can be estimated, and translated into the approximate amount of sib mating and mate competition in each generation. Also, both the number of foundresses within a particular fig and the sex ratio produced in that fig can be measured very accurately in a wild population.

There is much evidence suggesting that females can control the sex of their offspring by the fertilization process (Flanders 1956). So selection acting on the sex ratio produced by individual females under different ecological conditions may result in a sex-ratio pattern at the individual level. Thus quantitative predictions about the relationship between the amount of local mate competition and the sex ratio produced can be tested.

CHAPTER II
ASYMMETRIC RELATEDNESS AND LOCAL MATE COMPETITION--THEORY

Introduction

Numerous factors may affect the sex ratio, as outlined in Chapter I. This chapter is a theoretical exploration of only two of these factors--asymmetric relatedness of parents to sons versus parents to daughters, and local competition among related males for mates. The motivation for reviewing and extending this theory is to facilitate the study of fig wasp sex ratios. However, by following Hamilton's (1979) elegant approach to this problem, very general results can be obtained. From these general results, specific predictions about haplodiploid (e. g., fig wasp) sex ratios, as well as sex ratios for many other types of genetic control, can be obtained. So, the problem will be studied by considering the general phenomena of asymmetric relatedness of parents to sons versus parents to daughters and local competition for mates among related males. With each development of the general theory a specific prediction relevant to fig wasps will be given as an example. These specific predictions will be applied to my empirical work on fig wasps in Chapter III.

Intuitive Development of Concepts

The first factor that will be discussed is the ratio of relatedness coefficients son-parent:daughter-parent, where 'parent' is the parent with the ability to control the sex ratio, and the relatedness is at the locus controlling the sex ratio. A more precise definition of this ratio, denoted by R , will be given later. As an illustration, consider a randomly mating diploid organism. If the sex ratio is controlled at an autosomal locus, son and daughter are each related to mother by $1/2$ and to father by $1/2$, so $R = 1$. If the sex ratio is controlled by a locus on the X chromosome, son and daughter are each related to mother by $1/2$, so $R = 1$ when mother controls the sex ratio; son is related to father by 0 and daughter by $1/2$, so $R = 0$ when father controls the sex ratio.

How does R affect sex ratios? If R is less than one, the parent controlling the sex ratio is more related to daughters than sons, so proportionately more daughters are predicted than if $R = 1$. More precisely, if the predicted sex ratio for autosomal, diploid control ($R = 1$) is x (males/total), then the predicted sex ratio for other types of genetic control (i. e., when R is different from one) is Rx .

The second factor to be discussed is the relatedness among males competing with each other for mates. As an example, let us examine the situation of a female adding an offspring to a deme, where the population is divided into randomly mating demes, and there is no mating between demes. Consider a locus controlling the sex ratio with alternative alleles A and a . (Or equivalently, consider two sex-ratio types, A and a . The use of alleles to denote alternative phenotypic traits is, to a certain extent, a heuristic device. See later section

on genetic models.) If all the males in the deme are AA, what ratio of males and females should an AA female add to this deme? Since all of the sperm that will be produced in the deme contain A, adding sons will not increase the success of the A allele (or A type). Adding a daughter with an A allele will increase the number of A alleles produced, because a daughter will add A-containing progeny directly to the next generation. In general, if a deme will produce a higher proportion of A sperm than the population will produce, the success of an AA female is greatest when producing a female-biased sex ratio. The A sperm of a son must compete for mates with related A sperm within the deme, while A daughters increase the success of the A allele directly by producing progeny with this allele. The name 'local mate competition' is used for the situation in which males compete with relatives for mates. Under these conditions female-biased sex ratios are predicted, and often observed (see Hamilton 1967). The argument would be exactly the same if there were competition within either sex for a limited resource. For example, competition among daughters for a mother's territory has been offered as an explanation for male-biased sex ratios (called 'local resource competition,' Clark 1978). Only local competition for mates is addressed directly in this thesis. However, it appears to be a straightforward step to develop the arguments within the more general framework of within-sex competition for limited resources (cf. Taylor 1981, Charnov 1982, chapter 5). The effect of within-sex competition on sex ratios can be measured by the difference between the proportion of A alleles within the deme and the proportion of A alleles within the population. The degree of differentiation of the deme from the population will be represented by P_{dt} (see next section for details).

In summary, asymmetries in parent-son:parent-daughter relatedness at the locus controlling the sex ratio is represented by R , and the intensity of local mate competition is reflected in P_{dt} . These two factors can be combined to give a general form (Hamilton 1979) for the sex ratio problems that will be discussed:

$$(2.1) \quad r^* = (1/2)RP_{dt}$$

where r^* is the predicted sex ratio (males/total) produced by each female in the population.

Formal Arguments and Major Results

Current work in this area of sex-ratio theory can be divided into two approaches. The first approach was outlined in the previous section, and is based on the work of Hamilton (1979, unpublished). This first approach is followed in this chapter. The second approach is characterized by Taylor and Bulmer (1980). They wrote equations for the fitness of various sex-ratio alleles under certain conditions, and solved for the sex ratio conferring the greatest fitness under these conditions. Further discussion of this approach can be found in Appendix A.

Hamilton (1979, unpublished) derived Equation 2.1 by uniting his own work on coefficients of relatedness and inclusive fitness (Hamilton 1964, 1972) with Price's (1970, 1972; Hamilton 1975) hierarchical analysis of natural selection. Assumptions of this model follow.

(1) The population is divided into an infinite number of demes, each colonized by N females. (Upper case will be used to denote constants, and lower case will be used to denote random variables.)

(2) Females mate within their demes and store the sperm.

(3) Mating is random within the deme (i.e., no correlation of genes identical by descent among uniting gametes within the deme, see Wright 1969).

(4) The deme disintegrates after one generation; the mated females disperse and colonize new demes along with $N-1$ other foundresses to start the next generation.

(5) Each female lays exactly K eggs.

(6) The sex ratio, r^* , is controlled at a single locus. This locus may be autosomal, sex-linked, or cytoplasmic.

(7) Each alternative allele at the sex-ratio locus causes its host to produce a particular sex ratio, when located in the parent with control over the sex ratio.

(8) Problems of heterozygote sex ratios are ignored, and individuals are treated as sex-ratio types (Hamilton unpublished, see later section on genetic models).

(9) The cost of producing a son equals the cost of producing a daughter, hence sex ratio and investment ratio are equivalent.

(10) The only type of within-sex competition is local competition for mates among males.

This set of assumptions will be referred to as 'assumptions A.' Several of these assumptions are violated in any natural population. I will present the predicted sex ratios when certain of these assumptions are not met.

Ideally, one would like to obtain a proof for Hamilton's formula (Equation 2.1) under less restrictive and more realistic assumptions. Such a proof is desirable because Hamilton's approach allows the predicted sex ratio to be obtained directly for various types of genetic control of the sex ratio. Also, it identifies two biologically meaningful phenomena for the study of sex ratios--patterns of parent-offspring relatedness and genetic differentiation among groups. Unfortunately, a general proof has not been obtained yet. I have obtained the predicted sex ratio for haplodiploids with female control of the sex ratio under a more general set of assumptions than those listed above. These results were derived using the techniques of the second approach to sex-ratio models (e.g., Taylor 1981, Taylor and Bulmer 1980). In each case examined, the results I obtained were exactly those of Hamilton's formula under the relaxed assumptions. Since Hamilton's approach is preferable due to its generality and intuitive appeal, I present the results as following from Hamilton's formula. In Appendix A proofs of these results using the methods of the second approach are presented. First some formal definitions.

Definitions and Previous Results

The definition of R for relatedness of offspring to parents is

$$(2.2) \quad R = \frac{2B_{ps}}{B_{ps} + B_{pd}}$$

where B_{ps} and B_{pd} are the "complete" coefficients of relatedness (sensu Hamilton 1972) at the sex-ratio locus of the parent who controls

the sex ratio to son or daughter, respectively. This definition allows R to be determined for any type of genetic control active in either parent (see Table 2.1).

P_{dt} is a measure of the degree of genetic differentiation of a deme from the total population at the sex-ratio locus. It is defined as

$$(2.3) \quad P_{dt} = E(v_d)/V_t$$

where v_d and V_t are the genetic variances (or variance of types) within the deme and within the total population, respectively, and E is the expectation operator (see Appendix A for further discussion of variances). P_{dt} is Wright's index of panmixia for hierarchical population structure (Wright 1969). So, we will be able to take advantage of some useful results for the panmictic index given by Wright (e. g., see Equation 2.21).

Wright's fixation index, F , will be used often in this chapter. F is the correlation between homologous genes of uniting gametes relative to the gene frequencies in the whole population (Wright 1969). If F is positive, the frequency of identical genes (alleles) co-occurring in a diploid zygote will be greater than expected under random mating Hardy-Weinberg frequencies. In other words, the portion of homozygotes will be greater than expected under random mating. For example, if allele A occurs with probability q , and allele a with probability $1-q$, then the expected proportion of AA homozygotes is $q^2 + Fq(1-q)$. Note that when $F = 0$, this yields the standard Hardy-Weinberg frequency under random mating.

Table 2.1. "Complete," or "life-for-life" coefficients of relatedness of parents to offspring. These coefficients are proportional to the inclusive fitness benefit to a parent of an act directed towards a son or daughter. A genetic element on a diploid autosome in a parent is equally likely to be in a son or daughter, so inclusive fitness benefits are equal for acts directed at sons and daughters, and $R = 1$. For elements on a Y chromosome in heterogametic males (or a patrilineally inherited element), $B_{fs} = 1$ and $B_{fd} = 0$, so $R = 2$. For elements on a Y chromosome in heterogametic females (or a matrilineally inherited element), $B_{ms} = 0$ and $B_{md} = 1$, so $R = 0$. The values of B for elements on an X chromosome can be obtained from the table. For example, if females are XX, males XY or XO (as in haplodiploids), and the sex ratio is maternally controlled, then $B_{ms} = 1/2$ and $B_{md} = (1+3F)/(2+2F)$, and $R = (1+F)/(1+2F)$. Definitions and values for B are in Hamilton (1972); applications for sex ratios of differing values of R under different types of genetic control are in Hamilton (1979).

		OFFSPRING	
		XY or XO	XX
CONTROLLING	XY or XO	F	1+F
PARENT	XX	1/2	$(1+3F)/(2+2F)$

Hamilton (1979) derived the predicted sex ratios for various types of genetic control under assumptions A, plus the assumptions that the settling of foundresses into demes is random. The information needed to calculate R as a function of F is given in Table 2.1. Since settling is random, variance within a deme, v_d , is a sampling variance, s^2 , for a random sample from the population. A standard result is $E(v_d) = [(N-1)/N]V_t$, so $P_{dt} = (N-1)/N$. With a value for F we are in a position to obtain Hamilton's (1979) predictions for sex ratios under various types of genetic control, and under assumptions A and random settling. Under assumptions A the mating in each generation is a mixture of random and sib mating, and the entire population mixes randomly between generations. A well-known result in population genetics (Li 1976, p. 245) is that

$$(2.4) \quad F = w/(4-3w)$$

where w is the proportion of sib mating in each generation. Under assumptions A, $w = 1/N$, and $F = 1/(4N-3)$. The relevant result for this thesis is for haplodiploid organisms with maternal control of the sex ratio. Haplodiploidy can be considered equivalent to sex linkage with XX females and XO males, and all loci on an X chromosome (Li 1976). From the above information, $R = (4N-2)/(4N-1)$, and

$$(2.5) \quad r^* = (1/2)[(4N-2)/(4N-1)][(N-1)/N].$$

This result has also been obtained by Taylor and Bulmer (1980) via the methods of the second approach. Their procedure is outlined in the appendix.

Relaxing the Assumptions

New results are obtained when assumptions (1), (5), and (7) are relaxed. These results will be presented first, then I will discuss the effect of changing some of the other assumptions.

To obtain predicted sex ratios from Equation 2.1, we need values for R and P_{dt} . With respect to the R term from the general model, Table 2.1 remains valid in all cases and we need only re-evaluate F from Equation 2.4. First, the unrealistic assumption (1) that all demes are each of the same size N is relaxed. Let demes be of size n with probability d_n ($n = 1, 2, 3, \dots, L < \infty$), and the sum of d_n over all n be equal to one. Maintaining the remainder of assumptions A, the proportion of colonizing sib-mated females contributed to the population from demes of size n is

$$(2.6) \quad w_n = \frac{d_n K(1-r)}{\sum_{n=1}^L d_n K(1-r)} = \frac{d_n}{\sum d_n} = \frac{d_n}{\bar{n}} .$$

Hence, the proportion of sib-mated females in each generation from all demes is

$$(2.7) \quad w = \frac{\sum d_n}{\sum d_n} = \frac{1}{\bar{n}} .$$

For convenience, all summations will be over n , from 1 to L , unless otherwise noted. With this value for w , which is the reciprocal of the

arithmetic mean of the deme sizes, we can obtain F from Equation 2.4, and solve for the predicted sex ratio

$$(2.8) \quad r^* = \frac{1}{2} \frac{(4\bar{n}-2)}{(4\bar{n}-1)} \left(1 - \sum_{n=1}^L \frac{d_n}{n}\right).$$

Notice that the variance term is obtained by replacing N with the harmonic mean of n . The variance term is the expected variance over the distribution of deme sizes (see the note on the expectation of conditional variances in Appendix A).

I now relax assumptions (5) and (7). Assumption (7) states that a particular allele at the sex-ratio locus in the controlling parent causes that parent to produce a certain sex ratio. This implies that the sex ratio is a genetically fixed trait. However, there is much evidence that the sex ratio in parasitic wasps is a phenotypically plastic trait under maternal control (Charnov 1982 and references, Frank 1983, Werren 1983). This phenotypic plasticity may be incorporated into the population-genetic model being developed in the following way. Replace assumption (7) by the assumption that a sex-ratio allele in the active parent produces sex ratios denoted by the vector $\underline{r} = (r_1, r_2, \dots, r_L)$, where r_n is the sex ratio produced in a deme of size n (i. e., the sex ratio is conditional on deme size). It is unlikely that a single allele could have such an effect; however, population-genetic models are truly idealized representations, and if carefully constructed are quite robust against departures from unlikely assumptions of this sort. This is an important point, and will be mentioned again in the discussion at the end of the chapter.

Assumption (5) states that all females produce K offspring. Let us now assume that females produce K_n offspring in a deme of size n , where K_n is a constant. Under these new assumptions, the value for w can be obtained directly from Equations 2.6 and 2.7.

$$(2.9) \quad w = \frac{\sum_d \frac{K_n (1-r_n)}{n}}{\sum_d \frac{K_n (1-r_n)n}{n}}$$

In this new model we are seeking the sex ratio within a deme of size n , so the P_{dt} term we need is actually the expected variance within a deme of size n divided by the population variance. The expected value for this conditional variance is $(n-1)/n$ (see Appendix A). With this information, the predicted sex ratios for each deme size can be calculated. For haplodiploidy and maternal control of the sex ratio

$$(2.10) \quad r_n^* = \frac{1}{2} \frac{(2a-2b)(n-1)}{(2a-b)n} \quad n=1,2,3,\dots,L$$

where r_n^* is the predicted sex ratio in a deme of size n , and a and b are

$$(2.11) \quad a = \sum_d \frac{K_n (1-r_n)n}{2}$$

$$b = \sum_d \frac{K_n (1-r_n)}{4} .$$

From Equation 2.9, w may now be expressed as $2b/a$. Noting that the R term is the same for all r_n^* ($n = 1, 2, 3, \dots, L$), and that R is a function of r_n^* , a quadratic equation in r_n^* can be obtained. Using the quadratic formula, and choosing the root between 0 and 1 yields

$$(2.12) \quad r_n^* = \frac{1}{2} \frac{(x - \sqrt{x^2 - 4yz}) (n-1)}{y} \frac{(n-1)}{n} \quad n=1, 2, 3, \dots, L$$

$$(2.13) \quad \begin{aligned} x &= \sum_n d_n K_n (6n^2 - 4n + 1) / 4n \\ y &= \sum_n d_n K_n (n-1) (4n-1) / 4n \\ z &= \sum_n d_n K_n (2n-1)n / 4n . \end{aligned}$$

One final extension of the theory will be made here. The previous equation assumes that all n females produce exactly K_n offspring in a deme of size n . Clearly, this is unrealistic. Let k_{ni} be the number of offspring by the i^{th} female ($i = 1, 2, \dots, n$) in a deme of size n . Further

$$(2.14) \quad K_n = \frac{1}{n} \sum_{i=1}^n k_{ni}$$

$$(2.15) \quad h_{ni} = k_{ni} / K_n$$

$$(2.16) \quad w_n = \sum_{i=1}^n h_{ni}^2 / n^2$$

where Equation 2.14 is the average number of offspring per female in a deme of size n , Equation 2.15 is the contribution of foundress i relative to the average of all foundresses in a deme of size n , and Equation 2.16 is the expected proportion of sib-mated daughters produced from a deme of size n .

Since the contribution of the i^{th} female is likely to be a stochastic event, let a particular array of (h_{nij}) occur with probability g_{nj} ($j = 1, 2, 3, \dots$) such that the sum of the g_{nj} over all j is one. Thus, the expected proportion of sib-mated daughters produced from a deme of size n is

$$(2.17) \quad \sum_{i=1}^n \sum_{j=1}^{\infty} g_{nj} h_{nij}^2 / n^2 = 1/n' = t_n/n$$

where n' is an effective deme size ($1 \leq n' \leq n$), and t_n is a standardized measure of the expected variance in offspring number per female in a deme of size n . So, the proportion of sib mating in the population is

$$(2.18) \quad w = \frac{\sum_d \frac{K}{n} \frac{(1-r)}{n} \frac{t_n}{n}}{\sum_d \frac{K}{n} \frac{(1-r)}{n} n} = \frac{2b'}{a} .$$

The variance term is obtained by taking the expectation of the conditional variance over the distribution of h_{nij} (see Appendix A), yielding

$$(2.19) \quad P_{dt} = 1 - \frac{\sum_{i=1}^n \sum_{j=1}^{\infty} g_{nj} h_{nij}^2}{n^2} = \frac{n'-1}{n'}$$

which may be interpreted as the expected sampling variance for the effective deme size, n' . From the previous equations it can be seen that r_n^* is now obtained by replacing b with b' and n with n' in Equation 2.10. Solving for r_n^* yields the same result as Equation 2.12 when n is replaced by n' in the variance term and x, y , and z of Equations 2.13 are replaced by

$$(2.20) \quad x' = \sum_{n=1}^L \frac{d K_n \{6n' - 2n'(t_n + 1) + t_n\}}{4n'}$$

$$y' = \sum_{n=1}^L \frac{d K_n (n'-1)(4n' - t_n)}{4n'}$$

$$z' = \sum_{n=1}^L \frac{d K_n (2n' - t_n)n'}{4n'}$$

Charnov (1982, p. 72) presented the same solution for diploids under the assumptions of constant deme size, and variable contributions of foundresses.

Further Extensions of the Theory

The methods employed in this chapter and in Appendix A may be used to extend the theory further. The model developed above (Equations 2.12 and 2.20) assumes that the sex ratio of all foundresses is constant within a deme size. If females varied their sex ratios as well as their contributions (Equation 2.15), a new situation arises. There appear to be two cases; (i) the females within a deme may vary their sex ratios, but they have no information about the sex ratios of the $n-1$ other foundresses; and (ii) the females within a deme may vary their sex ratios, and one or more females have some information about the sex ratios of the other foundresses (Hamilton 1967 recognized these two cases). In case (i) it seems likely that all females within a deme should produce the same sex ratio according to Equations 2.20. This problem is probably easier to analyze using the methods of the second approach described in the appendix. Case (ii) is an interesting problem since it parallels Kolman's (1960) statement concerning no selection of the variance in sex ratio for individuals within the population, only selection of the overall mean population sex ratio. Sex ratios of foundresses with information are best treated as conditional sex ratios. It is clear that in the case of conditional sex ratios, the mean sex ratio in a deme is not the same as in the case of unconditional sex ratios (Werren 1980a). In other words, the mean sex ratio in a deme does not have the same stability properties as the mean sex ratio in a randomly mating population. A general solution for case (ii) has not been obtained; the methods of the appendix are suggested as a starting place.

An implicit assumption in the models with phenotypic plasticity which I have developed is that the foundresses can accurately assess the number of other foundresses. Hence, these models are in fact conditional sex-ratio models. However, notice that when offspring number per foundress was allowed to fluctuate, the expected variance was used to derive Equations 2.20. Deciding whether the predicted sex ratios ought to be conditional on the expected value of a parameter, or on local levels of that parameter, is a difficult problem. The extent to which a model accurately describes a natural situation depends on how well the model reflects which information an animal is actually able to assess and use. These models suggest the kinds of information that are valuable to an organism with respect to its fitness, and thus which information an organism "ought" to assess if it can. Only by empirical research can we discover whether sex ratios are conditional on a certain parameter in any given species.

Discussion

The work in this chapter is synthesized and extended in four sections. (i) Asymmetric relatedness is briefly discussed, including the problem of expected versus conditional values of R . (ii) Local mate competition is discussed within the larger framework of within-sex competition for limited resources. Some generalities implicit in the models derived in this chapter are mentioned, and avenues leading to fruitful extensions of the theory are suggested. (iii) The robustness of population-genetic models for the study of behavior patterns is examined. (iv) The theoretical work in this chapter is related to

previous work on similar sex-ratio problems. Throughout the discussion several of assumptions A and other latent assumptions are examined.

Asymmetric Relatedness

'Asymmetric relatedness' describes the ratio of inclusive fitness to an individual for a unit of investment in male offspring versus female offspring (Table 2.1). Using the notation in this chapter, $(1/2)R = B_{ps} : B_{pd}$; or, R is the ratio of inclusive fitness of sons to daughters for the allele at the sex-ratio locus in the controlling parent. R embodies two types conflict. First, mother and father (or mother and daughter in social Hymenoptera, Trivers and Hare 1976) will have different expected fitnesses for the sex ratio of their offspring. For example, a haploid male gains nothing when his mate produces a son, and he passes his entire genome to daughters. Second, different parts of the genome have different fitness valuations for sons and daughters, as Table 2.1 clearly expresses. Both of these points are well known (Hamilton 1972, 1979, Charnov 1982, Frank 1983, Beckenbach 1983). What I wish to stress here is that when R varies, it varies as a function of F, the probability of alleles on uniting gametes being identical by descent (F = 0 implies random mating). This leads to two interesting observations.

In Equation 2.10, $R = 2(a-b)/(2a-b)$. When all the deme sizes are very large, R is very close to one, and $r_n^* = (1/2)(n-1)/n$. This is the standard result for diploid organisms (Hamilton 1967, confirmed for variable deme sizes in this chapter), so when F is small, haplodiploid females are similar to diploid females in their valuations of sons and daughters. Two interesting predictions emerge. (a) If the deme sizes

are usually large, but occasionally a small deme occurs, haplodiploid foundresses in a small deme should behave like diploid foundresses, since the expected value of R is near 1. (b) If the demes sizes are usually quite small, but occasionally a larger deme occurs, the foundresses in a large deme are predicted to produce an extremely female-biased sex ratio, since the expected value of R is close to its minimum value of $2/3$. Prediction (b) is especially interesting since it describes quite well the deme structure of Melittobia (van den Assem et al. 1982 and references), and provides a partial explanation for the extremely female-biased sex ratios of Melittobia that have been a mystery (see also the section on population structure below).

The second observation follows from the above discussion of conditional versus unconditional sex ratios. The value for R discussed so far has always been the expected value of R . Clearly, R is a random variable. If the individual controlling the sex ratio can obtain some information about its own R , then it should produce sex ratios conditional on this information. Care must be taken when considering the mechanism, since there are inherent conflicts over the values of R within the genome. Seger (1976) discusses some general implications of self-assessment of F , and possible mechanisms (e. g., average relative homozygosity within an individual). The methods described in this chapter are probably easily adapted to modeling conditional sex ratios of this sort.

Much has been written about sibling interactions as determinants of sex ratios (e. g., Taylor 1981, reviewed by Charnov 1982). From this discussion it is clear that sib mating may affect sex ratios through R by making F greater than O . Sib mating does not affect sex ratios when

R is independent of F (as in diploids). Taylor (1981) and Charnov (1982, p. 70) noted that sib mating is not essential for biased sex ratios. The important point is that sib mating is just one way to obtain an F greater than 0.

Within-Sex Competition for Limited Resources

The arguments in the previous section demonstrated that one component of biased sex ratios is F, the probability of alleles on uniting gametes being identical by descent. The effect of F is described in the general model (Equation 2.1) by R. In this section within-sex competition for limited resources, which is the second component of the general model, is discussed.

When the genetic variance among a group of competitors within a sex is less than the genetic variance in the population, the sex ratio is likely to be affected. Under local mate competition the fecundity of a daughter is independent of the total number of daughters in the deme, while sons compete with a relatively homogeneous group of suitors for a fixed number of females. The degree of relative homogeneity among males determines the ratio of fitness valuations of a son to a daughter for the controlling parent. For example, if all males in the group are identical, then when there are enough males to inseminate all the females, the fitness valuation of further sons is 0. Local resource competition is a similar situation with the roles of males and females reversed (Clark 1978, reviewed by Charnov 1982).

The relative genetic homogeneity among competitors (actually, its expected value) was described in the general model by P_{dt} . Equations 2.12 and 2.20 were derived assuming random settling of foundresses, and thus P_{dt} is less than one due to the sampling variance bias (equivalently, due to competition among both brothers and unrelated males within small groups). Note that this is only one way of obtaining a P_{dt} value less than one, and competition among brothers is not required.

This general approach (Hamilton's use of the Price equation) can be expanded to cover cases of (i) local resource competition or (ii) local resource competition and local mate competition. The necessary change in the current model is a redefinition of the fitness of a sex-ratio allele as a function of the sex ratio it produces (see Hamilton 1975, unpublished, for details of the Price equation). With this new fitness function, the solution of the predicted sex ratio will likely be a function of P_{dt} .

Three more points about P_{dt} . (i) Population-wide random settling between generations is unlikely. By applying Wright's (1969) detailed study of population structure, some interesting insights emerge. (ii) P_{dt} in the above models represents the expected variance within a deme. This variance is a random variable in nature. Sex ratios conditioned on the actual within-group variance will differ from the unconditional sex ratios of these models. (iii) The above models assume that interactions among competing males for mates are random within a deme, and that no other form of intra-sex or inter-sex competition or cooperation exists. If there are other types of intra- or inter-sex cooperation or competition, then the predictions must be modified since

the reproductive values of sons and daughters will be affected. These three cases are relevant to both local resource competition and local mate competition. I discuss them mainly in the context of local mate competition.

Numerous useful results about population structure and mating systems are summarized by Wright (1969, chapter 12). I discuss only one here. When P_{dt} is less than one, some differentiation among demes exists. Greater resolution of the population structure may be obtained, since

$$(2.21) \quad P_{dt} = P_{ds} P_{st}$$

where the subscript 's' represents subpopulations -- a level of organization between demes and the population. For example, settling of foundresses into demes from a small, local subpopulation may be random or correlated, and in addition the subpopulations may be quite distinct due to either selection or drift. This differentiation among subpopulations would further increase the skew in the sex ratio. This population structure very likely describes Melittobia, adding an additional component that aids in explaining their extremely skewed sex ratios. See Alstad and Edmunds (1983) for a relevant discussion of adaptation to a host and associated differentiation of demes in scale insects, and Wade (1978, 1982 and references) and Wilson (1980) for further discussion and empirical evidence of population structure and its expected effects. Also, note that the value of F discussed under 'asymmetric relatedness' will be affected by population structure. Thus, when R is a function of F , R and P_{dt} are not independent.

P_{dt} is the ratio of the expected variance within a deme to the population variance (Equation 2.4). The models of Equations 2.12 and 2.20 assume that a foundress can accurately assess the number of other foundresses, and use this information to estimate the expected variance within the deme. She then produces a sex ratio conditional on this expected variance. If a foundress can assess some cues that will provide a better estimate of the actual genetic variance in her deme than number of foundresses, she "ought" to produce a sex ratio based on this conditional estimate (Hamilton 1967 suggested this in slightly different terms). Genetic recognition is a complex subject, since different loci within a genome will be asymmetrically related to the same loci in another genome (Blaustein 1983 and references). In summary, different subsets of the genome will disagree over the genetic variance within the deme, and hence the best sex ratio.

An implicit assumption in the above models is that the intensity of competition among males (or within a sex) is independent of relatedness. If this assumption is violated, then the above models need to be altered. For example, suppose a lone male has very little chance of successfully mating, while groups of brothers obtain most of the matings. Clearly, the sex ratio will be affected (Alexander and Sherman 1977). This phenomenon might be modeled by adjusting the fitness valuations of sons and daughters, which is a function of the sex ratio.

Some Comments on Genetic Models

Population-genetic models that examine a certain behavioral trait (or "strategy" Verner 1965, Hamilton 1967) usually assume that the trait is genetically determined at one or a few loci. The allele determining the specific strategy that has a fitness greater than or equal to all other alleles which determine alternative strategies is said to be "unbeatable" (Hamilton 1967), or an "ESS" (Maynard Smith and Price 1973). However, genetic variability for the sex ratio appears to be rare (reviewed by Charnov 1982); and when it is found, the associated genes are often in conflict with the majority of the genome (outlaws in the sense of Alexander and Borgia 1978, see Frank 1983). Also, it seems unlikely that an effect of a single allele would be the ability to assess the number of other foundresses, or genetic variance within a group, or other important cues, and then adjust the sex ratio of its host accordingly (i. e., an allele that codes for phenotypic plasticity, or a "conditional strategy" in the sense of Dawkins 1980, as in Equations 2.12 and 2.20). With this difficulty in mind, of what value are such models? More specifically, how robust are these models against various sorts of departures from the one allele for one strategy assumption?

The robustness can be analyzed within the framework of the levels of selection. Hamilton (1967) noted that number of grandchildren is a true measure of the propagation of an autosomal gene. That is, the number of copies of an autosomal gene is directly proportional to the number of grandchildren. Hence, all autosomal alleles have identical fitnesses with respect to sex-ratio strategies, and the fitness of an autosomal allele equals the fitness of the individual bearing that

allele (in the sense of Frank 1983). Thus, I prefer to view autosomal-allele models as equivalent to individual reproductive-success models, or, that the "realized" level of selection is the individual.

Many of the implicit assumptions when equating single-locus models with individual-level models have been summarized in this chapter. The most obvious one is non-autosomal control of the sex ratio (Hamilton 1967). The different values of R obtained from Table 2.1 express inherent conflicts within the genome. Also, there are conflicts between the parents, or between parent and offspring, over the value of R . The resolution of these two conflicts seems to depend on whichever genetic entity, or which individual, can usurp control of the sex ratio (e. g., Crow 1979 on genetic control of sex ratio in Drosophila; Trivers and Hare 1976, Alexander and Sherman 1977 on parent-offspring conflict in social Hymenoptera). Also, R is usually taken as expected relatedness. If some form of genetic recognition occurs (and sex ratios are conditional on this information), different portions of the genome are favored asymmetrically--even different autosomes will not gain the same benefits in terms of inclusive fitness (Hamilton 1964). There has been much discussion about this subject recently (e. g., Alexander 1979, Blaustein 1983, Dawkins 1982, Holmes and Sherman 1982). If genetic recognition is used as a cue for sex ratios, then the expected relatedness over a portion of the genome (e. g., over all autosomes) may be used, or some smaller subset of the genome may be able to usurp control of the sex ratio to its own advantage.

In the models developed in this chapter females are treated as sex-ratio types (assumption 8), rather than being either a homozygote or heterozygote at the sex-ratio locus. Taylor and Bulmer (1980) showed

settling model. However, in order for the Price equation approach to yield the correct solution, types must be assumed in order to obtain the proper variance factor (P_{dt}) (Hamilton 1979, unpublished). Thus, the variances in this chapter are all variances of the proportion of types of foundresses per deme, not truly genetic variances at a single locus. Perhaps a reason that this assumption is necessary is that by using expected values for relatedness and variances throughout the derivations, the realized level of selection is either a cytoplasmic particle, a chromosome, or the individual; hence types are more appropriate than alleles at a locus for calculating variances.

Relating this Study to Previous Work

Throughout this chapter I have alluded to previous work on related sex-ratio problems. Here I review the relevant literature and its relationship to this study.

Three sets of existing theory apply, (i) within-sex competition, (ii) conflicts over the sex ratio (R), and (iii) conditional sex ratios.

Within-sex competition. Charnov (1982, chapter 5) reviews and extends a theory he classifies as "sex ratio in spatially structured populations." The defining characteristic of this family of theory is that within-sex competitive or cooperative interactions are not random with respect to genotype. [This is my definition based on the ideas summarized in this thesis; this definition is equivalent to the ideas expressed in Alexander and Sherman (1977)]. This definition may be extended to cases in which between-sex interactions will also affect sex

ratios (Taylor 1981, Pickering 1980).] Within this family several specific models have been generated; Charnov (1982) places these specific models into three groups.

(i) Local mate competition. There has been some controversy (and confusion) over just what is the essential feature of local mate competition that causes female-biased sex ratios. The original formulation identified the problem by recognizing a latent assumption in Fisher's (1930) equal investment theory (Hamilton 1967). This assumption was that there exists population-wide (random) competition for mates among males. The problem was discussed within the following scenario. Mating within local groups founded by a few females, followed by dispersal of mated females to found new colonies (Hamilton 1967). In this scenario, both competition among related males and inbreeding occur. Hamilton (1967) implicitly stated that biased sex ratios were due to mate competition, and that an ecological correlate of mate competition is inbreeding. Maynard Smith (1978) provides a model with a correlation between inbreeding and biased sex ratios. However, inherent in this model is a correlation between inbreeding and competition among related males for mates. Taylor and Bulmer (1980), Colwell (1981), Taylor (1981), and Charnov (1982), show that inbreeding is not required for biased sex ratios in these types of models. Colwell (1981) and Wilson and Colwell (1981) suggest that group selection is responsible for biased sex ratios in the local mate competition models. [Hamilton (1975, p. 26) explicitly mentioned that the biased sex ratios in his 1967 paper can be viewed as group selection, and his 1979 approach is explicitly a group selection approach.] Whether group selection (i.e., differential productivity of groups) is essential for female-biased sex

ratios in this scenario is largely a semantic issue. Alexander and Sherman (1977, p. 495-96) capture the simple essence of local mate competition. "Local mate competition refers to mating rivalry among genetic relatives. Its effects may derive from such competition not only between siblings or between parent and offspring, but also among more distant relatives, for mates that may be either related or unrelated to those competing for them." [Charnov (1982, p. 69) and Colwell (1981, p. 402) both incorrectly cite Alexander and Sherman (1977) as stating that the effect of local mate competition is due to inbreeding.]

(ii) Local resource competition. Clark (1978) considered a case in which daughters inherit a mother's territory, hence there is competition among daughters for a local resource. This situation is qualitatively the same as local mate competition, with the roles of males and females reversed.

(iii) The haystack model. In these models (Bulmer and Taylor 1980, Wilson and Colwell 1981) one or more fertilized females found a patch, followed by several generations of no dispersal and mating within the patch. If resources are unlimited, there is no competition among females (i. e., no local resource competition), but there is competition among males (local mate competition). If resources are limiting, both local mate competition and local resource competition occur.

Conflicts over the sex ratio. There are two types of conflict, within a genome (Table 2.1) and between individuals (e. g., father versus mother, Table 2.1).

Different parts of the genome have different values of R (ratio of relatedness parent-son:parent-daughter). The major theoretical contributions to intragenomic conflict and the sex ratio are due to Hamilton (1967, 1979), and have been dealt with directly in the development and discussion of the models in this chapter. Some further theoretical considerations and references can be found in Frank (1983) and Charnov (1982).

Conflicts between parents over the sex ratio are described in Hamilton (1979) and in the development of the theory in this chapter. Another type of conflict between individuals over the sex ratio is between mother and daughter in social Hymenoptera. Trivers and Hare (1976) pointed out that mothers are equally related to sons and daughters, hence a mother favors a 1:1 investment ratio. Daughters are related to sisters by $3/4$ and brothers by $1/4$, and so favor a 1:3 (males:female) investment ratio. Alexander and Sherman (1977) criticize Trivers and Hare on several points, two of which are germane to the theory in this chapter. Trivers and Hare (i) assume that females only mate once in order to derive coefficients of relatedness, and (ii) they assume no local mate competition. Alexander and Sherman's arguments are strictly qualitative. Quantitative predictions follow directly from Equation 2.1, since R is the relatedness factor discussed by Trivers and Hare which depends on singly mated females, and P_{dt} is the effect of local mate competition on the sex ratio. Table 2.1 provides R for singly mated mothers; values of R for multiply mated mothers and for daughter-daughter and daughter-son are required to derive specific quantitative predictions. These quantitative predictions will aid in evaluating Trivers and Hare's hypothesis about power asymmetries between

mother and daughter, and Alexander and Sherman's criticisms due to the neglected effects of multiple matings and local mate competition.

Conditional sex ratios. A conditional strategy is a set of rules (evolved or learned) that govern the behavior of an individual which depend on information from the environment. Essentially, if condition A holds, then follow strategy a, and if condition B holds then follow strategy b, describes conditional behavior (Dawkins 1980). Trivers and Willard (1973) were the first to apply this sort of thinking to sex ratios. They noticed that under certain local conditions males are sometimes favored over females, and at other times females are favored over males. For example, in polygynous species with strong male-male competition, a male with median reproductive success will have a lower reproductive success than a median female. This is due to almost all of the male reproductive success being concentrated in a few highly successful males, while most females enjoy some reproductive success. So, if local conditions are poor, females are favored over males, and if conditions are excellent, males are favored over females.

Good evidence for conditional sex ratios was found in an experiment on Lariophagus distinguendus (Pteromalidae) (Charnov et al. 1981) in which males were favored in relatively small hosts, and females in relatively large hosts. The authors suggested that this shift is due to a greater increase in fecundity with size for females than in mating success with size for males. Charnov (1982) reviews other studies on conditional sex ratios (see also Werren 1980a).

Summary

Several testable models have been derived in this chapter. When applying the theory to a natural population, care should be taken to tailor a testable model specifically to the natural population under study. Throughout the chapter many suggestions for alterations of the basic models were suggested. Many of these extensions of the theory provide a suitable forum for testing some of the more subtle and exciting hypotheses of current evolutionary theory. For example, if genetic recognition is occurring in a certain organism, then the predicted sex ratios will often vary in a manner different from sex ratios not conditioned on genetic-recognition information. Another exciting area for research is the inherent conflicts within a genome over the sex ratio, and how these conflicts are "fought" and resolved. Perhaps the greatest advantage in studying sex ratios is that the possible outcomes are easily specified, they are relatively easy to measure empirically when compared to other traits related to fitness, and identifying a likely functional relationship between the fitness of a genetic entity and its expressed sex ratio is also not difficult. The approach outlined in this chapter has the advantage of being general while maintaining the ability to generate very specific, testable hypotheses. In the following chapter this theory is applied to my empirical work on fig wasps.

CHAPTER III
SEX RATIOS OF THE FLORIDA FIG WASPS

Introduction

This chapter is divided into six sections, (i) a review of relevant literature on local mate competition and sex ratios, (ii) the goals and structure of the empirical research, (iii) the natural history of fig trees and fig wasps, (iv) experiments and discussion of the breeding structure of fig wasps as it relates to sex ratios (Chapter II), (v) sex-ratio data collected for fig wasps from Florida, and (vi) a discussion of these data in light of the theory presented in Chapter II. The motivation for the empirical work is the theory of Chapter II and the previous studies reviewed in the next section.

Previous Reports on Local Mate Competition

Hamilton (1967) and Charnov (1982) reviewed the literature and reported cases in haplodiploids in which sib mating and female-biased sex ratios occur. These cases are usually taken as qualitative support of local mate competition theory. In a few studies, data are presented in which correlates of the amount of inbreeding and the amount of local mate competition (F and P_{dt} , respectively, from Chapter II) within a population are reported with sex-ratio data (Salt 1936 for Trichogramma evanescens; Wylie 1965, Velthuis et al. 1965, Walker 1967, and Werren

1980a,b, 1983 for Nasonia vitripennis; Hamilton 1979 for Blastophaga sp.; Zaher et al 1979, Wrensch and Young 1978, for Tetranychus spp.). Only Hamilton (1979) and Werren (1980a, 1980b, 1983) actually interpreted their data with respect to local mate competition theory. Hamilton (1979) found only a very slight increase in the sex ratio (males/total) with increasing deme size. Werren (1983) interpreted his results as being qualitatively consistent with local mate competition theory for haplodiploids (Hamilton 1979, Taylor and Bulmer 1980). The above within-species studies [except Hamilton (1979), see below] were conducted in the lab, and the number of female wasps or mites per host was used as an indication of the local level of inbreeding and mate competition. In addition to a lab study Werren (1983) also found a positive correlation between wasp density (and presumably local mate competition) and sex ratio in field samples, as predicted. In this chapter empirical studies on natural population of fig wasps addressing local mate competition theory will be described.

Previous Reports on Fig Wasp Sex Ratios

Hamilton (1979) studied the sex ratio of the pollinator wasps (Blastophaga sp.) occupying a fig belonging to the section Americana of the subgenus Urostigma. He reared the pollinator wasps from 37 maturing figs, measured the sex ratio, and estimated the number of foundresses (ovipositing females in a fig) by examining their remains within the fig (see Chapter I and below for life cycle of figs and wasps). The overall mean sex ratio was 0.09 (males/total), with a slight, although steady, increase in the sex ratio with increasing foundress number (mean sex ratio for one-foundress figs was 0.05, for five-foundress figs, 0.12;

Hamilton 1979, Fig. 6). These sex ratios were significantly less than predicted by his theory based on local mate competition and asymmetric relatedness. Hamilton proposed three hypotheses to explain the discrepancy between theory and observation. (i) The foundresses produce unequal clutch sizes, as proposed in Equations 2.20. (ii) There is a disadvantage to an allele of not being able to recombine when in a haploid male, thus reducing the reproductive value of males and lowering the predicted sex ratio. (iii) The sex ratio is controlled by a symbiont with ovariole (matrilineal) transmission. There are insufficient data for a detailed analysis of these hypotheses.

Hamilton (1967) cites Grandi (1929, not seen) as reporting 22 males out of 257 total progeny for a sex ratio of 0.086 in Blastophaga psenes, the pollinator of the edible fig, Ficus carica. Joseph (1958) reports that the sex ratio of B. psenes normally varies between 9 and 18 males per 100 females (sex ratio of 0.083-0.153). Ramirez (1970a) states that males usually comprise less than 15% of the total progeny in New World Blastophaga. Galil and Eisikowitch (1971) report a mean sex ratio of 0.4 for Blastophaga quadraticeps (an Old World Blastophaga). They provide some possible "good for the survival of the species" explanations of the sex ratio. Neither Grandi, Joseph, Galil, nor Ramirez discuss a correlation between number of foundresses and sex ratio.

Goals and Structure of the Empirical Research

The ideal structure of empirical research is often given as (i) assumptions, (ii) a priori hypothesis (or model), (iii) collection of data, and (iv) inference. The goal is falsification or (temporary)

acceptance of the model. My a priori intention was to test sex ratios conditional on deme sizes against a model (Equation 2.5) that I incorrectly supposed predicted such conditional sex ratios. Hamilton (1979), Charnov (1982), Werren (1983), and Frank (1983) all incorrectly apply the unconditional model to sex ratios conditional on deme size, although Frank (1983, note 10) pointed out the discrepancy. A posteriori I recognized that Equation 2.5 was quantitatively incorrect since it was an unconditional prediction, although qualitatively quite similar to the appropriate conditional models derived in Chapter II. So, using the data presented in this chapter to test the quantitative predictions of the conditional models of Chapter II is formally an a posteriori test. The data may also be used to test the a priori qualitative prediction of Equation 2.5 that sex ratio increases with increasing deme size. In summary, the empirical evidence that will be presented in this chapter is strong support from a natural population of the qualitative predictions of local mate competition theory as developed by Hamilton (1979). In addition, the data collected are used to help build a more realistic model and explanation of the observed variability in fig wasp sex ratios. This a posteriori model building is an attempt to both understand the biology of Florida fig wasps and their sex ratios and to improve our ability to predict the sex ratios of the approximately 900 species of fig wasps as yet unstudied. Also there exist numerous other organisms that exhibit the postulated population breeding structure and with various sorts of conditional strategies and genetic control over the sex ratio.

An ideal quantitative test of the theory in Chapter II requires data or assumptions on the following: (i) the value of F for calculating R ; (ii) the level of local mate competition, measured as P_{dt} ; (iii) the type of genetic control over the sex ratio; (iv) the local levels of the cues which the wasps assess and to which they adjust their sex ratio, i.e., whether the sex ratio is unconditional or conditional on cues correlated with R and/or P_{dt} ; and (v) the conditional sex ratios of the wasps under different conditions of the cues assessed.

Organization of this Chapter

The remainder of this chapter is organized as follows. (i) The natural history of the Florida fig trees and fig wasps is reviewed. (ii) The general methodology used to study fig wasp breeding structure and sex ratios is presented. (iii) An experiment examining species recognition of fig-tree hosts by pollinators is related. (iv) Data on the number of foundresses (= colonizing female wasps) per fig (= deme) in the wild, unmanipulated populations used for experimentation are reported. (v) Three choice experiments are described which determine whether a strong preference by foundresses exists for either (a) entering an unoccupied receptive fig versus entering a receptive fig that has already been entered by another foundress, (b) entering an unparasitized fig versus a parasitized fig, or (c) entering a fig containing foundresses that are genetic relatives versus a fig containing non-relatives. (vi) An experiment on male wasps to determine if they prefer to search for mates within the fig in which they were

born or another fig when presented with a choice is discussed. (vii) Data on sex ratios from wild, unmanipulated figs are reported. (viii) Manipulation experiments yielding data on sex ratio and foundress number are presented. (ix) Data from a manipulation experiment in which some of the foundresses in each fig were sibs is contrasted with the manipulation experiment (viii) in which no co-foundresses were sibs. These data are analyzed in light of the qualitative prediction of sex ratios conditioned on genetic recognition mentioned in Chapter II.

Natural History

Knowledge of the unique and complex life cycles of figs and their pollinator wasps and parasites is crucial for understanding the sex ratio of the pollinator wasps. In this section the natural history of the Florida figs and fig wasps is summarized, and references are given to more complete discussions of other species.

There are approximately 900 species of fig trees comprising the genus Ficus (Moraceae). Each species of fig tree appears to have its own unique species of pollinator wasp (Agaonidae) (Wiebes 1979, in press, Janzen 1979a). Ficus has been subdivided taxonomically (e. g., subgenera, sections, etc.), as has Agaonidae (Ramirez 1974, Wiebes 1979, 1982). Since each fig species-fig wasp species symbiosis is a delicately intertwined and unique relationship, there is tremendous diversity and a potentially rich source of comparative information within the fig-fig wasp complex. The biological diversity of the 900 symbiotic relationships corresponds roughly to the taxonomic subdivisions of both the trees and the wasps (Ramirez 1974).

There are two species of native figs in south Florida, Ficus aurea Nuttall and F. citrifolia P. Miller. These two species are pollinated by the agaonids Pegoscapus jimenezi Grandi and P. assuetus Grandi, respectively (Wiebes in press). [The two species of agaonids were previously considered one species, Secundeisenia mexicana Ashm. (Butcher 1964).] A botanical description and review of the literature for both F. aurea and citrifolia can be found in Condit (1969), and in Tomlinson (1980). Both species range through the West Indies, and perhaps to Central or South America, although fig taxonomy is notoriously confusing. The range maps for the two species in Florida given by Brockman et al. (1968) agree with my observations.

Life Cycles of Florida Figs and Wasps

The life cycles of F. aurea and F. citrifolia and their respective pollinators are identical except for the species of parasites attacking each fig tree-fig wasp species pair. So, this description applies to both species of fig trees unless otherwise mentioned (see Ramirez 1974 for diversity of life cycles among fig species). In order to describe the life cycle, let us arbitrarily begin at the stage in which already mated female fig wasps are searching for receptive figs to enter. 'Fig wasps' refers to the agaonid pollinators, and will sometimes be denoted by 'pollinators' or 'pollinator wasps.' 'Fig' refers to the inflorescence of fig trees, which contains hundreds of staminate (= male) and pistillate (= female) flowers lining a hollow, central cavity.

A single, narrow passage leads from the outside of the fig to the central cavity, and is called the 'ostiole.' Young figs appear as bud-like structures on newer twigs. After about 15-20 days, these young figs have developed to a stage in which the female flowers are ready to be pollinated, the central cavity is well defined, the male flowers are still immature, and the ostiole is passable to prospective pollinators. This stage of fig development will be referred to as 'receptive.' When a fig wasp finds a receptive fig, she antennates the ostiole. If she decides to enter, she forces her way through the narrow ostiole, often losing her wings and antennae in the process. After the first foundress (= pollinator that has entered a receptive fig) enters, the ostiole begins to tighten and is impassable in 24-48 hours (pers. obs.). A fig typically has 1-4 foundresses, with an average of about 2 (see below for details). If no foundresses enter a fig, the fig remains receptive to prospective foundresses for about 10 days (pers. obs.).

The ostiole is a one-way passage, so once inside the fig, a foundress can never leave. The stigma of the pistillate flowers are intertwined to form a continuous mat on which a foundress walks. She probes down the styles of the pistillate flowers with her ovipositor. The pistillate flowers exhibit a dimorphism in style length; the morphs are referred to as 'short-styled' and 'long-styled.' If she can reach the ovary of a flower, she lays a single egg in that flower. The developing larva eats the tissue in the ovary, so that flower never gives rise to a seed, and ultimately harbors one wasp. Among the long-styled morph, the foundresses are rarely able to reach the ovary and oviposit, so these flowers usually develop seeds.

In aurea, the central cavity is 1.5-2.5 mm in diameter, and the entire fig is 4-6 mm in diameter at this receptive stage. There are 40-120 short-styled flowers per fig (judging from the rearing data given below), and probably about the same number of long-styled flowers per fig. These estimates are approximate 80% confidence intervals based on visual estimates of thousands of figs (see Hill 1967 for actual counts of the number of long- and short-styled flowers in several species of Hong Kong figs). There are about 20-30 staminate flowers per aurea fig. In citrifolia, the central cavity is 2-3.5 mm in diameter, and the entire fig is 5-8 mm in diameter at the receptive stage. There are 175-350 short-styled flowers and about the same number of long-styled flowers per fig, and approximately 25-40 staminate flowers.

After each oviposition a foundress removes pollen, which she collected in the fig in which she was born, from two thoracic (mesosternal) pockets. This pollen is dusted from the forelegs directly onto the subgenital plate, and then the plate is dragged over the stigma as she begins to search for a new flower in which to oviposit. The complex pollination ritual and associated morphology of the two species will be published elsewhere (Frank in prep.). The pollination ritual of the Florida species is quite similar to the descriptions of two Costa Rican species given by Galil et al. (1973), although clear differences exist. For example, the Costa Rican wasps Blastophaga (= Pegoscapus) toduzi and B. esterae deposit pollen on the stigma by rubbing their foretibia together without actually touching the subgenital plate (Galil et al. 1973).

Several foundresses may simultaneously oviposit, pollinate, and search for new flowers within the small, enclosed cavity. By cutting open figs at the proper stage, I have observed two or more foundresses ovipositing in the same fig. On one occasion I cut an aurea fig in half in which 14 foundresses were all attempting to oviposit. Just after cutting this fig in half, several foundresses seemed to be engaged in what appeared to be "pushing matches," but after one minute all of the foundresses seemed to be focused only on finding a flower for oviposition. On no other occasion did I see any behavior that might be interpreted as interfering with another foundress. Ramirez (1970a) reports that New World Blastophaga (= Pegoscapus, see Wiebes in press) which are laying eggs are very aggressive towards other foundresses. To my knowledge he did not observe wasps in Florida, so the contradiction between our observations may be due to watching different species, or to different methods of observation. I have also seen one or more foundresses dead within a fig while other foundresses are still actively ovipositing and pollinating. The dead foundresses' abdomens are usually shrunk in such a way as to suggest that the majority of their eggs were laid before they died.

After laying their eggs and pollinating, the foundresses die within the central cavity. From the period just before egg laying until about 10 days afterwards, approximately 10 different species of parasitic wasps (Hymenoptera; Chalcidoidea) lay eggs in the fig flowers by drilling through the fig epidermis with their long ovipositors. Some species oviposit in both aurea and citrifolia, whereas others are specialists. J. T. Wiebes is currently working on the taxonomy of these wasps (see also Butcher 1964). The parasites develop in the

ovaries of the pistillate flowers, and often kill the pollinator wasp if in an ovary with a developing pollinator (Joseph 1958). Each ovary produces only one wasp, either a pollinator or a parasite. Figs collected from the wild usually harbor parasites. In a sample of six aurea figs, the % parasites reared were 5, 18, 26, 31, 34, 37; and in five citrifolia figs 0, 0, 7, 48, 52. In addition to hymenopterous parasites, nematodes and a Cecidomyiidae (Diptera) were found in F. citrifolia. The nematodes will be discussed towards the end of this section, and the cecidomyiid will be discussed in the section on sex ratio experiments on citrifolia.

Approximately 28 days after the foundresses entered the fig, the male agaonids emerge from the ovaries in which they developed. The CO₂ content within the fig is quite high at this time (Galil et al. 1973). Although the females have metamorphosed into adults, they remain quiescent in this CO₂-rich environment. (The fig is still sealed at this stage.) The males are wingless, have reduced eyes, and are almost wholly without pigmentation. They search within the fig for flowers containing a female. When they find such a flower, they chew a small hole in the ovary of the flower, insert their long, telescoping genitalia, and mate with the quiescent female.

After nearly all of the females have been mated, one or more males will chew a tunnel through the wall of the fig. (There are some interesting questions about which males chew the tunnel, and when they chew it. See the section on male-mating preference below.) This hole causes the CO₂ concentration within the fig to drop, and the females become active (Galil and et al. 1973 give CO₂ concentration data for Ficus religiosa). They emerge from the flower ovary in which they

developed, walk to an anther which is loaded with pollen at this stage, and fill their pollen pockets. The females then exit through the tunnel chewed by the males, and quickly take to wing (predaceous ants of the genus Pseudomyrmex are abundant on figs at this stage). These mated females, loaded with pollen, search for receptive figs to begin a new generation. The figs ripen within 1-2 days, and are eaten by birds and mammals which disperse the seeds.

Parasitic nematodes found in several fig wasp species plausibly affect sex ratios. They may do so either by causing differential mortality of the sexes, or since they are transmitted only through female fig wasps, they may bias the primary sex ratio (see Frank 1983). Nematodes were common in citrifolia, but never observed in aurea. Nematodes have been observed in other species of agaonid pollinator wasps (Martin et al. 1973, Hamilton 1979, Bouček et al. 1981). No detailed study of the biology of these nematodes has been made. Based on Martin et al. (1973) and my observations, a possible life cycle of the nematodes is as follows. Female pollinators entering a receptive fig harbor juvenile nematodes within their body. After a foundress carrying nematodes dies, the nematodes inside her feed on her body and emerge. I occasionally observed several entwined nematodes emerging from between the thoracic plates (pronotum and scutum) of a dead foundress, approximately 2-8 days after she died. The nematodes are large at this stage (0.5-1.0 mm in citrifolia), and this is probably the adult stage. These adult nematodes produce abundant progeny. On a few occasions, I observed thousands of juvenile nematodes that were moving quickly over the surface of the ovaries containing seeds and wasps, about 2-5 days before males would have emerged to begin mating.

(Cutting open a fig for observations prevents further development of the wasps.) As Martin et al. (1973) suggest, these juveniles probably gain access to female pollinators by entering the ovary in which the pollinator develops through the hole chewed by a male for mating.

General Methods

In this section locations, dates, and details of the methodology common to most of the data collection or experimental protocols are presented. The specific details of each experiment precede each unit in the remainder of this chapter.

Locations and Dates

There are two major study areas. The first is Key Largo, Florida. The site is located on the east coast of the island to 700 m inland, at approximately $25^{\circ} 17'$ north latitude and $80^{\circ} 17.3'$ west longitude according to U. S. Geologic Survey map NG 17-8, series V501. Further reference to Key Largo, Florida will be to this site. The second major site is in Everglades National Park, Florida. According to the same Geologic Survey map, this site is located $25^{\circ} 22.4'$ - $25^{\circ} 23.3'$ north latitude and $80^{\circ} 22.1'$ - $80^{\circ} 24.3'$ west longitude. This site will be referred to as Everglades, Florida. Finally, some collections were made near Mrazek Pond in the Everglades, which is approximately $25^{\circ} 10.5'$ north latitude and $80^{\circ} 54'$ west longitude on the same Geologic Survey map. This site will be referred to as Mrazek Pond, Florida.

Preliminary observations were made in December 1980 and May 1981. The remainder of the work was conducted from 25 June to 25 August 1981 and from 1 May to 15 July 1982. Specific dates will be presented in each unit.

Rearing Pollinator Wasps for Experiments

Pollinator wasps were used for several experiments, for example when the number of foundresses per fig was experimentally controlled. The wasps were reared by collecting a fig at the stage in which males were searching for mates (determined by swelling and softening of the fig) and placing it in a vial covered with a fine mesh cloth (tergal). A fig can be cut in half to mobilize the females, or one can wait until the males chew an exit tunnel. (The time interval until a tunnel is chewed ranges from immediately to two days after collection, and sometimes not at all.) The wasps will typically live 24-48 hours (and up to 72 hours) in the vials if kept in the dark and below 25° C.

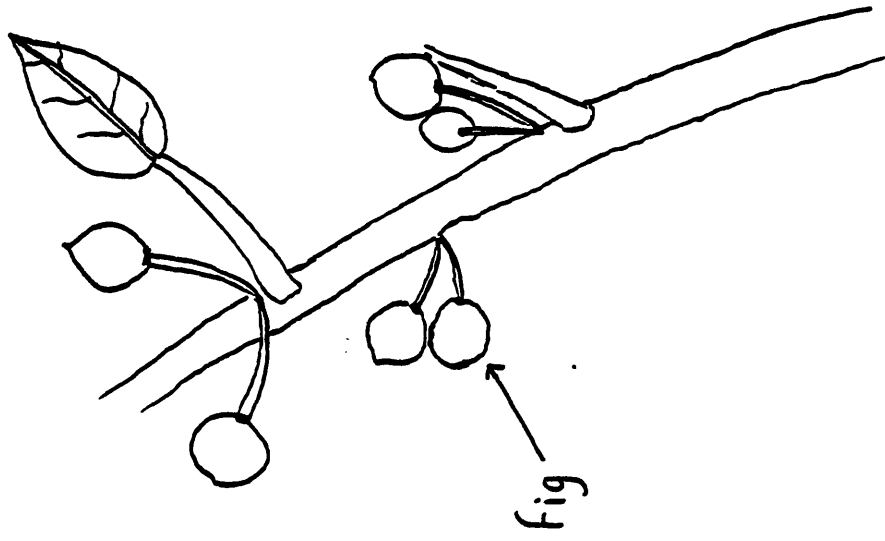
Maintaining a supply of wasps for experimentation required daily collecting and rearing. The usual procedure was to collect 30-50 figs of the desired species (i.e., F. aurea or citrifolia, or both if needed) between 15:00 and 20:00 hours. Figs at the correct stage (active males inside but no exit tunnel yet) swell and soften, so they can be identified fairly easily. Each fig was placed in a vial and covered. Typically, about one-half would have exit holes and emerged wasps by 8:00 the following morning; the remaining figs were often cut in half to allow the females to escape. These vials with wasps were then kept in a cooler at 8-15°C, and used for experiments during that day. The

wasps become active almost immediately upon being removed from the cooler and warming up (ambient temperature is usually above 30°C at the time of year the studies were conducted). Due to the difficulty of maintaining a supply of wasps for experimentation, (i) figs were sometimes collected, cut in half, and the wasps used the same day, and (ii) wasps were sometimes kept over a second night after collecting and used the following day (in this case the wasps were stored overnight in the dark at 23-28°C). In all cases the major criterion for using a wasp was that it appeared healthy and active. Unless otherwise noted the wasps were reared in the same locale in which the experiments were conducted.

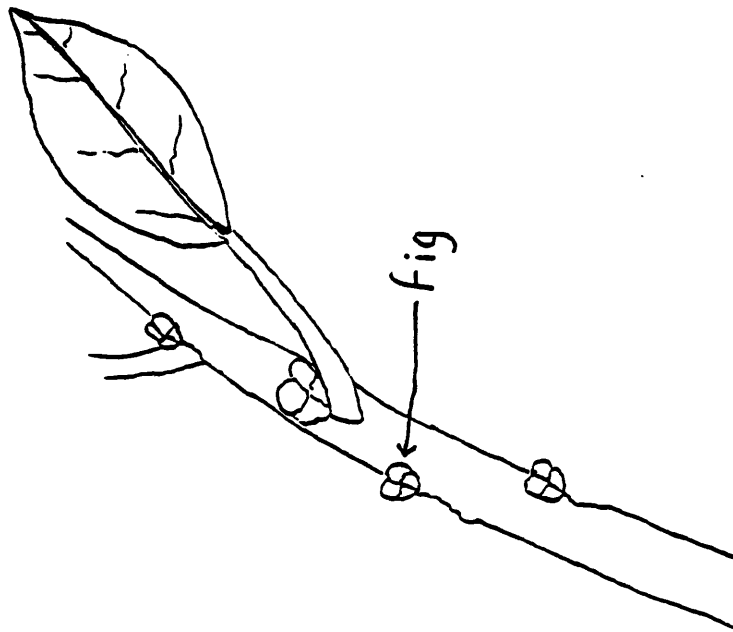
Preparing Trees for Study

In all of the experimental manipulations that will be described figs at a receptive stage (i.e., can be entered by a foundress) with no foundresses inside were used. These figs were obtained by covering figs about five days after they first appear with a fine-mesh tergal cloth. This bagging procedure prevents access by either pollinator or parasitic wasps. The figs became receptive 8-20 days after bagging, and remained receptive for about 4-10 days afterwards. F. aurea and F. citrifolia differ somewhat in structure (Fig. 3.1) and methods of bagging. (i) F. aurea figs are sessile, and a fig pair is just anterior to where the petiole of a leaf attaches to the twig (or just anterior to a leaf scar, typically about one-half of the figs on a twig are associated with leaf scars). To protect a pair of young figs with cloth, the leaf (if it were there) was first snipped off near the twig.

Figure 3.1. (a) A twig of Ficus aurea bearing (sessile) figs in the receptive stage. (b) A twig of F. aurea bearing figs (on peduncles) in the receptive stage.



(b)



(a)

A piece of tergal cloth (about 6 X 7 mm) was wrapped around the fig pair and fastened to the twig at each end with a twist of steel wire. (ii) F. citrifolia figs are borne on a 5-13 mm peduncle. Young figs were protected by bagging an entire twig bearing 10-50 figs. No leaves were clipped. All experiments were conducted in the field on naturally occurring trees.

Collecting Figs for Sex-Ratio Counts

Maturing figs were identified by a characteristic softening and swelling, signalling male activity and mating. This occurred 26-31 days after the first foundress entered the receptive fig. Figs collected without exit tunnels during the male-active stage contain all the progeny of the foundresses, since the only escape is the exit tunnel. Each fig was put in a separate vial and covered with tergal cloth. During the next two days an exit tunnel was usually chewed by the males, and the females emerged. If no exit tunnel was chewed, the fig was cut in half, and the females were allowed to escape. The entire fig and all wasps reared were then placed in 80% ethanol. The alcohol was changed one or two times during the first 24 hours since the tissues of the fig contain much water, which lowers the tonicity of the alcohol solution. Sex ratios are obtained by counting all individuals that were reared, and then carefully dissecting the fig and especially the ovaries of the flowers in search of wasps that did not eclose.

Handling the Wasps

Several times during this chapter I will refer to 'placing a wasp.' For females wasps this is accomplished by letting the wasp walk onto my finger, then putting my finger next to the area I want the wasp to be, and waiting for her to walk off my finger. Male wasps are moved by using the tip of an insect pin, which they will grasp with their legs.

Species Recognition of Fig-Tree Hosts by Pollinators

It has been suggested that the trees produce a species-specific chemical attractant for their respective pollinating species when bearing receptive figs (see Janzen 1979a). I have observed an aurea tree bearing receptive figs with many thousands of female fig wasps on the leaves and actively antennating the figs, while on a neighboring aurea tree with young figs at very nearly the same stage I could not find any fig wasps. This supports the chemical-attractant portion of the hypothesis. Wiebes (1979) and Janzen (1979a) reviewed the literature on species specificity of the pollinators. It is generally agreed that there is usually one species of pollinator per fig species.

To test the species-specificity of the two species of Florida fig wasps, a simple experiment was conducted in the Everglades during June 1982. I examined the preference of female wasps reared from F. citrifolia for entering F. aurea and F. citrifolia, and the preference of wasps reared from F. aurea for entering the two fig species. The null hypothesis tested was that a wasp's preference for aurea and citrifolia was independent of the tree from which it was reared. The alternative hypothesis was that there was a positive correlation between

the tree from which a wasp was reared and its preference for the two species of figs.

A receptive pair of aurea figs was chosen. These figs had been bagged with a fine mesh cloth (tergal) to exclude pollinator and parasitic wasps. An aurea-reared wasp was placed just below the fig pair (see Fig. 3.1). Four behaviors were considered: (i) attempting to enter a fig (entry behavior is characterized by a flattening of the antennae against the fig epidermis while searching for the ostiole, and when the ostiole is found an attempt is made by the wasp to push her head under the topmost tissue layer), (ii) walking off the fig, (iii) flying off, (iv) and antennating the fig for at least five seconds before leaving. If a wasp antennated the figs for at least five seconds, I considered her to have "assessed" the figs. After assessing, a wasp would be scored either as attempting to enter, or flying off after assessing the figs. If a wasp flew off before assessing the figs, she was not counted as having completed a trial. If a wasp walked off the figs, she was replaced just below the figs where she began the trial. In summary, a wasp either flew off without assessing, in which case she was not counted, attempted to enter, or assessed the figs and then flew off. When a wasp began entry behavior, she was not allowed to enter and was discarded. After 13 trials with aurea-reared wasps, 11 trials with citrifolia-reared wasps were conducted on the same aurea fig pair. Then the same experiment was conducted on a citrifolia fig pair with both citrifolia- and aurea-reared wasps, except citrifolia-reared wasps were tested first. The results are summarized in Table 3.1, and indicate that a wasp exhibits a strong preference for entering the same species of fig from which it was reared.

Table 3.1. The tendency of pollinating wasps to enter the same species of fig from which they were reared. The rows indicate the fig species from which a wasp was reared, and the columns indicate the number of wasps that attempted to enter a fig and the number of wasps that antennated a fig for at least 5 seconds and then flew off, respectively. The null hypothesis tested is that a wasp's preference for aurea and citrifolia is independent of the tree from which it was reared versus the alternative hypothesis that there is a positive association between the tree from which a wasp was reared and its preference for the two species of figs. For the trials on F. aurea, using the standard chi-square contingency test, $X^2_{(1)} = 24.0$, $p < 10^{-5}$, and for the trials on F. citrifolia $X^2_{(1)} = 12.69$, $p < 0.0002$.

 receptive figs tested -- F. aurea

	No. of Wasps	
	entering	assessing and flying off
aurea-reared wasps	13	0
citrifolia-reared wasps	0	11

 receptive figs tested -- F. citrifolia

aurea-reared wasps	0	10
citrifolia-reared wasps	9	3

A few anecdotal comments are relevant. In approximately five trials in the Everglades during August 1981 (definitions of trials and details were not recorded, but similar to experiment described above), one aurea-reared pollinator wasp actually entered a citrifolia fig. On another occasion, one of two aurea-reared pollinators entered a citrifolia fig. In this second case, 12 minutes elapsed from the time the wasp antennated the fig until it successfully found the ostiole and penetrated the topmost layer of tissue to begin entry. On this same citrifolia tree, 20 citrifolia-reared pollinators were introduced by the same technique described, and the average time per wasp to find the ostiole and begin entry was approximately one minute.

In summary these experiments and observations tentatively support the hypothesis that there is a species-specific attractant released by the trees and used as a cue by a specific species of pollinator wasp. Anecdotal observations also suggest that at least occasional mistakes occur such that a particular species of pollinator enters the "wrong" tree species.

Breeding Structure

The goal of this chapter is to examine two factors affecting sex ratios -- asymmetric relatedness, R , and the level of local mate competition, P_{dt} . Both depend on the breeding structure. This section is a summary of what was learned about the breeding structure of natural populations of Pegoscapus assuetus and P. jimenezzi in south Florida.

Characterization of the breeding structure will be in the spirit of Wright (1969, chapter 12), as in Chapter II. The essence of the approach is represented symbolically by

$$(3.1) \quad P_{it} = P_{id}P_{ds}P_{st}.$$

For the qualitative discussion in this section, the following intuitive definitions are sufficient. P_{it} is a measure of the relatedness among mating individuals with respect to the total population, or the expected level of inbreeding over the entire population. (Alternatively, P may be used to characterize the relatedness among males competing for mates. Relatedness among mates is important when discussing asymmetric relatedness, R , and relatedness among competing males is important when discussing local mate competition.) A value of 1 represents random mating, and 0 implies perfect assortative mating. P_{id} represents the relatedness among mating individuals with respect to members of their deme. There may be a high proportion of sib mating within a deme, but if females mate with a sib in the same proportion that the sibs exist within the deme, then P_{id} is 1 and mating within the deme is random. Due to the unique natural history of fig wasps, demes can be readily identified with individual figs. P_{ds} measures the degree of genetic differentiation among demes within the subpopulation. The more homogeneous demes are with respect to their subpopulation, the smaller the value of P_{ds} . If settling of foundresses out of the local subpopulation of fig wasps is random, then P_{ds} is $[(n-1)/n]P_{st}$. If the foundresses assort themselves into demes from the subpopulation by genotype, P_{ds} will differ from $[(n-1)/n]P_{st}$ (positive assortment lowers the value).

Subpopulations are much harder to delimit than demes for fig wasps. Since subpopulation structure of these wasps is unknown, I offer only the vague definition of "a cloud of pollinators together in both time and space." P_{st} measures genetic differentiation among these subpopulation clouds.

In the remainder of this section (i) qualitative observations relevant to subpopulation structure will be discussed; (ii) data and experiments on deme size and patterns of settling from local subpopulations will be presented; and (iii) an experiment and some comments on patterns of mating within a fig (deme) will be discussed.

Subpopulation Structure

Nothing is known about subpopulation structure of any fig-wasp species. My only purpose in this section is to make some relevant qualitative comments about Florida figs and some predictions about large-scale population structure and fig wasp sex ratios. The important factors that must be considered are the spatial distribution of the host fig trees, their flowering phenology, and the patterns of movement of the wasps.

F. aurea is occasional to moderately abundant throughout its distribution on the mainland. In a survey of trees and shrubs in six hardwood hammocks in the Everglades, F. aurea obtained importance value ranks (a combination of relative density, dominance, and frequency; a rank of 1 is "most important") of 13 in a hammock with 17 species, 91 of 18, 11 of 17, absent, 6 of 17, and 17 of 17 (Olmstead et al. 1980).

Individuals range from 2 m to over 30 m in height, with typical individuals bearing between 10^3 and 10^5 figs per crop. The flowering phenology for figs is usually given as well-synchronized within a single tree, and asynchronous within the population (Janzen 1979a). F. aurea does not follow this pattern; the flowering within a tree is sometimes quite asynchronous. On several occasions I observed a single tree bearing both maturing and receptive figs. Synchrony within a crown was also observed, so no clear rule can be stated.

Occasional frosts also have an effect on the population structure of the pollinators. A frost occurred over much of southern Florida in early February 1981. About 11 weeks later, I traveled through the area, and noted that many aurea trees had lost all of their leaves and figs. Most trees were in the early stages of putting out new leaves, and some trees were bearing figs in the receptive stage. These receptive figs mostly had no pollinators inside, and occasionally one pollinator. No data were recorded. My after-the-fact estimate is 10-30% of the figs had a single pollinator. This anecdote supports Janzen's (1979a) predictions that severe conditions at the edge of a distribution of figs will lead to occasional extreme bottlenecks or actual extinctions followed by recolonization, and that in harsh environments intra-crown asynchrony of flowering is more likely in order to maintain a local subpopulation of pollinator wasps. Such extinctions and recolonization events are conducive to genetic differentiation among subpopulations (Wright 1969).

F. citrifolia is less common on the mainland than F. aurea, and is confined to a more southerly distribution than aurea. Olmstead et al. (1980) did not report any F. citrifolia in the six hammocks

studied, although citrifolia is more often found in the extensive pinelands than is aurea (pers. obs.). citrifolia is occasional in the Everglades Park and throughout the Florida Keys, but is rarely, if ever, common. The mainland citrifolia have a pronounced flowering peak from May to July, and appear to flower only occasionally the remainder of the year. Individual trees are sometimes well synchronized, while other individuals bear figs at all stages of development. I have no observation on the phenology of citrifolia in the Keys.

Nothing is known about the movement of the wasps between trees, or how long they live in the wild. The wasps live less than 72 hours at 25° C when kept in vials without water or a sugar source. Anecdotal observations reviewed by Ramirez (1970b) suggest that the wasps are capable of traveling a long distance; however, it is likely that these were rare events. Janzen (1979a) also discusses longevity and movement of pollinator wasps. Experiments similar to those of Dobzhansky and Wright (1943, 1947; see Wright 1978 for a review) examining the movements of marked Drosophila individuals from a release site would be interesting. The key would be to extract some of the chemical attractant from flowering trees for baiting traps, and then performing release-recapture experiments.

In summary, little is known about the large-scale population structure of the pollinator wasps for any fig species. From Equation 2.21 it can be seen that any structuring at the level of subpopulations (P_{st} less than one) will decrease the predicted sex ratios. Also, F from Equation 2.4 was calculated assuming random mixing of the entire population between each generation. If P_{st} is less than one, F will be greater than predicted by Equation 2.4; for haplodiploidy this

implies R is lower and the predicted sex ratio is lower than when P_{st} is assumed to be one. A qualitative prediction for fig wasp sex ratios was given by Frank (1983). Across fig wasp species, as the degree of isolation in the host fig tree flowering increases in space and time, the predicted sex ratios for a given set of conditions will become more female biased.

Settling of Foundresses from Local Subpopulations

An assumption relied upon to derive specific quantitative predictions (e. g., Equation 2.12) for haplodiploids is that the wasps settle randomly into demes in each generation (Hamilton 1967, 1979). I treat this assumption in two parts: (i) there is no subpopulation structure ($P_{st} = 1$, see above section), and (ii) settling from the local cloud (= subpopulation) is random [$P_{ds} = (n-1)/n$; see Chapter II]. In this section I examine the settling of foundresses into demes (figs) from the local cloud. Two aspects are addressed. First, the distribution of deme sizes in wild populations is studied for both Ficus aurea and citrifolia, and this information is related to previous studies on other species. Second, three preference experiments are described. These experiments were designed to determine whether strong preferences of foundresses for certain attributes of a receptive fig exist. In addition to providing clues about the breeding structure, these experiments also provide some information about the attributes of a receptive fig assessed by prospective foundresses. This information will be of interest when discussing the introduction of an experimentally determined number of foundresses into receptive figs for sex-ratio studies (see 'sex-ratio data' section).

Distribution of the number of foundresses per fig. Janzen (1979b) studied the distributions of foundress numbers in four species of Costa Rican figs, and concluded that the distributions were significantly different from a Poisson distribution. He hypothesized that the departure from the Poisson distribution is due to ". . . active selection of unoccupied figs by newly arriving female wasps" (Janzen 1979b, pp. 127-28), since there were fewer unoccupied figs than expected (Table 3.2). I collected data on the distribution of foundresses per fig in both F. aurea and F. citrifolia (Tables 3.3 and 3.4). These data also differ significantly from a Poisson distribution, with fewer unoccupied figs than expected. However, I suggest that this departure from a Poisson distribution is due in part to the ostiole being sealed off by the fig soon after a wasp has entered rather than solely due to active selection of unoccupied figs by wasps. This suggestion is based on (for aurea and citrifolia) (i) qualitative observations that an unoccupied fig remains receptive to wasps for about 10 days, whereas an occupied fig closes one to two days after being entered (pers. obs.); (ii) the experiments I performed suggesting that the wasps do not discriminate between unoccupied figs and figs entered for the first time an hour previously, but that wasps do not attempt to enter figs that have been occupied for 22 hours (see next section); and (iii) the fact that the tree benefits from having fewer foundresses since proportionately more males are produced with increasing foundress number (sex-ratio data below), and only female wasps benefit the tree since only they carry pollen (Hamilton pers. comm.; this point is discussed later).

Table 3.2. The distributions of the number of pollinating wasps per fig for three fig species in Costa Rica (data of Janzen 1979b). X is the observed number of foundresses per fig and n is the total sample size. Y is the number of wasps entering each fig after the first wasp has entered ($Y = X-1$), and n' is n minus the number of observed zeros. $E(X)$ and $E(Y)$ are the expected number of wasps per fig and the expected number of wasps entering a fig after the first wasp has entered, respectively, based on the Poisson distribution (the values in the table are rounded to the nearest whole number, calculations are based on values rounded to three decimal places). X^2 values are for the standard goodness-of-fit test. If there are sufficient degrees of freedom, the categories are lumped such that the expected numbers are at least 5 in each cell. Janzen also reported data for F. insipida that fit neither the expected X nor Y distribution well.

X	n	0	1	2	3	4	5	6	X^2	df	p
$Y = X-1$	n'		0	1	2	3	4	5			
<u>F. cotinifolia</u>	275	325	50	257	17	1					
E(X)			132	119	54	21			254	2	<0.001
E(Y)				257	218	1			0.3	1	0.60
<u>F. morazaniana</u>	109	130	21	58	39	12					
E(X)			35	46	30	13	6		17	3	<0.001
E(Y)				61	35	12			0.5	1	0.54
<u>F. ovalis</u>	154	175	21	80	49	15	7	2	1		
E(X)			38	58	44	23	12		29	3	<0.001
E(Y)				74	54	20	6		5.1	2	0.08

Table 3.3. The distributions of the number of pollinating wasps per fig for four samples from F. aurea. Each sample was taken from a different tree on a different day. Samples 1-3 are from Key Largo, Florida, during June 1981, and sample 4 is from Everglades, Florida, during August, 1981. The structure of the table is the same as Table 3.2.

X	n	0	1	2	3	4	5	6	7+	χ^2	df	p
Y = X-1	n'		0	1	2	3	4	5	6			
sample 1	94	100	6	29	32	15	10	5	3			
E(X)			11	24	27	20	11	7		5.5	4	0.23
E(Y)				24	33	22	15			4.2	2	0.12
sample 2	99	100	1	32	37	19	6	1	1	3		
E(X)			11	24	27	20	11	7		17	4	0.002
E(Y)				29	36	22	13			1.0	2	0.61
sample 3	101	102	1	48	40	6	5	2				
E(X)			18	31	27	16	10			38	3	<.001
E(Y)				48	36	17				1.5	1	0.22
sample 4	183	205	22	141	38	4						
E(X)			67	75	42	16	5			103	3	<.001
E(Y)				142	36	5				0.2	1	0.65
Totals	477	507	30	250	147	44	21	8	4	3		

Table 3.4. The distributions of the number of pollinating wasps per fig for six samples from *F. citrifolia*. Each sample was taken from several neighboring trees. All samples are from Everglades, Florida, during July 1981. The structure of the table is the same as Table 3.2.

X	n	0	1	2	3	4	5	6	7	X ²	df	p
Y = X-1	n'		0	1	2	3	4	5	6			
sample 1	126	126	0	59	41	13	7	6				
E(X)			19	36	34	21	10	5		39	4	<0.001
E(Y)				52	46	20	8			8.5	2	0.014
sample 2	105	105	0	59	24	15	6	1				
E(X)			19	33	28	16	10			42	3	<0.001
E(Y)				51	37	17				7.1	1	0.008
sample 3	133	135	2	54	44	21	9	3	1	1		
E(X)			18	37	37	24	12	7		26	4	<0.001
E(Y)				48	49	25	11			2.6	2	0.27
sample 4	76	76	0	11	31	20	6	7	1			
E(X)			6	15	19	17	11	9		17	4	0.002
E(Y)				15	25	20	11	6		5.2	3	0.16
sample 5	120	124	4	102	15	3						
E(X)			41	45	25	13				115	2	<0.001
E(Y)				101	18	2				1.8	1	0.18
sample 6	146	149	3	90	33	15	7	0	1			
E(X)			31	49	38	20	11			63	3	<0.001
E(Y)				80	49	18				7.5	1	0.006
Totals	706	715	9	375	188	87	35	17	3	1		
d _n = X/n'				.53	.27	.12	.05	.02	0	0		

Each sample was obtained by collecting 100-200 figs from several aurea or citrifolia trees on a single day. These figs were no longer receptive to entering wasps. The figs were cut open and the number of dead foundresses inside each fig, designated X, were counted. If a fig were considered to be still receptive to entering wasps after it was cut open (e.g., pollinators still actively ovipositing), it was discarded from the sample. The data are reported in Tables 3.3 and 3.4, along with dates and locations. Each sample was compared to a theoretically generated Poisson distribution by the χ^2 goodness-of-fit test. As Janzen (1979b) found, the observed distributions are significantly different from Poisson. However, when the number of wasps entering a fig after the first wasp entered, Y, (i.e., $Y = X-1$) was compared to a Poisson by a goodness-of-fit test, the fit was much closer for Y than for X, for both the data I collected in Florida and for some of Janzen's data (Tables 3.2-3.4). Hamilton (1979) reported the number of foundresses per fig for a Brazilian Ficus species that fit neither the expected X nor Y distribution consistently well. In summary, it appears that for F. aurea and F. citrifolia the deviation from a Poisson distribution for the number of wasps per fig, X, is largely explained by the relatively long period (about 10 days) that a fig remains receptive to a wasp when it has not been entered versus the short period (about 1 day) of time a fig is receptive to entering wasps after the first wasp has entered the fig.

Preference of foundresses for occupied versus unoccupied figs. An occupied fig is defined as a fig receptive to entering wasps that already contains at least one foundress--an unoccupied fig contains no

foundresses. The null hypothesis that Pegoscapus spp. females enter occupied and unoccupied receptive figs at the frequency at which they are encountered (no preference) was tested against the alternative that unoccupied figs are entered at a proportion greater than the frequency at which they are encountered [preference for unoccupied figs was suggested by Janzen (1979b)].

Trials were conducted on F. aurea by placing a wasp just below a pair of receptive figs that were unoccupied (they had been bagged to exclude all wasps). The first wasp entered one of the pair of figs, and this fig was then designated occupied. When a wasp enters a fig, she loosens the top piece of tissue of the ostiole, and leaves her wings tucked just under this top flap such that the wings are visible from the outside of the fig. Another wasp was placed below the pair of figs and allowed to begin entry on either of the pair of figs. If a wasp flew off, or did not attempt to enter either fig over a 5-minute period, a new trial was begun with a new female; thus 10 trials implies 10 trials in which an entry was attempted on one of the two figs. When the entry behavior pattern began, the female was removed and the fig she chose (occupied or unoccupied) was recorded. This procedure was repeated 10 times on two different F. aurea pairs of figs. The data are reported in Table 3.5. Trials were conducted on F. citrifolia by holding a pair of unoccupied receptive figs together so that a wasp could easily walk from one fig to the other directly, and then placing a wasp on either the left or right fig. The first wasp was placed on one fig (choice of left or right was random), and she entered one of the figs which was then designated occupied. Ten wasps were then placed alternately on the left and right fig, and scored for beginning entry behavior as above.

Table 3.5. Preference of pollinating wasps for entering occupied versus unoccupied figs. The experimental methods are described in the text. The 95% upper confidence bound for p , the probability of a wasp entering an unoccupied fig given a choice between one occupied and one unoccupied fig, is reported where the sample size is at least 20 (see Lindgren 1976, p. 429, for the technique used to estimate the small-sample confidence intervals for p). Although 8 trials are insufficient for estimating a confidence bound for p in the case of a 22 hour delay between the first entry and the experimental trials (right-most column, see text for experimental details), the probability of 8 successes in 8 trials, over all values of p less than 0.71, is less than 0.05 (this is obtained by integrating over the likelihood density function). Thus, the likelihood of less than 71% preference for an unoccupied fig versus a fig occupied for 22 hours is low. 'citr' is an abbreviation for 'citrifolia.'

	aurea			citr	aurea & citr	citr with 22 hr. delay
	trial 1	trial 2	total			
unoccupied entries	3	6	9	7	16	8
total entries	10	10	20	10	30	8
unoccupied/total entries, \hat{p}			0.45	0.70	0.53	1.0
95% confidence bound for p			<0.63		<0.68	>0.71

Data are in Table 3.5. This experiment was repeated on F. citrifolia, except that 22 hours were allowed to elapse between the time the first wasp entered one of the two figs to establish an occupied fig, and the time that 8 preference trials were performed. Data are in Table 3.5.

The most striking result is that Pegoscapus assuetus (citrifolia-reared) clearly prefers an unoccupied fig over a fig that has been occupied for one day. Statistical analyses of the other results indicate that strong preference for unoccupied figs over recently entered figs seems unlikely (Table 3.5).

Preference for parasitized versus unparasitized figs. I have observed parasites of several species drilling into unoccupied figs with their ovipositor. Whether eggs are laid into unoccupied figs is unknown. The pollinator wasps (Pegoscapus spp.) often antennate a fig for 1-5 minutes before entering a fig or leaving in search of other receptive figs. An interesting question is whether the pollinator wasps respond to cues of parasitism, such as puncture holes made by a parasite ovipositor. Although an association between a response to parasitism and sex ratios is not clear, this experiment adds some information about the attributes of a receptive fig assessed by prospective foundresses. The null hypothesis that Pegoscapus spp. females entered unoccupied figs with Idarnes carme (a common parasite of the family Torymidae) ovipositor puncture holes as frequently as they entered unoccupied figs that have not been punctured by a parasite ovipositor was tested against the alternative hypothesis that pollinator wasps chose unpunctured figs more frequently than punctured figs.

The experiment was conducted on F. citrifolia, using Pegoscapus assuetus (citrifolia-reared) females. A fig pair was bagged. These figs were uncovered when receptive to entry by pollinators, and I. carme females were released on the figs and allowed to perform their oviposition behavior, which includes puncturing the outside of the fig with their long ovipositor. It is not known whether an I. carme female lays an egg when she drills into an unoccupied fig with her long ovipositor; this is an interesting problem since Idarnes spp. may only be able to develop in the ovary of a flower in which there is a pollinator wasp egg (see Joseph 1958 on Philotrypesis caricae, a torymid parasite of figs). On one of the two figs, 7-12 punctures were made by I. carme females, and this fig was designated 'parasitized.' The parasites were not allowed to puncture the other fig of the pair, and this was the unparasitized fig. The two figs were held together so that a pollinator wasp could walk back and forth between the figs. A recently reared pollinator wasp was placed on one of the two figs and allowed to antennate them. Eventually a wasp would either fly off, walk off the figs, or begin entry behavior on one of the figs. If a wasp flew off, it was not included in the data. If a wasp walked off the fig pair, it was replaced on the fig on which it began the trial; returning the wasp to the fig on which it began the trial was continued for about 5 minutes. If a wasp continued to walk off the fig pair for more than 5 minutes without beginning entry behavior, she was discarded and not recorded in the data. If a wasp began entry behavior on a fig, an entry was scored for that fig and the female was removed before she actually entered the fig. A different wasp was used for each trial. It is assumed that ignoring wasps that flew off, or continue to walk off the

figs for more than 5 minutes, does not introduce a bias into the estimate of the probability that a pollinator wasp will choose to enter an unparasitized versus a parasitized fig.

In 12 of 22 trials, the pollinator wasps began entry on the unparasitized fig. The estimated probability of entering an unparasitized versus a parasitized fig is 0.545, with a 95% upper confidence bound of 0.714 (this value was obtained following the method given in Lindgren 1976, p. 429). Thus, strong preference for a fig without puncture holes made by the parasite I. carme versus a fig with puncture holes seems unlikely.

Preference for figs containing genetic relatives versus nonrelatives. Since the degree of bias in the sex ratio depends, in theory, on the genetic differentiation among mating groups, the pattern with respect to genetic relatedness in which the wasps settle into figs out of the local subpopulation of wasps is of particular interest. The null hypothesis that pollinator wasps enter figs with genetic relatives at the frequency in which they are encountered (random settling) was tested against the alternative hypothesis that wasps enter figs with genetic relatives at a frequency different from the frequency that such figs are encountered (either assortative or disassortative settling).

The experiment was conducted on F. citrifolia, using Pegoscapus assuetus (citrifolia-reared) wasps. Two maturing figs were collected, and the dispersing pollinator wasps were reared from these mature figs. A branch of a citrifolia tree bearing receptive figs that had been protected from pollinator and parasite wasps by bagging was uncovered, and a pair of receptive figs selected. One pollinator wasp from one of

the two mature figs (arbitrarily designated the left mature fig) was introduced into the left fig (left receptive fig), and one wasp reared from the other mature fig (right mature fig) was introduced into the right receptive fig. Then a wasp reared from one of the two mature figs was placed on one of the two receptive figs (starting fig was alternated from trial to trial), and allowed to walk back and forth between the two receptive figs. If she flew off, she was not counted; if she walked off the fig pair, she was returned to the receptive fig on which she started the trial; if she began entry behavior, she was removed before actually entering, and both the fig she was reared from and the fig she attempted to enter were recorded.

Before analyzing the data, a problem inherent in the design of this experiment needs to be addressed. Two female wasps reared from the same fig are not necessarily sisters, since more than one foundress may have laid eggs within the fig (see above section on the distribution of pollinating wasps per fig). Therefore, on many of the trials experimental wasps chose between two figs that were both occupied by non-sibs. Keeping this caveat in mind, the data are: 52 trials in which 27 experimental wasps entered a receptive fig containing a wasp that was reared from the same fig as the experimental wasp, yielding an estimated probability of 0.519, with a 95% confidence interval of (0.387, 0.650). The difficulty is that if the alternative hypothesis is true (i. e., there is assortment of the foundresses based on genotype), then for some of the trials of the experiment the experimental wasp and the wasps already in the receptive figs are non-sibs, so the expected probability of entering either fig is 0.5; on the remainder of the trials the experimental wasp and one of the wasps in the receptive figs

were sibs, so the expected probability of entering either fig is different from 0.5. Thus, under the alternative hypothesis the expected probability varies from trial to trial, and an accurate confidence interval for the probability of entering a particular fig is not attainable. Nevertheless, the closeness of the observed probability to 0.5 suggests that strong assortment by genotype is unlikely (see the discussion below).

Discussion of three preference experiments. Summarizing the pattern of settling of foundresses from a local cloud, there is no evidence that settling is other than random. A slightly stronger statement can be made. Within the limitations of the experimental designs, strong preference for either (i) unoccupied figs versus figs just entered (less than two hours), (ii) unparasitized figs, or (iii) genetic relatives can be ruled out with a high probability.

Some improvement in the experimental designs can be achieved. (i) When comparing occupied versus unoccupied figs, a fresh fig pair could be used for each trial. In other words, one trial would consist of allowing a female to enter one of two unoccupied figs, then allowing a second to choose between these two figs. The next trial begins on a new fig pair. This would control the problem of chemical cues left at the ostiole by a wasp that has begun entry behavior. (ii) For parasitized versus unparasitized figs, comment (i) applies. Also, parasites other than Idarnes carme may be used. (iii) The greatest difficulty in testing for genetic recognition at the ostiole is that the relationship among females reared from the same fig is unknown. Females reared from a fig in which it is known that there was only a single foundress could be used, thereby guaranteeing sisterhood. The technique for obtaining a

generation of females reared from a single-foundress fig is described in the sex-ratio experiments below.

Male Pollinator Wasp Mating Preference

An assumption of the quantitative models (e.g., Equation 2.12) is that mating occurs randomly within a local group ($P_{id} = 1$). This assumption is difficult to test directly since the fig, which forms the local mating group, must remain sealed until the males emerge and begin to mate. During mating the movement of the males may be observed by cutting open a fig; however at this stage no method has been developed to determine which males and females are sibs. In order to test for a pattern in the searching of the males, a simple experiment was performed. The null hypothesis tested was that there was no correlation between male movement while searching for females and any cues within the fig (e.g., the location of male and/or female sibs, or areas rich in unmated females). Since there was no a priori reason to suspect positive or negative correlation, the alternative hypothesis was that a correlation between male movement and some cue within the fig existed (this experiment was suggested to me by W. D. Hamilton).

Two citrifolia figs with males searching for mates were selected randomly, brought into the lab, and cut in half. Only one-half of each of the two figs was used in each trial. From one-half of each of the two figs a male was randomly chosen and marked with Testor's enamel hobbyist paint. Each marked male was then either returned to the half-fig from which it was taken (= own fig), or each marked male was placed in the fig-half from which the other marked male was obtained (=

not own fig). The two half figs, each containing one marked male and many unmarked males, were then joined with insect pins for 50-70 minutes. After this time elapsed, the location of these marked males was determined as either own fig or not own fig. The data are reported in Table 3.6. The standard contingency table χ^2 test yields $\chi^2_{(2)} = 6.4889$ ($p = 0.039$) when testing the null hypothesis that the location of the males at the end of the trial was independent of the fig from which that male was reared versus the two-sided alternative that either a positive or negative correlation between final location and the fig from which a male was reared exists. The data suggest that males spend significantly more time in the fig-half from which they were reared than in a fig-half from which they were not reared.

An independent control was performed to test the efficacy of the design. First, it was noted that the probability of switching fig halves during the experiment, when averaged over both types of starts (N,N or 0,0), was 38/102, or 37%. The experimental design was repeated, except that the two marked males were each returned to a randomly selected fig-half that belonged to neither male. The prediction was that a male would change fig halves 37% of the time, the average rate of exchange over both types of switches (N to 0 and 0 to N) in the first experiment. Switches were observed in 14 of 32 trials, or 43%. These two rates of exchange are not significantly different ($p = 0.52$, using a test for the equality of two percentages given by Sokal and Rohlf 1969, p. 607). This lends a degree of independent support to the validity of the results.

Table 3.6. Male pollinator mating area preference. Two Ficus citrifolia figs were cut in half, then a pair of half figs, not originally forming a single fig, were joined. The rows designate whether both experimental males began a trial in their own-half fig (0,0) or in the half-fig that is not their own (N,N) (see text for further details of the design). The columns are defined as follows: (i) if both males finished the trial in the same fig-half in which they began the trial, 'no switches' was recorded for that trial; (ii) if one male finished the trial in the same fig-half in which it began the trial and the other male finished in the opposite half from which it began, 'one switch' was recorded; and (iii) if both males finished the trial in the opposite fig-half from which they began, 'two switches' was recorded. For example, if a trial started (0,0) and no switches occurred, then the finish was (0,0); if one switch occurred, the finish was (0,N); and if two switches occurred, the finish was (N,N). The null hypothesis that there is no correlation between the tendency to switch and the position of the start was tested against the two-sided alternative that a correlation exists. $X^2_{(2)} = 6.49$, $p = 0.04$.

		no switches	one switch	two switches
start	0,0	15	9	3
	N,N	5	15	4

The results suggest that a male prefers his own fig-half. Exactly what cue a male is assessing is not clear. The implications of this behavior for sex ratios depend on whether (i) the cue is positively correlated to relatedness of mates, (ii) the cue is positively correlated to relatedness of other males searching for mates, or (iii) the cue is not correlated to relatedness.

If the cue is positively correlated to relatedness of mates, then the value of P_{id} is less than 1, and F can be considerably larger than under random mating. Adjustments would be necessary in both the R and P_{dt} terms of Chapter II. The predicted sex ratios for fig wasps would be below those given in Chapter II for haplodiploids under random mating. Experiments to test for correlation among mates would be facilitated by using figs in which the number of foundresses is known. (The technique for obtaining such figs is described in the section on sex-ratio experiments below.) For example, the tendency of a male to return to his own fig when (a) his fig had one foundress, and (b) his fig had many foundresses, could be compared. The problem with this design is that a stronger tendency of a male to return to his own fig when it is a single-foundress fig versus multi-foundress fig does not separate between correlation of mates and correlation of males. If the males show a stronger tendency to move to one-foundress figs, then a second experiment to test for correlation among males is required.

A test for correlation among males is as follows. A fig that had two foundresses (and therefore two sib-ships of males) is cut in half. The location of all males is noted, they are marked, and then their locations are randomized upon being returned to a different fig that had a single foundress. (The mating males from this second fig are first

removed.) The halves are joined, and an hour later the location of the marked males is noted. If there is correlation among the males, they will tend to aggregate in the positions relative to each other as they were found in their original fig. If the males tend to move in sib groups, then implications for sex ratios are not clear (this is discussed again later). Movement in sib groups may be a form of cooperative mating among brothers. Also, the problem of which male chews the exit tunnel, and when, is interesting when viewed with respect to male-mating patterns. As a final note, I see no clear implications for the sex ratio if the tendency of males to move to their own figs is uncorrelated to relatedness.

Summary of Breeding Structure

Random settling from the entire population between each generation and random mating within local groups are probably never realized in natural populations. For fig wasps, it is convenient to consider the breeding structure over three hierarchical levels--large-scale population structure, settling of wasps into figs from a local subpopulation, and mating patterns within the fig.

The large-scale population structure refers to the degree of genetic differentiation among subpopulations of fig wasps that are grouped together in time and space over several generations. Most theoretical models conclude that genetic differentiation among subpopulations can occur only under extremely unlikely circumstances (Wade 1978). However, the models themselves are quite unrealistic. Several authors, while admitting the difficulties with the formal

theory, suggest that genetic differentiation among subpopulations may not be uncommon (Wright throughout his works, see 1969-1978, Wilson 1980, Wade 1978). Indeed, the use of Wright's index of panmixia, P , for the sex-ratio models in Chapter II imply that these are group-selection models. Hamilton (1967, 1975, 1979) recognized sex ratios as one of the few phenomena in nature for which there was impressive evidence supporting the action of group selection. Colwell (1981), Wilson and Colwell (1981), Charnov (1982), and Frank (1983) also discuss local mate competition in a group-selection framework.

Wright (1978) has summarized and discussed the empirical literature on variability among subpopulations. Edmunds and Alstad (1978) present evidence for genetic differentiation of scale insect subpopulation among host trees, and suggest how such differentiation is maintained by selection. Wade (1982) has inferred the action of group selection at a rate much greater than predicted by theoretical models in a series of experiments on Tribolium. This is perhaps evidence that selectable genetic differentiation among subpopulations is not as rare as previously supposed.

The study of differentiation among subpopulations of fig wasps will require a multi-faceted approach. Possible techniques include electrophoresis, DNA hybridization, chromosomal studies, genetic markers with release-recapture experiments, and studies of the phenology of host trees. Also, comparisons of the sex ratios between insular versus nearby mainland subpopulations within a population, and comparisons of sex ratios among species in which host-tree distributions differ may provide a tentative assay for differentiation (differentiation is predicted to be a negative correlate of the sex ratio, see earlier discussion).

The next lower level of fig wasp population structure is the settling of foundresses into figs from a local cloud (subpopulation) of wasps. There is no evidence that settling is other than random with respect to genotype. Implications of assortative and disassortative settling for group selection (and sex ratios in the case of fig wasps) have been discussed often in the literature (Hamilton 1967, 1971, 1975; Wilson 1980 and references).

The final level to complete the hierarchy is the mating patterns of the wasps within a fig. An interesting empirical result was obtained in this study. Males, when given a choice, prefer to search for mates in the fig in which they were born versus an alien fig. Whether this male preference is correlated to assortative mating, or to males tending to move in sibships as they mate, is unknown. Important implications for sex ratios exist if either of these cases are true. These implications were discussed above.

The theory developed in Chapter II demonstrates that empirical studies of local mate competition are studies of two factors--breeding structure, and if and how an organism uses correlates of the breeding structure to adjust the sex ratio it produces (conditional sex ratios). This section summarized empirical evidence on fig wasp breeding structure. In the following section, data correlating sex ratios of fig wasps and their breeding structure are presented.

Sex Ratios in Florida Fig Wasps

Data were collected (i) to test the hypothesis that sex ratios of fig wasps are conditional on number of foundresses per fig (deme size) and increase with increasing number of foundresses (Equation 2.12 in a qualitative sense), and (ii) to test the hypothesis that fig wasp sex ratios are conditional on the amount of genetic variability within a fig for a given number of foundresses--the prediction is that the sex ratio will decrease with decreasing variability. These qualitative hypotheses were developed a priori. The data will also be used for a posteriori comparisons to the quantitative theory of Chapter II.

The data and discussions are organized as follows. (i) Sex ratios from wild, unmanipulated figs are presented for both Pegoscapus jimenezi (from Ficus aurea) and P. assuetus (from F. citrifolia). These data provide some baseline information against which experimental results may be compared. (ii) Sex ratios and foundress numbers are reported for P. assuetus (citrifolia). These data were gathered from figs in which the number of foundresses was controlled experimentally and parasites were excluded. (iii) Sex ratios in (ii) were found to be conditional on foundress numbers. These data are examined in an attempt to discover the mechanism underlying the sex-ratio adjustment. (iv) Sex ratios in P. assuetus were studied in figs in which some of the foundresses were sibs. These data are compared to the data of (ii) in which no foundresses in a given fig were sibs. The prediction was that the sex ratio would be lower in figs with sib-foundresses. No difference was detected, and an improved experimental design is suggested. (v) Sex-ratio experiments for P. jimenezi (aurea) are briefly mentioned. (vi) The data on sex ratios and breeding structure are compared to the

theory of Chapter II. The results are discussed and future directions are suggested.

Data from Unmanipulated Figs--F. citrifolia and aurea

Ripening figs that have not been experimentally manipulated can be collected before an exit hole is chewed by the pollinator males. The parasite and pollinator wasps from these figs can be reared and counted, and the flowers within the fig dissected in order to insure that all wasps within the fig are counted. Thus a very precise estimate can be made of the sex ratio of the pollinator wasps within a maturing fig. The sex ratios of the pollinator wasps and the numbers of parasites per fig for six aurea and five citrifolia figs are reported in Table 3.7.

The theory that this thesis addresses is concerned with the relationship between both the amount of inbreeding, F , and the genetic differentiation among mating groups (= figs), P_{dt} , and the sex ratio within each group. Two problems exist with respect to the data in Table 3.7. (i) A major component of both the level of inbreeding, and the level of genetic differentiation, is a function of the number of foundresses per fig. Thus, to estimate the magnitude of F and P_{dt} , the number of foundresses must be estimated. Since the foundresses die inside the fig, their number may be estimated by searching inside the fig for the chitinous remains of the foundresses. While an estimate can be obtained in this manner, I have little confidence in this technique since the chitinous parts of one or more of the original foundresses may be scattered throughout the fig, making it difficult to reconstruct precisely the original number of foundresses. Also, it is impossible to

Table 3.7. The proportion of males of pollinator wasps Pegoscapus jimenezi and assuetus in naturally occurring figs of F. aurea (= a) and F. citrifolia (= c), respectively. The total numbers of parasites per fig are also reported. All figs of both species were collected from Everglades National Park, Florida.

Fig species	males/total	% males	No. parasites
a	19/89	19	32
a	12/57	21	3
a	67/176	38	39
a	31/80	39	36
a	34/79	43	47
a	35/81	43	42
c	4/63	6	58
c	14/116	12	0
c	15/89	14	0
c	21/89	19	94
c	36/168	22	13

determine whether the foundresses entered the fig at approximately the same time, and therefore probably produced about the same number of progeny, or some of the foundresses entered very early and some very late, and therefore disproportionate contributions of the foundresses were possible. (ii) The second problem is that the theory predicts the sex ratio of the progeny produced by the foundresses, and the parasitic wasps probably kill a proportion of the progeny [This has not actually been demonstrated for any of the parasites of F. aurea or F. citrifolia; however, Idarnes carme may kill a pollinator wasp during its development, see Joseph (1958) and Gordh (1975).] Thus, the observed pollinator sex ratio is only an estimate of the actual progeny sex ratio since a proportion of the progeny have been killed by parasites. This estimate of the sex ratio within a fig is probably unbiased (i.e., there is no differential parasitism of males and females), however the variance of this estimate increases as the proportion of pollinator wasps killed by parasites increases, which makes it more difficult to estimate sex ratios precisely.

Sex Ratio versus Foundress Number--F. citrifolia

Theory (Chapter II) predicts that conditional sex ratios of haplodiploids in a deme-structured population are favored over unconditional sex ratios. For example, Equation (2.12) suggests that sex ratios conditional on the relative genetic homogeneity within a deme are favored. So, if the foundresses adjust their sex ratio according to the genetic homogeneity within their fig relative to the population, as the model predicts, then the number of foundresses within a fig is a

likely proximate cue for sex-ratio adjustment. Foundress number is correlated to relative genetic homogeneity (see section on breeding structure above).

An experiment was conducted on F. citrifolia in Everglades National Park, Florida, in order to obtain sex-ratio data at experimentally determined foundress numbers. Twigs bearing 10-50 figs too young to have been entered by pollinating wasps were bagged with a fine mesh cloth (tergal). When the figs became receptive to entering pollinator wasps, the bag was removed and 1-8 pollinator wasps were introduced into each fig. The pollinator wasps were obtained for introductions by rearing wasps from maturing figs on trees within approximately a 10 km radius of the site of the experimental trees. The introductions were accomplished by placing a wasp on a receptive fig and observing whether the wasp entered the fig, walked off, or flew off. If the wasp entered the fig, an entry for that particular fig was recorded; if she walked off the fig, she was replaced on the fig repeatedly for about 5 minutes, and then if she still had not entered, she was discarded; and if the wasp flew off, a new wasp was placed on the fig (potential difficulties of this technique are discussed below). This procedure was repeated until the desired number of wasps had entered a particular fig. All introductions into a single fig were performed during a 90-minute period. Also, an important point is that each wasp that entered a particular fig was reared from a different maturing fig, guaranteeing that no two foundresses within an experimental fig were sibs. (This point will be discussed more fully below, where an experiment is described in which only wasps reared from the same maturing fig were introduced into each receptive fig.) After pollinator

wasps were introduced into the receptive figs, the tergal bag was replaced over the twig to exclude additional pollinator wasps and all parasitic wasps from the figs. The bag was kept in place for 15-20 days after introductions, and then removed.

After 27-32 days following the introductions, the experimental figs began to mature. These figs were collected about one day before the exit tunnel was chewed by the pollinator males, guaranteeing that all male and female pollinator wasps of the progeny generation were collected with each fig. The wasps were reared, and the sex ratios counted. No parasitic wasps were reared since the figs were protected by a tergal cloth.

The data are reported in Table 3.8. The sex ratio rises steadily with foundress number. Three technical difficulties must be mentioned before discussing the data.

(i) Not all experimental figs reached maturity (35 of 80 in a representative sample), and the pollinator wasps did not develop to a stage at which they could be counted accurately. A variety of factors, including disease and cecidomyiid gall midges (see below) kept these figs from reaching maturity. Also, in 11 of 68 experimental figs that were collected, very few wasps emerged from the fig, thus most of the wasps were still in the ovaries of the pistillate flowers where they developed. This occurred when the figs were collected too many days before the males would have eclosed, so that no mating occurred. If the fig failed to rear any wasps, or only very few wasps actually emerged from the fig, (for citrifolia, less than about 40 wasps, for aurea less than about 20), the sex ratio of that fig was not counted.

Table 3.8. Sex ratios of the progeny generation of Pegoscapus assuetus within Ficus citrifolia figs for an experiment in which foundress numbers were controlled and all parasitic wasps were excluded. 'm' is the total number of pollinator wasps reared in the progeny generation; 'rank m' is the rank of m within a given number of foundresses. 'galls' describes whether the central cavity of the fig was crowded with cecidomyiid galls at rearing (++) , had a few galls (+) , had 0 or 1 gall (-) , or the condition of the fig was not recorded (NR). The experiment was conducted Everglades National Park, Florida, May-July 1982. Each foundress introduced into a receptive fig was reared from a different maturing fig collected in the Everglades.

foundresses	sex ratio	m	rank m	galls
1	0.047	85	6	++
1	0.051	39	2	+
1	0.058	52	3	+
1	0.059	68	5	+
1	0.077	91	7	++
1	0.113	62	4	+
1	0.122	98	8	NR
1	0.131	130	9	NR
1	0.261	23	1	-
2	0.073	191	6	+
2	0.096	250	8	-
2	0.097	124	3	-
2	0.108	157	4	+
2	0.115	113	2	-
2	0.132	197	7	+
2	0.133	83	1	+
2	0.133	158	5	+
2*	0.241	137	9	+

Table 3.8 -- continued

foundresses	sex ratio	m	rank m	galls
3	0.106	188	6	+
3	0.110	335	11	-
3	0.114	149	4	-
3	0.118	272	10	++
3	0.140	243	9	+
3	0.141	198	7	+
3	0.151	239	8	+
3	0.160	106	2	+
3	0.182	154	5	+
3	0.188	128	3	+
3	0.228	101	1	-
4	0.134	276	7	-
4	0.139	317	10	+
4	0.163	313	9	++
4	0.165	334	11	-
4	0.174	195	4	+
4	0.194	217	6	+
4	0.197	213	5	++
4	0.197	152	3	++
4	0.209	296	8	+
4	0.211	133	2	+
4	0.219	105	1	++

Table 3.8 -- continued

foundresses	sex ratio	m	rank m	galls
5	0.155	399	12	+
5	0.172	297	9	+
5	0.186	177	4	+
5	0.189	132	1	-
5	0.207	319	10	-
5	0.219	224	8	+
5	0.223	215	7	-
5	0.242	198	6	+
5	0.243	181	5	+
5	0.286	175	2	+
5	0.294	176	3	++
5	0.355	391	11	-
8	0.218	234	4	-
8	0.231	277	5	-
8	0.235	119	1	+
8	0.283	166	2	+
8	0.414	181	3	-

* This datum point was considered an outlier in all statistical analyses.

(ii) The second problem was that cecidomyiid gall midges, Ficiomyia birdi Felt (1934), were abundant in figs of F. citrifolia (these gall midges seem to be much more abundant during the summer months). These galls appeared in figs that were bagged when only about 3 mm in diameter, 10-14 days before these figs became receptive to entering pollinator wasps. This suggests that the cecidomyiid eggs are laid in the figs at a very early stage in the ontogeny of the fig, probably before the tissue forming the center cavity invaginates (also, the midges do not have a piercing ovipositor). The midge larva forms a large gall that protrudes into the center cavity; these galls can be found in receptive figs not yet entered by pollinator wasps. If the galls are numerous, they crowd the center cavity, possibly affecting a foundress' assessment of the number of co-foundresses. Also, the crowding by these galls may cause some mortality among the pollinator-wasp progeny. A crude coding for the degree of crowding in the central cavity due to cecidomyiid galls, at the time of rearing and counting the sex ratio, is reported along with sex ratio and total number of progeny per fig in Table 3.8. These sex-ratio data will be related to theory (Chapter II) in a discussion section below.

(iii) The behavior of the female wasps during introductions was highly variable and (to me) unpredictable. As stated above the introductions were accomplished by placing a wasp on a receptive fig and observing whether the wasp entered the fig, walked off, or flew off. If the wasp entered the fig, an entry for that particular fig was recorded; if she walked off the fig, she was replaced on the fig repeatedly for about 5 minutes, and then if she still had not entered, she was discarded; and if the wasp flew off, a new wasp was placed on the fig.

I did not successfully quantify these behaviors. Based on my qualitative observations, I suspect the following factors affect whether a wasp enters a fig, walks off, or flies off: the number of wasps in the vial from which a female was taken from; light intensity; wind; length of time since the wasp eclosed; the manner in which I got her to walk on my finger; the quickness with which I moved my finger to the receptive fig and got her to walk on it; chemical attractiveness of the receptive fig; and if she walked off, the smoothness with which I got her to walk onto my finger again and replaced her on the receptive fig. The goal of this experiment was to elucidate the correlation between foundress number and sex ratio. No information was obtained concerning the above factors and the wasps' behavior during introductions. However, the introduction technique was uniform with respect to foundress number. Hence if there is a correlation between any of the above factors listed and sex ratio, the only effect would be to increase the variance in the sex ratio at each foundress level. In other words the effect would be to increase the experimental error, decreasing the precision of the experiment, and possibly preventing a true correlation between foundress number and the sex ratio from being detected. As a final note, the foundress-preference experiments discussed in the breeding structure section were designed in part to examine entry behavior--no notable patterns were detected (see above).

Mechanism of Sex-Ratio Adjustment

Although the sex ratio rises steadily with foundress number (these data are discussed in detail below), the mechanism underlying the increase in sex ratio with foundress number is not clear. Three possible mechanisms are: (i) a foundress lays proportionately more males among her earlier eggs, and as foundress number increases the number of eggs per foundress decreases; (ii) when a foundress lays a small clutch of eggs relative to her co-foundresses, she increases the proportion of males laid [i.e., the sex ratio adjustment is active rather than an effect of decreasing total clutch size as in (i)]; and (iii) most, or all, of the foundresses adjust their sex ratio according to the number of co-foundresses.

Some information relevant to the three possible mechanisms underlying sex-ratio variability can be gleaned from the data by examining correlates of the observed sex ratio in figs within each foundress number. Two possible correlates are listed in Table 3.9--the rank of m , which is the rank within a given foundress number of the total number of progeny reared from a fig, and the presence (+ or ++ in Table 3.8) or absence (-) of cecidomyiid galls in the fig. There are three pairwise correlations among the following three variables; (i) the rank of the sex ratio within a given foundress number, designated r , (ii) the rank of m within a given foundress number, and (iii) the presence or absence of galls. The trends are summarized in Table 3.9. The correlation between rank m and r was tested using Kendall's tau (Hollander and Wolfe 1973). For the null hypothesis that K is zero (no correlation) versus the alternative that K is different from zero, $K = -0.259$, $p = 0.014$ (two-tailed test). A test of the null hypothesis that

Table 3.9. The correlations among the three variables (i) absence of galls (coded as a low quantity) or presence of galls (coded as a high quantity), (ii) rank m, which is the rank, within a given foundress number, of the number of progeny reared from a single fig, and (iii) r, which is the sex ratio in that fig. Statistical analyses of these relationships are described in the text and in Appendix B. The values in parentheses approximate the probability that the correlation is different from zero. Single-foundress figs were not included in the analysis, since the theory does not make a clear prediction about the sex ratio in such figs. From data presented in Table 3.8.

	----- rank m	----- r
galls	-(0.84)	+(0.75)
rank m		-(0.99)

there is no correlation within a given foundress number between the absence (coded as a low quantity) or presence (coded as a high quantity) of galls and rank m versus the alternative of a negative correlation indicates a weak negative trend ($p = 0.16$, one-tailed test, see appendix for details of the statistical procedure). A test of the null hypothesis that there is no correlation within a given foundress number between galls (coded as above) and rank of sex ratio, r , versus the alternative that there is either positive or negative correlation indicates a weak positive trend ($p = 0.25$, two-tailed test, see appendix). The average number of progeny per fig at a given foundress number are also valuable summary statistics for interpreting the relations among r , rank m , and presence or absence of galls. These statistics are reported in Table 3.10.

Each of the three mechanisms (mentioned above) that possibly underlie the strong correlation between sex ratio and foundress number are discussed in turn. (i) The first mechanism suggested was that a foundress lays proportionately more males among her earlier eggs, and that as foundress number increases the number of eggs per foundress decreases, and thus the sex ratio within the fig increases. The data relevant to this mechanism follow. (a) The weak negative correlation between rank m and rank r within a given foundress number does not contradict this mechanism, since as the total number of progeny produced in a fig, m , within a given foundress number increases, either the relative contributions of the foundresses with the fewer progeny are increasing or the contributions of the foundresses with more progeny are increasing. In other words, as m increases the additional progeny are, on average, later in the laying sequence of one or more of the foundresses. (b) The sex ratio within a fig, r , can be correlated with

Table 3.10. The average number of progeny per fig and average number of progeny per foundress at the foundress numbers studied. From data presented in Table 3.8 for P. assuetus from F. citrifolia.

foundresses	1	2	3	4	5	8	totals*
number of figs	9	8	11	11	12	5	47
mean number of progeny per fig	72.0	159.1	192.1	231.9	240.3	195.4	208.5
s. e. of the mean	10.9	18.8	22.3	24.2	25.8	27.4	11.4
mean number of progeny per foundress	72.0	79.6	64.0	58.0	48.1	24.4	58.5
s. e. of the mean	10.9	9.4	7.4	6.1	5.2	3.4	5.9

* All totals exclude one-foundress figs, since no sex-ratio predictions were made for these figs.

the average number of progeny per foundress within a fig, x . The regression line obtained from the data (excluding single-foundress figs, and the datum point for the 5-foundress fig with a sex ratio of 0.355) is $r = 0.282 - 0.00178x$, yielding a coefficient of determination, R^2 , of 0.503. (c) The evidence in both (a) and (b) do not contradict mechanism (i); however, neither do they separate clearly mechanism (i) from mechanism (ii) and (iii). The information provided by the single-foundress figs is the best test of mechanism (i), since mechanism (i) predicts that the sex ratio of a foundress' progeny becomes more female-biased as the total brood size increases, independent of all other factors, such as number of co-foundresses. In other words, males are laid early in the laying sequence. The data for r and rank m for single-foundress figs are in Table 3.8. Correlating rank r and rank m with Kendall's tau, K equals 0.222 ($p = 0.238$). If the datum point $m = 23$, $r = 0.261$, is considered an outlier, $K = 0.56$, $p = 0.031$, conditional on the removal of the outlier. Hence, there appears to be a mild positive correlation between the sex ratio and the brood size, which suggests that males are laid more frequently at the end of a laying sequence rather than at the beginning, thus contradicting mechanism (i). In summary, mechanism (i) seems unlikely based on the observed tendency of males to be laid later in the laying sequence in single-foundress figs.

The second mechanism (ii) proposes that foundresses laying small clutches relative to their co-foundresses will lay a relatively high proportion of males. The data available provide little information on this hypothesis. However, an experiment could be performed that would probably yield sufficient information to test this hypothesis. Most of

the egg-laying in a fig occurs within 24-36 hours after the first foundress enters the fig, and in all of the experimental figs reported in this thesis, all the wasps were introduced within a 90-minute period, and most often during a shorter interval. An experiment could be performed in which the time between introductions was varied for a fixed number of total foundresses. For example, two foundresses would be introduced within a short time interval, then the time until the third introduction could be varied. If mechanism (ii) is correct, there should be a positive correlation between time until the third entry, and sex ratio within the fig, since the relative contribution of the third foundress is likely to decrease with increasing time between entries. Mechanism (iii) is a likely possibility, however it is difficult to conceive of what sort of easily obtained evidence would clearly implicate this mechanism over mechanisms (i) and (ii). A genetic marker, as Werren (1980a,b) used, would be extremely useful, but none are known among fig wasps.

Genetic Recognition and the Sex Ratio--F. *citrifolia*

The number of foundresses within a fig is a correlate of the relative genetic homogeneity, P_{dt} . However, other cues, such as odors, may provide a better estimate of the genetic homogeneity. To test whether sex ratios are conditional on cues of genetic homogeneity other than foundress number, an experiment was performed on P. *assuetus* (from *citrifolia*). The data presented in Table 3.8 represent the sex ratio in figs into which a given number of foundresses were introduced, each foundress having been reared from a distinct maturing fig. Thus,

the foundresses represent a random sample from the local subpopulation. To create a comparison with the random sample of foundresses, five females all reared from the same maturing fig were introduced into each of three receptive figs (i.e., 15 females reared from one fig were introduced into three figs, yielding three replicates). Since some of these females are sibs, the expected genetic variance within these figs is lower than in figs where the foundresses are a random sample. Three a priori hypotheses follow from the theory. (i) The location of the population of sex ratios (a statistical population) per fig will be lower in figs with foundresses reared from a common fig than in figs with foundresses reared from different figs. (ii) The distribution of sex ratios per fig will have a stronger left-skew in figs with foundresses reared from a common fig than in figs with foundresses reared from different figs, since the sex ratio is predicted to decrease as the number of sibs increases within a fig with five foundresses. (iii) The variance in the sex ratio among replicates taken from a single maturing fig is less than the variance in the sex ratio among figs where foundresses were reared from different maturing figs, since some maturing figs would have had few foundresses and therefore have reared a high proportion of sibs, while other maturing figs would have had many foundresses and thus reared a low proportion of sibs. The data for this experiment are in Table 3.11.

Analysis of the three hypotheses are discussed in turn. (i) Denoting figs with five foundresses taken from different maturing figs as 'control figs,' and figs with five foundresses taken from the same maturing fig as 'experimental figs,' the median of the sample of control figs is 0.221 ($n = 12$) and the median of the sample of experimental figs

Table 3.11. The sex ratio among the progeny generation in figs of F. citrifolia to which five pollinating wasps reared from the same maturing citrifolia fig were introduced. Females introduced into figs with the same maturing fig code number were reared from the same maturing fig, and thus form replicates. 'm' refers to the total number of progeny reared from each experimental fig, and 'galls' refers to the absence (-), presence of a few (+), or many (++) cecidomyiid galls. '(NR)' indicates that the presence or absence of galls was not recorded. The experiment was conducted in the Everglades National Park, Florida. See text for statistical analysis.

maturing fig code	sex ratio	m	galls
1	0.107	159	+
1	0.163	313	+
1	0.167	221	+
2	0.156	205	-
2	0.171	170	+
2	0.240	146	NR
3	0.202	188	+
3	0.223	166	++
3	0.287	167	++
4	0.195	338	-
4	0.245	319	-
5	0.186	242	++
5	0.249	193	++
6	0.223	247	+
6	0.224	223	-
7	0.135	444	+
7	0.205	161	+
8	0.242	207	+
8	0.246	236	+

Table 3.11 -- continued

maturing fig code	sex ratio	m	galls
9	0.203	138	++
9	0.232	276	+
10	0.268	183	-
10	0.292	120	+
11	0.219	146	-
11	0.234	158	-
12	0.126	190	+

is 0.221 ($n = 26$). No commonly used statistical procedure applies since the control population is not a priori symmetrically distributed (a binomial distribution with $p < 0.5$ has a right skew). However, it is quite clear that no detectable shift in the location occurred, since the medians are equal. (ii) The only standard procedure available for testing shift in skew between two populations when the control is not a priori symmetric, is the Kolmogorov-Smirnov two-sample test (procedure is outlined in Hollander and Wolfe 1973). The alternative hypothesis is one-sided since the prediction is that the experimental population will exhibit a greater right-skew than the control population. The test statistic is $J_2' = 0.533$, $p = 0.43$. Thus, there is insufficient evidence to reject the null hypothesis. However, the Kolmogorov-Smirnov test is notoriously weak (i. e., high type II error rate), and if a real difference between the control and experimental populations exists, it is likely to be small. (iii) The hypothesis that the variance among replicates from the same maturing fig is less than the variance over all samples can be tested by a Kruskal-Wallis test (Hollander and Wolfe 1973). The test statistic is $H = 16.14$. An approximate significance level can be obtained by comparison to the chi-square distribution (i. e., let $X^2_{(11)} = 16.14$). This procedure yields an approximate attained significance level of $p = 0.14$. The only inference that can be drawn from this approximate procedure is that strong within-group homogeneity was not detected.

A more precise experiment can be designed for detecting genetic recognition and an associated sex-ratio shift. In the experiment described above, the number of foundresses in the maturing figs from which the wasps for introductions were reared was unknown. Wasps reared

from figs in which the foundress numbers were experimentally controlled could be used, thus the expected number of sibs introduced into an experimental fig could be controlled. The proportion of sibs among the foundresses could be manipulated, and the total number of foundresses could be varied, allowing comparisons over a broad range of genetic variances within figs. Such an experiment should indicate whether the proximate cue used by the foundresses is genotypic recognition or foundress number.

Sex Ratio versus Foundress Number--*F. aurea*

An experiment excluding parasites and controlling foundress numbers identical to the experiment described above for *F. citrifolia* was performed on *F. aurea*, using *Pegoscapus jimenezi* wasps. Table 3.12 reports sex ratio and foundress number for an experiment conducted in Key Largo, Florida. In this experiment each foundress introduced into a receptive fig was reared from a different maturing *F. aurea* fig collected on Key Largo. Table 3.13 reports sex ratio and foundress number for a similar experiment on *F. aurea* conducted in Everglades National Park, Florida, with each foundress introduced into a receptive fig reared from a different maturing fig collected in the Everglades. An experiment was also performed on *F. aurea* in the Everglades in which three or four foundresses, each reared from the same maturing fig, were introduced to a receptive fig. The data are in Table 3.14. Sample sizes are too small for inference, however (i) sex ratios appear to increase with increasing foundress number, and (ii) the sex ratios appear higher in the Everglades than on Key Largo.

Table 3.12. Sex ratios of the progeny generation of F. aurea within figs for an experiment in which foundress numbers were controlled and all parasitic wasps were excluded. 'm' is the total number of pollinator wasps reared in the progeny generation. The experiment was conducted in Everglades National Park, Florida. Each foundress introduced into a receptive fig was reared from a different maturing fig collected in the Everglades.

foundresses	sex ratio	m
1	1.000	27
1	0.103	39
2	0.125	16
2	0.188	64
3	0.273	44
3	0.433	30
3	0.463	108
4	0.207	58
4	0.257	74
4	0.260	50
4	0.300	90
4	0.654	133
5*	0.044	45
5*	0.065	46
5	0.408	49

*The progeny generation in these figs was not well reared, which makes counting the sex ratio difficult, thus it is probably legitimate to consider these data points outliers.

Table 3.13. Sex ratios of the progeny generation of *F. aurea* within figs for an experiment in which foundress numbers were controlled and all parasitic wasps were excluded. 'm' is the total number of pollinator wasps reared in the progeny generation. The experiment was conducted on Key Largo, Florida. Each foundress introduced into a receptive fig was reared from a different maturing fig collected on Key Largo.

foundresses	sex ratio	m
1	0.000	9
1	0.000	30
1	0.000	33
1	0.045	66
1	0.055	55
1	0.056	89
1	0.068	44
1	0.065	46
1	0.069	87
1	0.077	39
1	0.077	52
1	0.079	76
1	0.088	68
1	0.091	110
1	0.116	43
1	0.121	58
1	0.129	31
1	0.159	63

Table 3.13 -- continued

foundresses	sex ratio	m
2	0.000	25
2	0.000	32
2	0.010	75
2	0.047	64
2	0.080	75
2	0.082	73
2	0.093	75
2	0.125	120
2	0.179	106
3	0.130	54
4	0.174	69

Table 3.14. The sex ratios among the progeny generation in figs of F. aurea to which three or four pollinating wasps reared from the same maturing aurea fig were introduced. 'm' refers to the total number of progeny reared from each experimental fig. The experiment was conducted in Everglades National Park.

foundresses	sex ratio	m
3	0.143	35
4	0.175	40
4	0.189	74
4	0.241	108
4	0.250	48
4	0.319	116
4	0.344	64

Discussion of Data and Theory

In this section the theory of Chapter II is compared with the data on fig wasps. As stated earlier, the quantitative theory of Chapter II was developed a posteriori with respect to this data set. This a posteriori data analysis is aimed at increasing our understanding of fig wasp sex ratios and improving our predictive power in future studies. First, a detailed quantitative study of the data for Pegoscapus assuetus (from F. citrifolia) is reported. Second, a qualitative discussion of the data for P. jimenezi (from aurea) is presented. Finally, an overview of the fig wasp data and the theory is discussed, and future directions for empirical work are outlined.

Quantitative Analysis of P. assuetus Sex Ratios

Data have been obtained that allow a direct check of the predictions of Equation 2.12. The underlying assumptions of this model are assumptions A of Chapter II, with the following additions and changes. (i) The settling of foundresses from the population is random in each generation. (ii) The demes are of size n with probabilities d_n . d_n is estimated by taking the average values over the data in Table 3.4. (iii) The clutch size for each foundress in a deme of size n is K_n ($n = 1, 2, 3, \dots$), instead of K . K_n is estimated from Table 3.10, using $K_6 = 40$ and $K_7 = 32$ (by interpolation). (iv) Sex ratios are conditional on deme size. These changes were outlined in the development of Equation 2.12 in Chapter II.

Equation 2.12 gives the predicted sex ratio as $r_n^* = (C/2)[(n-1)/n]$ ($n = 1, 2, 3, \dots$), where C is the term from the quadratic equation. Using the estimates for d_n and K_n in equations 2.13, $C = 0.815$. The predicted values for r_n^* from Equations 2.13 are given in Figure 3.2 (solid curve), along with the sex-ratio data from Table 3.8. The qualitative trends of predicted and observed sex ratios are similar (both increase with foundress number at about the same rate), but the observed sex ratios are significantly more female-biased than predicted.

Several additional factors affecting sex ratios were mentioned in Chapter II; however, no data are available on these additional factors. How these factors affect the predicted sex ratio will be explored briefly. The factors are: (i) differential contribution of the foundresses within a fig, represented by t_n (Equations 2.14-2.20), which would lower the predicted sex ratio; (ii) large-scale population structure, represented by P_{st} , which also leads to more female-biased predictions; and (iii) patterns of mating and local mate competition within a fig, which may cause the predicted sex ratios to increase or decrease. Each is discussed in turn.

Differential contributions of foundresses. The foundresses within a fig certainly do not all lay the same number of eggs. Equations 2.15-2.20 describe the predicted sex ratio when the contributions of the foundresses differ. The parameter used to measure the expected variability in clutch sizes is t_n . t_n may be written as $\sigma_n^2/\mu_n^2 + 1$ (similar to Charnov 1982, p. 72), where σ_n^2 is the expected variance in clutch sizes and μ_n^2 is the square of the expected clutch size in figs of size n ($n = 2, 3, 4, \dots$; $t_1 = 1$). The predicted sex

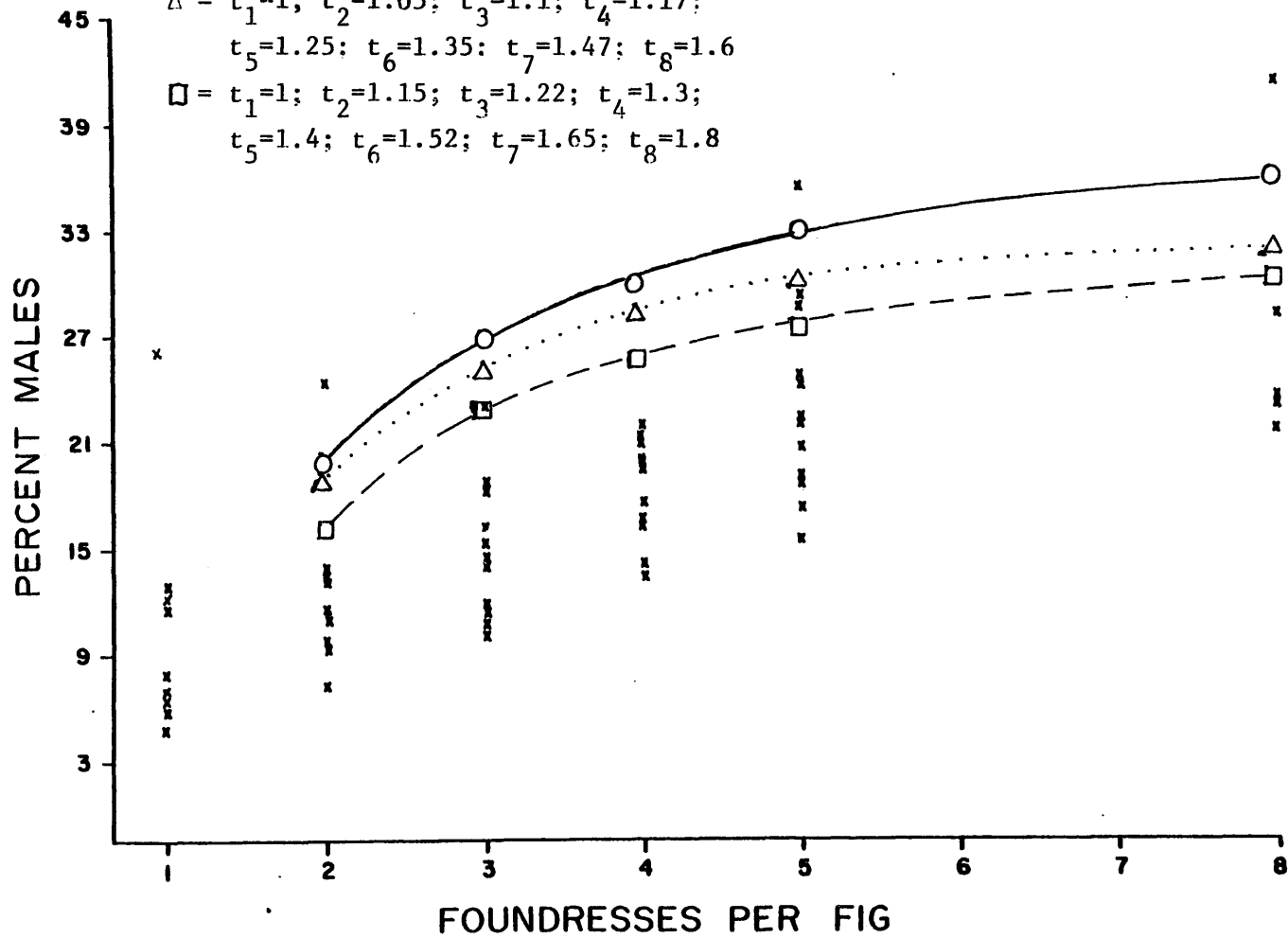
Figure 3.2. Correlation between sex ratios and foundresses per fig for Pegoscapus assuetus from Ficus citrifolia. The upper curve is the predicted sex ratio according to Equation 2.12, using the data in Table 3.4 to estimate d , the data in Table 3.10 to estimate K , and assuming that clutch sizes of all foundresses within a fig are equal ($t_n = 1$ for all n). The lower two curves are from Equation 2.20, using t_n ($n=1,2,3,\dots,8$) as listed in the figure. t_n is defined in Equation 2.17. The two sets of t_n 's represent what I consider to be a reasonable range for the expected variance in clutch sizes within figs with n foundresses. These values for t_n were arrived at by trial and error.

x = datum points from Table 3.8

o = t_n equals 1 for all $n=1,2,3,\dots$

Δ = $t_1=1$; $t_2=1.05$; $t_3=1.1$; $t_4=1.17$;
 $t_5=1.25$; $t_6=1.35$; $t_7=1.47$; $t_8=1.6$

\square = $t_1=1$; $t_2=1.15$; $t_3=1.22$; $t_4=1.3$;
 $t_5=1.4$; $t_6=1.52$; $t_7=1.65$; $t_8=1.8$



ratios using the P. assuetus data for d_n and K_n are given for two sets of values for t_n ($n=1,2,3,\dots,8$) (Fig. 3.2, dotted and dashed curves). These two sets were arrived at by trial and error, such that the true values of t_n very likely fall between them (i. e., the two sets form an upper and lower for t_n). This moves the predicted sex ratios closer to the observed values, and seems entirely justified on logical grounds. However a qualitative discrepancy still exists.

Large-scale population structure. The effects of large-scale population structure on fig wasp sex ratios has been discussed by Frank (1983). However, his quantitative interpretation (1983, Fig. 8) is incorrect. Since $P_{dt} = P_{ds}P_{st}$, large-scale population structure (i. e., P_{st} less than one) reduces the predicted sex ratio. The P_{dt} term is easily adjusted. For example, if $P_{st} = 0.85$, and settling from the local subpopulation is random, $P_{dt} = [(n-1)/n](0.85)$. However, the R term is also affected by large-scale population structure, since R is a function of F, the inbreeding coefficient. If there is differentiation among subpopulations, the correlation of uniting gametes that are identical by descent with respect to the entire population increases. So, F increases, and for haplodiploids, R decreases (Table 2.1), and predicted sex ratios decrease. The exact quantitative relationship between P_{st} and R for haplodiploids under assumptions A, and random settling from local subpopulations, is unknown. However, for haplodiploids (with maternal control of the sex ratio), R is bounded within the interval $[2/3, 1]$ (see Table 2.1), so the effect of small changes of P_{st} (e. g., $P_{st} = 0.85$) will probably be reflected by insignificant changes of R. Thus,

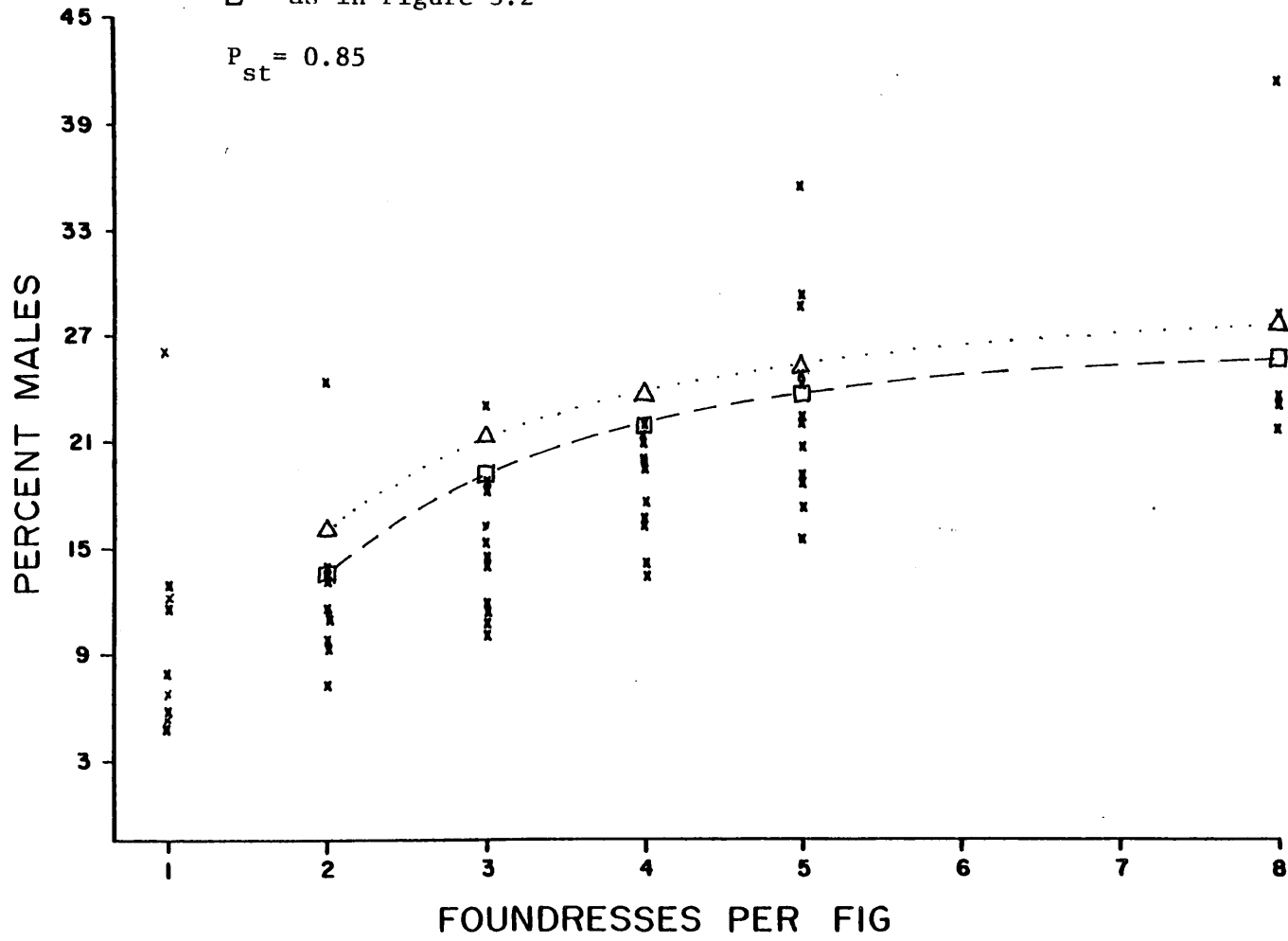
Figure 3.3. Correlation between sex ratios and foundresses per fig for Pegoscapus assuetus. The two curves are identical to the lower two curves of Figure 3.2, except that P_{st} is set at 0.85. See text for further explanation.

x = datum points from Table 3.8

Δ = as in Figure 3.2

\square = as in Figure 3.2

$P_{st} = 0.85$



an upper bound for the predicted sex ratios can be obtained by changing P_{st} and ignoring the small associated reduction of R . With this in mind, the upper bounds for the predicted sex ratios using the two sets of t_n 's from Figure 3.2, d_n and K_n from the P. assuetus data, and P_{st} set at 0.85, are plotted in Figure 3.3. A value of $P_{st} = 0.7$ yields a close fit to the observed sex ratios, however in the absence of supporting data such a value seems extreme.

Given the population dynamics of fig wasps, and the probable bottlenecks that occur in the winter and at the fringes of the distribution, a value for P_{st} of 0.85 is not unrealistic. Wright (1978, chapter 7) reviews values of differentiation for several species based on electrophoretic data. It is difficult to compare these values to what might be expected of fig wasps, since the population dynamics of the species studied electrophoretically varies widely. The only reasonable conclusion that can be made from the data summarized by Wright (1978) is that a value for P_{st} of 0.85 does not appear to be extreme. [My use of P_{st} is nearest to $1-F_{DS}$ of Wright (1978, chapter 7).]

The quantitative analysis of the P. assuetus data has used the empirical distribution of the number of foundresses per F. citrifolia fig (d_n) estimated from the data in Table 3.4. These data were collected during the months of July and August. F. citrifolia has a pronounced flowering peak from May through August (the summer wet season), and flowers infrequently during the winter months (the dry season). Therefore, it is possible that the fig wasp population drops considerably during the winter, and single-foundress and occasional double-foundress figs predominate. To explore the effect of population

Figure 3.4. Correlation between sex ratios and foundresses per fig for Pegoscapus assuetus. The upper curve is the average over the two lower curves of Figure 3.2, where $P_{st} = 1$ and F is estimated from the data in Tables 3.4 and 3.10. The middle curve is the average over the two curves of Figure 3.3, where $P_{st} = 0.85$ and F is estimated from the data. The middle curve is also approximately the predicted values for the two sets of t_n 's from Figure 3.2, with $P_{st} = 1$ and $F = 1$. The lower curve is the average over the two sets of t_n 's from Figure 3.2, with $P_{st} = 0.85$ and $F = 1$. See text for further explanation.

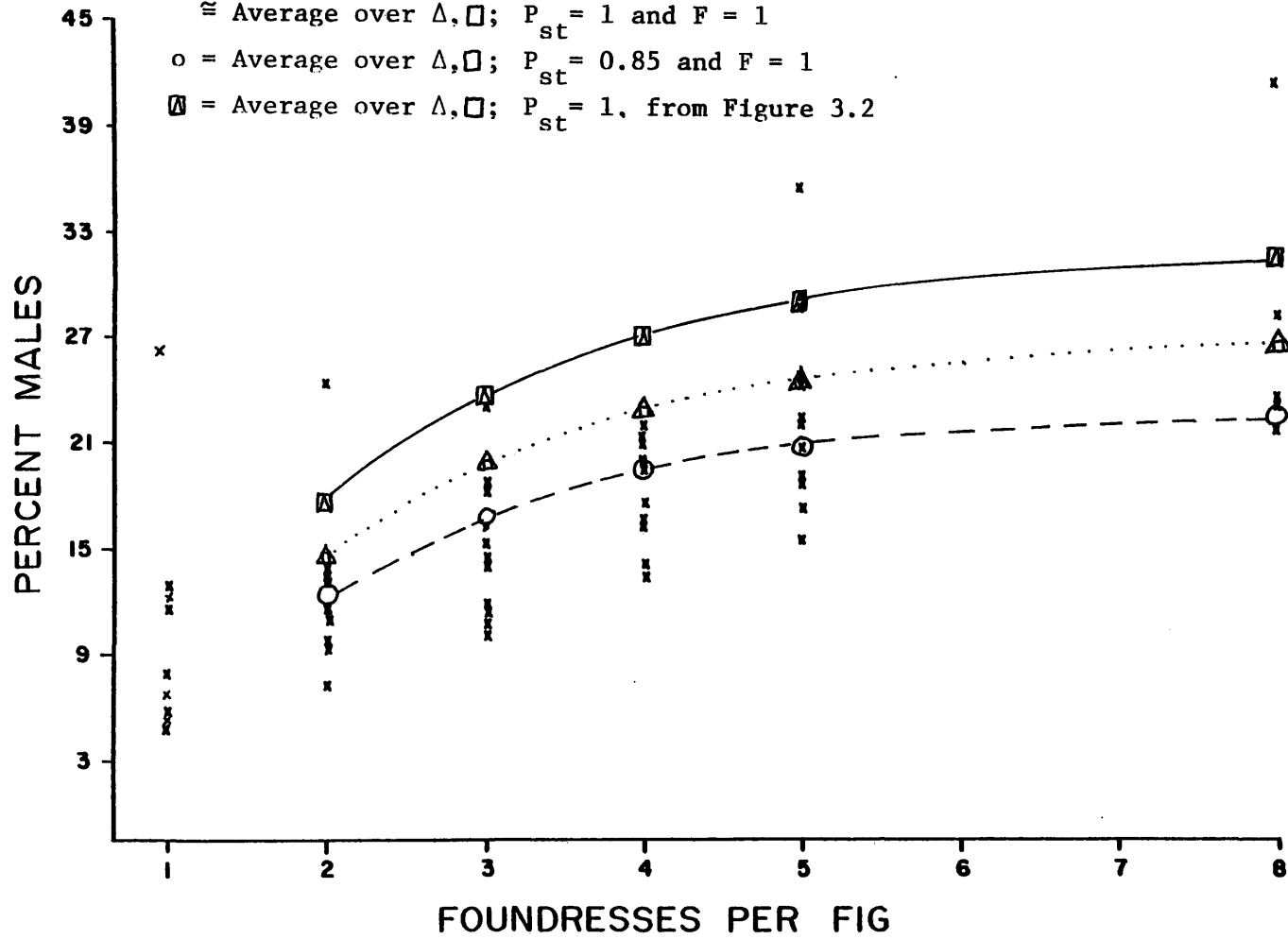
x = datum points from Table 3.8

△ = Average over Δ, □; $P_{st} = 0.85$, from Figure 3.3

≅ = Average over Δ, □; $P_{st} = 1$ and $F = 1$

○ = Average over Δ, □; $P_{st} = 0.85$ and $F = 1$

▣ = Average over Δ, □; $P_{st} = 1$, from Figure 3.2



bottlenecks on the predicted sex ratio, let us consider the extreme case. If all figs have only a single foundress, F will approach 1, and R approaches $2/3$ (Table 2.1). Figure 3.4 presents the predicted sex ratios for the four combinations of $P_{st} = 1$ or 0.85 , $F = 1$ or estimated from the empirical distribution of foundresses per fig (Table 3.4), and taking the average predicted sex ratio over the two sets of t_n 's given in Figure 3.2. These curves represent a reasonable range for the predicted sex ratios under the conditions discussed so far. (Most importantly, we are still assuming random settling from the local subpopulations, and random mating and mate competition within the fig.) A quantitative discrepancy between predicted and observed sex ratios still remains.

Patterns of mating and mate competition. An assumption underlying the quantitative analysis of the P. assuetus data above is that mating is random within the fig--i.e., $P_{id} = 1$. The experiment described earlier, in which males showed a preference for searching for mates in their own fig versus an alien fig, suggests that P_{id} is plausibly less than one. Violation of the random mating assumption clearly raises F , and thus lowers the R term. Exactly how the P_{dt} term is affected is not clear. When random mating was assumed, P_{dt} reflected the level of local mate competition. If mating is assortative, the degree to which males compete with relatives for mates would greatly increase. So, it seems that the level of local mate competition would be considerably greater, and the predicted sex ratio lower. In summary, assortative mating within the figs (P_{id} less than one) appears to lower the predicted sex ratio considerably, since it simultaneously lowers R and increases the level of local mate competition.

Another plausible interpretation of the male-mating preference experiment is that males tend to aggregate by relatedness. Typically, a fig has 1-3 foundresses, so the males of the progeny generation can be divided into 1-3 sibships. One can easily imagine possible benefits to individual males that move in brother-mating groups. For example, in a brother-mating group little time may be wasted on jostling with nearby males, so the overall sibship efficiency is increased. This sibship efficiency is critical, since a group of brothers can perhaps mate more quickly with most of the females, then chew an exit tunnel to end the mating period. It is conceivable that efficient sibship mating followed immediately by chewing an exit tunnel is a superior strategy for individuals over searching alone and encountering a good deal of pushing and competing for mates. Further behavioral observations on the males will be helpful. I am not certain how mate searching in sibships would affect predicted sex ratios.

Qualitative Discussion of P. jimenezi Sex Ratios

The total number of fig wasp progeny per fig in F. aurea is typically 40-80 (Tables 3.12-3.14), while in F. citrifolia it is 150-300 (Tables 3.8, 3.11). The small number of progeny per aurea fig greatly increases the variance in the sex ratio, and makes quantitative studies more difficult. For this reason, most of the empirical research focused on F. citrifolia. However, some data for P. jimenezi (from aurea) were obtained (Tables 3.12-3.14), although sample sizes are too small for statistical inference. Three interesting trends in the data are notable. (i) The sex ratios tended to increase with increasing

foundress number at both Key Largo (Table 3.13) and the Everglades (Table 3.12). (ii) Sex ratios from the Everglades (Tables 3.12 and 3.14) appear to be higher than sex ratios of Key Largo (Table 3.13). (iii) Within the Everglades, the sex ratios of P. jimenezi (from aurea, Tables 3.12, 3.14), appear higher than the sex ratios of P. assuetus (from citrifolia, Tables 3.8, 3.11). These trends are discussed in the next section.

Thesis Summary

The theory developed in Chapter II has provided a good qualitative explanation for the P. assuetus sex-ratio data. The following issues, which are amenable to future empirical studies, have arisen during the development of the theory and discussion of the quantitative predictions and observed sex ratios.

Large-Scale Population Structure

In theory, genetic differentiation among subpopulations of pollinator wasps leads to lower predicted sex ratios. A combination of direct genetic techniques (e. g., electrophoresis) and studies of population demography is the most promising approach. Demographic studies may include information on flowering phenologies of the host fig trees, dispersal studies on the pollinator wasps (see p. 64), and data on the distribution of pollinators per fig through space and time. Also, an among-species hypothesis is that as the distribution of the host fig species becomes more isolated in time and space, the lower the predicted sex ratios of the pollinating wasps.

Population Bottlenecks

The distribution of the number of pollinators per fig undoubtedly varies through time. A bottleneck in the population of pollinating wasps may occur during a low point in the flowering of the host tree population, or after a catastrophe such as drought or frost (see p. 63). Bottlenecks will very likely affect the large-scale population structure (see Wright 1969), tending to increase differentiation and thus lower the predicted sex ratios. Also, during a bottleneck, one-foundress and occasionally two-foundress figs will predominate, and F will increase towards one. This will lower R , and thus lower the predicted sex ratios. This prediction is empirically tractable; island versus mainland regions, or peripheral versus central localities of a distribution, will likely provide useful information since bottlenecks are more common in isolated regions. Note that the sex ratios of P. jimenezi (from aurea) are lower on an island (Key Largo) than on the mainland (Everglades), although the sample sizes are quite small.

Patterns of Mating and Mate Competition in the Fig

The quantitative models have assumed random mating and mate competition within a local group (e. g., a fig). If mating and mate competition are non-random, the predicted sex ratios will be affected. First, the inbreeding coefficient, F , will increase if mating is assortative, and thus lower R and the predicted sex ratios (and vice

versa for disassortative mating). Second, the effect of non-random mate competition on the predicted sex ratios will depend on the fitness valuations of a male offspring as a function of the sex ratio. The expected fitness valuation of a male when mating is random is proportional to F/M , the number of females divided by the number of males (i.e., each male has a probability of $1/M$ of mating with each female). In an experiment, P. assuetus males preferred to search for mates in the fig in which they were born versus an alien fig (see p. 78). This preference may be due to either positive genetic correlation of mates (assortative mating), positive spatial correlation of males searching for mates, or some other cue not correlated with genotype of mates or males. Experiments to distinguish among these three possibilities were outlined (p. 81).

Conditional Sex Ratios

When sex ratios vary according to some ecological parameter, the sex ratio may be considered conditional on this parameter. For example, Equation 2.12 explicitly develops the ESS sex ratio as a function of the number of foundresses in a deme (fig); that is, it predicts that the sex ratio of a fig wasp will be conditional on foundress number. In other words, sex ratios conditional on the level of the parameter confer a greater fitness than unconditional sex ratios. The observed sex ratios (Fig. 3.2) support the hypothesis that fig wasp sex ratios are conditional on foundress number.

Other parameters exist that may be used for adjusting the sex ratio to achieve greater fitness. One is genetic recognition among foundresses. The number of foundresses per fig yields information on the expected genetic variance within a fig. However, if some proximate mechanism exists for assessing more accurately the level of genetic variability within a fig, then sex ratios are predicted to be conditional on the assessed level of genetic variability. A simple experiment was constructed to test if fig wasp sex ratios are conditional on genetic recognition. Females reared from five separate figs were introduced into a single receptive fig, and the sex ratio produced was measured. For comparison, five females reared from the same fig were introduced into a single receptive fig, and the sex ratio was measured. Some of the foundresses within a fig will be sibs in the second set of figs, while in the first set foundresses are never sibs. So, genetic variability is lower within the second set of figs, and if sex ratios are conditional on assessing genetic variability, then the sex ratio is predicted to be lower in the second set. No difference in the sex ratios were detected. However, the design is quite crude. More refined methods for experimentation were outlined (p.106).

Another parameter which may be assessed and used for adjusting the sex ratio is differential contributions of the foundresses. Equation 2.20 is based on the expected variance in contribution of foundresses. However, the actual variance in contribution of foundresses within a fig may be used as a cue. This is perhaps testable by staggering the time between entry of foundresses, thereby changing their relative contributions. More information on time of entry and egg-laying behavior is needed before a rigorous experiment can be designed.

Some additional factors that may affect the sex ratio and some alternative explanations need to be addressed.

Sex Ratios Conditional on Number of Foundresses Expected versus Number Present

To illustrate this point, I will discuss a particular example. A first foundress enters a receptive fig, and begins to lay eggs. At this time, this is a one-foundress fig. As she lays her last eggs, the overall sex ratio of her progeny has nearly been determined. Should she (i) behave as if no other foundress will enter, or (ii) behave as if another foundress will enter with some specified probability? If (i), then she should produce a very low sex ratio, if (ii), then a slightly higher sex ratio. In either case, she should produce a lower sex ratio than if she were in a two-foundress fig (equivalently, if the probability of a second foundress arriving equals one) (Hamilton 1967). Assume the first foundress dies, and a second one enters the fig. Since the first foundress is dead, the second foundress adjusts her sex ratio conditionally according to being with a foundress that has produced a sex ratio below the simultaneous two-foundress predicted level (i.e., the expected sex ratio when two foundresses both can assess each other and adjust their sex ratios accordingly). An interesting property of this situation is that if the second female behaves so as to maximize her expected fitness, the overall sex ratio for both foundresses will be below the simultaneous two-foundress level (Hamilton 1967). To circumvent this difficulty, in all the experiments reported in this thesis the foundresses were introduced into a receptive fig within 90

minutes of one another. (A single female lays eggs for about 18-36 hours, a fig is receptive about 24-48 hours after the first foundress enters.) Entry of all foundresses within such a short time span under natural conditions is probably unusual. To study this problem, a combination of three approaches is needed. (i) A theoretical study of predicted sex ratios for various entry times of foundresses and for various sex-ratio strategies. (ii) Experiments in which the times between the entry of the foundresses is varied. (iii) Comparison among species in which the length of the egg-laying period per female and the length of time that a fig is receptive to new foundresses after the first has entered varies.

Control over the Sex Ratio

The predicted sex ratios for fig wasps as a function of foundress number were obtained by assuming the genetical control was autosomal and the mother had control over the sex ratio. There is a good precedent for maternal control of the sex ratio in Hymenoptera (Flanders 1956). However, if an extranuclear genetic element, or a parasite such as a nematode, were able to influence the sex ratio of the wasps, the predicted sex ratios would be different (Hamilton 1979). For example, matrilineally inherited particles are predicted to increase the female bias in the sex ratio to an extreme degree. The steady rise in the sex ratio with foundress number and the low variance within a given foundress number for P. assuetus (Fig. 3.2) argue against control other than autosomal and maternal.

Another interesting point is that there is an inherent conflict between a fig tree and its pollinator wasps over the wasps' sex ratio (Hamilton pers. comm.). The tree loses one seed for each fig wasp reared, and gains one pollen disperser only for each female fig wasp reared. So the tree is benefited by a low pollinator sex ratio, independent of foundress number. Since the wasps' sex ratio increases with increasing foundress number, this conflict between tree and wasp may help explain why the ostiole closes so quickly (24-48 hours) after the first foundress enters. Once the foundresses are inside, it does not appear that the tree can affect the pollinators' sex ratio, since the sex ratio rises steadily with foundress number. However, the inability of the tree to affect the pollinators' sex ratio is not certain, and remains a fascinating, open question.

Sex Ratio or Investment Ratio?

The predicted sex ratios depend on the assumption that the cost to a female of producing a son equals the cost of producing a daughter. This seems a reasonable assumption for fig wasps, since each progeny is laid in its own flower. If the cost of a female were greater, perhaps due to increased oviposition time required for fertilization, or increased nutrient content of eggs, the predicted sex ratios would increase (i.e., more males). If males were more costly, perhaps due to extra nutrients in the eggs to promote rapid development and an advantage in mate competition, the predicted sex ratios would be lower. I can think of no simple, direct methods for testing the cost of producing males versus females.

Efficiency of Theoretical Modeling Exceeds Efficiency of Adaptation?

Models can be made increasingly complex as more and more parameters are added. With enough parameters, agreement between theory and observation can easily be achieved. However, the reality of adding additional parameters must be questioned. Parameters such as large-scale population structure are plausible, and when added to the model a better fit of theory to observation is obtained (Fig. 3.3). Deciding which parameters to add to a model is a difficult task.

Future Directions

In this study of fig wasp sex ratios, an excellent qualitative agreement between theory and observation has been achieved. Methods for the quantitative analysis of fig wasp sex-ratio patterns have been clearly developed and applied in an a posteriori fashion. A great advantage of studying the fig tree-fig wasp complex is that with 900 species there is a rich source of comparative information. This allows an extensive test of the predictive powers of these quantitative theories. As more information on other species is obtained, the accuracy and precision of these quantitative models can be assessed in detail.

APPENDIX A
 MATHEMATICS FOR LOCAL MATE COMPETITION

There are three sections in this appendix, (i) an index of symbols used in Chapter II, (ii) a derivation of Equations 2.10, 2.12, and 2.20 by extending the methods of Taylor and Bulmer (1980), and (iii) a discussion of the variance terms, V_d , V_t and P_{dt} used in Chapter II, including derivations of the conditional variances used.

Index of Symbols Used

- B_{ps} , B_{pd} "Complete" coefficient of relatedness of the parent controlling the sex ratio to son or parent to daughter, respectively (Hamilton 1972).
- R ratio of relatedness coefficients, $R = 2B_{ps} : B_{pd}$
- P_{dt} Wright's index of panmixia (Wright 1969) represents the genetic (or phenotypic type) differentiation among demes within the population. A value of 1 denotes no differentiation among demes, while a value of 0 denotes complete differentiation.
- r^* predicted, unconditional sex ratio of each female in the population
- r_n^* predicted, conditional sex ratio for each female within a deme of size n ($n = 1, 2, 3, \dots, L$)

N	The number of already mated females founding a deme to start a new generation. Upper case is used to denote that all demes are of exactly the same size N .
n	as above, except that deme sizes vary from $n = 1, 2, 3, \dots, L$
d_n	the proportion of demes that are of size n
K	number of eggs laid by each female in the population
K_n	number of eggs laid by each female in a deme of size n
V_d	genetic variance within demes over the entire population
v_d	genetic variance within a deme, lower case used to denote that the variance within a particular deme is a random quantity
V_a	genetic variance among demes
v_a	genetic variance among demes, lower case used to denote that this quantity is dependent on conditions which are random
V_t	genetic variance within the population
F	Wright's fixation index (Wright 1969), the correlation between homologous genes of uniting gametes relative to the gene frequencies in the whole population.
w	proportion of sib mating in each generation over the entire population
w_n	proportion of sib mating in a deme of size n
a, b	defined in Equation 2.11
x, y, z	defined in Equation 2.13
k_{ni}	number of eggs by the i^{th} female in a deme of size n
h_{ni}	k_{ni}/K_n
g_{nj}	the probability of a particular array (h_{nij}) such that the sum of g_{nj} over j is one

t_n a measure of the variability in clutch sizes of foundresses within a deme of size n , a value of 1 means equal clutch sizes for all females, and a value of n means that all eggs are laid by a single foundress

n' n/t_n

x', y', z' Equations 2.20

Derivation of Results

In this chapter, Equations 2.10, 2.12, and 2.20 were developed from Hamilton's (1979) formula (Equation 2.1). At this time, the only proofs I can offer for these results follow as an extension of the methods of Taylor and Bulmer (1980). This section develops the methods and proofs of Equations 2.10, 2.12, and 2.20 in order.

Taylor and Bulmer (1980) found the solution for the constant deme size, equal contribution of foundresses, and random settling situation in haplodiploids. First, the methods they used are presented, then the necessary extensions for proving the new results developed in this chapter are provided.

Assume the following. The sex ratio is controlled at a single locus on an X chromosome. The allele R causes females to produce a sex ratio of r (males/total), and the allele S a sex ratio of s . R is assumed dominant, i. e., RS yields a sex ratio of r . The allele S is rare, such that there is never more than one of N females bearing an S allele per deme. Name the S allele a mutant type; let $x(i,j)$ ($0 \leq i \leq 2$, $0 \leq j \leq 1$, $i+j > 0$) be the relative frequency of demes in which there are $N-1$ non-mutant fertilized females and one female of type (i,j) with i S alleles in the female and j S alleles in her mate.

Letting x^+ be the relative frequency of deme types in the next generation

$$\begin{pmatrix} x^+(2,1) \\ x^+(2,0) \\ x^+(1,1) \\ x^+(1,0) \\ x^+(0,1) \end{pmatrix} = \begin{pmatrix} pu/(1-r) & 0 & \frac{1}{4N} & 0 & 0 \\ (1-p)u/(1-r) & 0 & \frac{2N-1}{4N} & 0 & 0 \\ 0 & pu/(1-r) & \frac{1}{4N} & \frac{1}{4N} & 0 \\ 0 & (1-p)u/(1-r) & \frac{2N-1}{4N} & \frac{2N-1}{4N} & 1 \\ (N-1)p & (N-1)p & \frac{N-1}{2N} & \frac{2N-1}{4N} & 0 \end{pmatrix} \begin{pmatrix} x(2,1) \\ x(2,0) \\ x(1,1) \\ x(1,0) \\ x(0,1) \end{pmatrix}$$

$$u = 1-s$$

$$p = s/\{s+(N-1)r\}$$

The eigenvalues of this equation are functions of r and s , so write $\lambda(r,s)$. First, note that $\lambda(r,r) = 1$, which is the dominant eigenvalue. Now, we seek $r \in [0,1]$ such that $\lambda(r,s) \leq 1$ for all $s \in [0,1]$. So, a necessary condition for $\lambda(r,s) \leq 1$ for all s is that $\partial\lambda/\partial s = 0$ at $r = s$.

Denoting the transition matrix as $A(r,s)$ and the characteristic equation of A as $f(r,s,\lambda)$, λ is obtained by solving $f(r,s,\lambda) = 0$ for λ as a function of r and s . Differentiating f with respect to s ,

$$\frac{\partial f}{\partial s} + \frac{\partial f}{\partial \lambda} \frac{\partial \lambda}{\partial s} = 0 .$$

Note that the condition $\partial\lambda/\partial s = 0$ at $r = s$ implies $\partial f/\partial s = 0$ at $r = s$, and that $\lambda(r,r) = 1$. So, taking

$$\partial/\partial s |A-I| = 0 \quad \text{at } r = s$$

one can solve for r^* , the value of r such that $(r^*, s) \leq 1$ for all s . Elementary row operations that do not affect the determinant simplify the work. Replace row 3 by row 3 + (1/2)row 4 + (1/2)row 5, and note that only the upper left 3 X 3 determinant need be evaluated. The result obtained is Equation 2.5.

Now, I extend Taylor and Bulmer's (1980) work. First, some of the unrealistic assumptions are relaxed. (i) Let demes of size n occur with probability d_n , such that the sum of d_n over all n ($n = 1, 2, 3, \dots, L$) equals one. (ii) Denote the number of offspring per female in a deme of size n as K_n , where K_n is a positive, finite integer. (iii) Denote the sex ratio produced by the R allele in demes of size n as $r_n \in [0, 1]$ for $n = 1, 2, 3, \dots, L$, and the sex produced by SS homozygotes as $s_n \in [0, 1]$.

Call the above transition matrix A_n for a deme of size n , and let $\underline{x} = x(i, j, n)$, i and j ranging as above and $n = 1, 2, 3, \dots, L$, be the relative frequency of demes of type (i, j) of size n . Then

$$\underline{x}^+ = \frac{1}{2a} \begin{pmatrix} d_1 K_1 (1-r_1) A_1 & d_1 K_2 (1-r_2) A_2 & \dots & d_1 K_L (1-r_L) A_L \\ 2d_2 K_1 (1-r_1) A_1 & 2d_2 K_2 (1-r_2) A_2 & \dots & 2d_2 K_L (1-r_L) A_L \\ 3d_3 K_1 (1-r_1) A_1 & 3d_3 K_2 (1-r_2) A_2 & \dots & 3d_3 K_L (1-r_L) A_L \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ \cdot & \cdot & \cdot & \cdot \\ Ld_L K_1 (1-r_1) A_1 & Ld_L K_2 (1-r_2) A_2 & \dots & Ld_L K_L (1-r_L) A_L \end{pmatrix}$$

where a is defined as in Equation 2.11. Let $\underline{r} = (r_1, r_2, r_3, \dots, r_L)$, and $\underline{s} = (s_1, s_2, s_3, \dots, s_L)$. It can be shown that $(\underline{r}, \underline{r}) = 1$ is the dominant eigenvalue, so we can mimic the above methods. Calling this transition matrix for variable deme sizes Q , it is easy to show by elementary row and column operations that

$$|Q-I| = \pm \left| \left[\sum_{n=1}^L \frac{nd_n K_n (1-r_n) A_n}{2a-I} \right] / 2a-I \right|$$

Evaluating $\partial |Q-I| / \partial s_n$ at $\underline{r} = \underline{s}$ yields Equation 2.10; hence the solution is as in Equation 2.12.

The second problem discussed in this chapter deals with unequal contributions of foundresses within a deme. Using the notation in the text,

$$A_n = \begin{pmatrix} \alpha & 0 & \frac{1}{4n'} & 0 & 0 \\ \beta & 0 & \frac{2n'-1}{4n'} & 0 & 0 \\ 0 & \alpha & \frac{1}{4n'} & \frac{1}{4n'} & 0 \\ 0 & \beta & \frac{2n'-1}{4n'} & \frac{2n'-1}{4n'} & 1 \\ \xi & \xi & \frac{n'-1}{2n'} & \frac{2n'-1}{4n'} & 0 \end{pmatrix}$$

$$\alpha = \sum_{i=1}^n \sum_{j=1}^{\infty} \frac{g_{nj} h_{nij}^2 s_n (1-s_n)}{(1-r_n) \{n^2 r_n + n h_{nij} (s_n - r_n)\}}$$

$$\beta = \sum_{i=1}^n \sum_{j=1}^{\infty} \left(\frac{g_{nj} h_{nij} (1-s_n)}{n(1-r_n)} - \frac{g_{nj} h_{nij}^2 (1-s_n) s_n}{n(1-r_n) \{n r_n + h_{nij} (s_n - r_n)\}} \right)$$

$$\xi = \sum_{i=1}^n \sum_{j=1}^{\infty} \frac{g_{nj} s_n h_{nij} \sum_{q \neq i} h_{nqj}}{n \{n r_n + h_{nij} (s_n - r_n)\}}$$

$$= \sum_{i=1}^n \sum_{j=1}^{\infty} \frac{g_{nj} s_n h_{nij} (n - h_{nij})}{n \{n r_n + h_{nij} (s_n - r_n)\}}$$

Following the steps outlined above, the solution for r_n^* given by Equations 2.20 is obtained.

Variance Terms

Classically, genetic variances at a locus with two alternative alleles are obtained as follows. Let allele A occur with probability p , and allele a with probability q , such that $p + q = 1$. Assign the value 1 to allele A, and 0 to allele a, and apply the properties of the binomial distribution. The variance of a population is given by pq . As

in classic analysis of variance procedures, the variability in the total population, V_t , is the sum of the total variability within groups (here, demes), V_d , plus the total variability between groups, V_a , and $V_t = V_a + V_d$ (infinite population size assumed). If phenotypic types are considered, as in the Price equation analysis of this chapter, let the probability of sex-ratio type A be p , and of sex-ratio type a be q . The variance of types within the population is pq . Under assumptions A and random settling of foundresses into demes from the entire population in each generation, a standard result is $V_a = pq/n$, which is the variance of an estimate of p for a sample size of n . From above, $V_d = V_t - V_a = pq - pq/n = pq(1 - 1/n)$, so $P_{dt} = V_d/V_t = 1 - 1/n$.

Equations 2.8, 2.10, and 2.19 use the expectation of a conditional variance to obtain P_{dt} . The general rule for the expectation of a conditional variance is (Lindgren 1976)

$$\text{Var } X = E_Y[\text{Var}(X|Y)] + \text{Var}(u_{X|Y})$$

$$\text{Var}(u_{X|Y}) = E_Y[(u_{X|Y} - u_X)^2]$$

where $u_{X|Y}$ is the conditional mean of X . In the case of random settling, $u_{X|Y} = u_X = p$, so this term can be ignored. However, with other forms of settling, this may not be the case.

Let X be the proportion, p , of a certain sex-ratio type in a deme of size n , and Y be a random variable denoting deme size. Since $E_{X|y}[\text{Var}(X|y)] = 1 - 1/n$ [this is the standard result for $E(s^2)$ for a random sample of size n], it follows in Equation 2.8 that

$$E_Y E_{X|Y}[\text{Var}(X|Y)] = \sum_{n=1}^L d_n (1 - 1/n)$$

which is the value for P_{dt} needed. In Equation 2.10, the P_{dt} term required is $E_{X|y}[\text{Var}(X|y)]$, which is $(n-1)/n$. In Equation 2.19, define X as the proportion of a certain sex-ratio type in the progeny generation, Z as a random variable denoting a particular array of (h^2_{nij}) with probability distribution given by the g_{nj} , and y as a fixed value denoting the deme size, n . Note that $v_d = V_t - v_a$, where v_a is the variance among demes (which depends on the values of y and z , and is therefore written in lower case), and $P_{dt} = 1 - E(v_a)/V_t$. The variance among demes in the variable X , given y and z as fixed values of the random quantities Y and Z , is

$$E_{X|y,z} \text{Var}(X|y,z) = (\sum_i h^2_{nij}/n^2)(v_t)$$

$$E_Z E_{X|y,Z} [\text{Var}(X|y,Z)] = (\sum_{j=1}^{\infty} \sum_{i=1}^n g_{nj} h^2_{nij}/n^2)(v_t)$$

So, $E(v_d)/V_t$ is reported in Equation 2.19.

APPENDIX B
STATISTICAL NOTE

Two sets of hypotheses were tested in Chapter III with a novel nonparametric procedure. These two sets of hypotheses were discussed on pp. 98 and in Table 3.10 during the analysis of the mechanism underlying the increase in sex ratio with increasing foundress number in Pegoscapus assuetus (from Ficus citrifolia). The hypotheses are (i) that there is no correlation within a given foundress number between the absence (coded as a low quantity) or presence (coded as a high quantity) of cecidomyiid galls and rank of the number of progeny in the fig, m , versus the alternative of a negative correlation; and (ii) that there is no correlation between galls (coded as above) and rank of sex ratio, r , versus the alternative that there is either positive or negative correlation.

Within a given foundress number the data can be divided into two groups, those with galls and those without. For hypothesis (i) the response can be considered rank m , and for (ii) the response can be viewed as rank r . Within a given foundress number we are looking for a shift in the location of the response between the two groups of figs with galls and without galls. A standard test for shift in location between two populations is the Wilcoxon Rank Sum Statistic, W (Hollander and Wolfe 1973). The special problem is that we wish to combine the information over all foundress numbers. Let W_i be the Wilcoxon Statistic for figs with i foundresses, where

$$W_i = \sum_{j \in NG} R_{ij}$$

and R_{ij} is the rank of the j^{th} response in figs with i foundresses and NG are figs with no galls. To combine the information in W_i for figs with exactly i foundresses, consider $W = \sum_{i=2}^8 W_i$. W can be obtained directly from Table 3.8 for both hypotheses (i) and (ii). For (i), $W = 88$; for (ii) $W = 67$. To obtain an approximate significance level for these statistics, let

$$W^* = \frac{W - E(W)}{\{\text{Var}(W)\}^{1/2}}$$

Note that

$$\begin{aligned} E(W) &= E(\sum W_i) = \sum E(W_i) \\ \text{Var}(W) &= \text{Var}(\sum W_i) = \sum \text{Var}(W_i) \end{aligned}$$

since the W_i 's are mutually independent under the null hypothesis. $E(W_i)$ and $\text{Var}(W_i)$ can be obtained by the standard formulae for the Wilcoxon Rank Sum Statistic (Hollander and Wolfe 1973). For (i) $W^* = 1.00$, and for (ii) $W^* = -1.21$. Under the null hypothesis, statistics of the form given for W^* are typically asymptotically $N(0,1)$ (or Z) random variables. In this case I have no proof of the asymptotic normality of W^* , nor have the small sample properties been analyzed by simulation. However, this procedure has historically been successful for obtaining approximate significance levels (see Hollander and Wolfe 1973 for examples), so it seems reasonable to apply it here. The significance levels reported in the text were obtained by comparing observed values of W^* to a $N(0,1)$ (or Z) table.

LITERATURE CITED

- Alexander, R. D. 1979. Darwinism and Human Affairs. Washington University Press, Seattle.
- , and G. Borgia. 1978. Group selection, altruism, and the levels of organization of life. *Annu. Rev. Ecol. Syst.* 9: 449-74.
- , and P. W. Sherman. 1977. Local mate competition and parental investment in social insects. *Science* 196: 494-500.
- Alstad, D. N., and G. F. Edmunds, Jr. 1983. Selection, outbreeding, and the sex ratio of scale insects. *Science* 220: 93-95.
- Assem, J. van den, H. A. J. in den Bosch, and E. Prooy. 1982. Melittobia courtship behaviour: a comparative study of the evolution of a display. *Netherlands J. Zool.* 32: 427-71.
- Beckenbach, A. 1983. Fitness analysis of the "sex-ratio" polymorphism in experimental populations of Drosophila pseudoobscura. *Amer. Nat.* 121: 630-48.
- Blaustein, A. R. 1983. Kin recognition mechanisms: phenotypic matching or recognition alleles? *Amer. Nat.* 121: 749-54.
- Boucek, Z. A. Watsham, and J. T. Wiebes. 1981. The fig wasp fauna of the receptacles of Ficus thonningii (Hymenoptera, Chalcidoidea). *Tijd. Ent.* 124: 149-233.
- Brockman, C. F., H. S. Zim, and R. Merrilees. 1968. Trees of North America. Golden Press, New York.
- Butcher, F. G. 1964. The Florida fig wasp, Secundeisenia mexicana (Ashm.), and some of its hymenopterous symbionts. *Fla. Ent.* 47: 235-38.
- Charnov, E. L. 1982. The Theory of Sex Allocation. Monographs in Population Biology No. 18. Princeton Univ. Press, Princeton.
- , R. L. Los-den Hartogh, W. T. Jones, and J. van den Assem. 1981. Sex ratio evolution in a variable environment. *Nature* 289: 27-33.
- Clark, A. B. 1978. Sex ratio and local resource competition in a prosimian primate. *Science* 201: 163-65.

- Colwell, R. K. 1981. Group selection is implicated in the evolution of female-biased sex ratios. *Nature* 290: 401-04.
- Condit, I. J. 1969. Ficus: The Exotic Species. University of California, Division of Agricultural Sciences.
- Crow, J. F. 1979. Genes that violate Mendel's rules. *Sci. Amer.* 240(2): 136-46.
- Darwin, C. 1871. *The Descent of Man, and Selection in Relation to Sex*. D. Appleton, New York.
- Dawkins, R. 1980. Good strategy or evolutionarily stable strategy? Pages 331-67 in G. W. Barlow and J. Silverberg, eds. *Sociobiology: Beyond Nature/Nurture?* AAAS, Westview Press, Boulder, CO.
- 1982. *The Extended Phenotype*. Freeman Press, San Francisco.
- Dobzhansky, T., and S. Wright. 1943. Genetics of natural populations: X. Dispersion rates in Drosophila pseudoobscura. *Genetics* 26: 23-51.
- 1947. Genetics of natural populations: XV. Rate of diffusion of a mutant gene through a population of Drosophila pseudoobscura. *Genetics* 32: 303-24.
- Edmunds, G. F., Jr., and D. N. Alstad. 1978. Coevolution in insect herbivores and conifers. *Science* 199: 241-45.
- Felt, E. P. 1934. A gall midge on a fig (*Ficiomyia birdi*, sp. nov.). *Ent. News* 45: 131-35.
- Fisher, R. A. 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- Flanders, S. E. 1956. The mechanism of sex-ratio regulation in the (parasitic) Hymenoptera. *Insectes Soc.* 3: 325-34.
- Frank, S. A. 1983. A hierarchical view of sex-ratio patterns. *Fla. Ent.* 66: 42-75.
- Galil, J. 1977. Fig biology. *Endeavour*, New Series 1(2): 52-56.
- , and D. Eisikowitch. 1971. Studies on mutualistic symbiosis between syconia and sycophilous wasps in monoecious figs. *New Phytol.* 70: 773-87.
- , W. Ramirez, and D. Eisikowitch. 1973. Pollination of Ficus costaricana and F. hemsleyana by Blastophaga esterae and B. tonduzi in Costa Rica (Hymenoptera: Chalcidoidea, Agaonidae). *Tijd. Ent.* 116: 175-83.

- Gordh, G. 1975. The comparative external morphology and systematics of the neotropical parasitic fig wasp genus Idarnes (Hymenoptera:Torymidae). Univ. of Kansas Sci. Bull. 50: 390-455.
- Grandi, G. 1929. Studio morfologico e biologico della Blastophaga psenes (L.). Boll. Lab. Ent. R. Istituto Superiore Agrario Bologna 2: 1-147.
- Hamilton, W. D. 1964. The genetical evolution of social behavior. I and II. J. Theor. Biol. 7: 1-52.
- 1967. Extraordinary sex ratios. Science 156: 477-88.
- 1971. Selection of selfish and altruistic behavior in some extreme models. Pages 57-91 in J. F. Eisenberg and W. S. Dillon, eds., Man and Beast: Comparative Social Behavior. Smithsonian Press, Washington, D. C.
- 1972. Altruism and related phenomena, mainly in social insects. Annu. Rev. Ecol. Syst. 3: 193-232.
- 1975. Innate social aptitudes of man: an approach from evolutionary genetics. Pages 133-155 in R. Fox, ed. Biosocial Anthropology. Wiley, New York.
- 1979. Wingless and fighting males in fig wasps and other insects. Pages 167-220 in M. S. Blum and N. A. Blum, eds. Sexual Selection and Reproductive Competition in Insects. Academic Press, New York.
- unpublished. Ess sex ratios for a sperm-storing animal with local competition for mates.
- Hill, D. 1967. The Figs (Ficus spp.) of Hong Kong. Hong Kong Univ. Press, Hong Kong.
- Hollander, M., and D. A. Wolfe. 1973. Nonparametric Statistical Methods. Wiley, New York.
- Holmes, W. G., and P. W. Sherman. 1982. The ontogeny of kin recognition in two species of ground squirrels. Am. Zool. 22: 491-517.
- Janzen, D. H. 1979a. How to be a fig. Ann. Rev. Ecol. Syst. 10: 13-51.
- 1979b. How many parents do the wasps from a fig have? Biotrop. 11: 127-29.
- Joseph, K. J. 1958. Recherches sur les chalcidiens, Blastophaga psenes (L.) et Philotrypesis caricae (L.), du figuier (Ficus carica L.). Annls. Sci. Nat., Zool. 20: 197-260.

- Kolman, W. 1960. The mechanism of natural selection for the sex ratio. Amer. Nat. 94: 373-77.
- Li, C. C. 1976. First Course in Population Genetics. Boxwood Press, Pacific Grove, California.
- Lindgren, B. W. 1976. Statistical Theory. Macmillan Publishing Co., New York.
- Martin, G. C. A. M. Owen, and J. I. Way. 1973. Nematodes, figs and wasps. J. Nemat. 5: 77-78.
- Maynard Smith, J. 1978. The Evolution of Sex. Cambridge University Press, Cambridge, England.
- , and G. R. Price. 1973. The logic of animal conflict. Nature 246: 15-18.
- Novitski, E. 1947. Genetic analysis of an anomalous sex ratio condition in Drosophila affinis. Genetics 32: 526-34.
- Olmstead, I. C., L. L. Loope, and C. F. Hilsenbeck. 1980. Tropical hardwood hammocks of the interior of Everglades National Park and Big Cypress Preserve. South Florida Research Center Report T-604.
- Pickering, J. 1980. Larval competition and brood sex ratios in the gregarious parasitoid Pachysomoides stupidus. Nature 283: 291-92.
- Price, G. R. 1970. Selection and covariance. Nature 227: 520-1.
- 1972. Extension of covariance selection mathematics. Ann. Hum. Genet. 35: 485-90.
- Ramirez, W. 1970a. Taxonomic and biological studies of neotropical fig wasps (Hymenoptera:Agaonidae). Univer. of Kansas Sci. Bull. 49: 1-44.
- 1970b. Host specificity of fig wasps (Agaonidae). Evolution 24: 680-91.
- 1974. Coevolution of Ficus and Agaonidae. Ann. Missouri Bot. Gardens 61: 770-80.
- Rhoades, M. M. 1933. The cytoplasmic inheritance of male sterility in Zea mays. J. Genet. 27: 71-93.
- Salt, G. W. 1936. Experimental studies in insect parasitism: IV. The effect of superparasitism on populations of Trichogramma evanescens. J. Exp. Biol. 13: 363-75.
- Seeger, J. 1976. Evolution of responses to relative homozygosity. Nature 262: 578-80.


- Shaw, R. F., and J. D. Mohler. 1953. The selective advantage of the sex ratio. *Amer. Nat.* 87: 337-42.
- Sokal, R. R., and F. J. Rohlf. 1969. *Biometry*. Freeman Press, San Francisco.
- Stalker, H. D. 1961. The genetic systems modifying meiotic drive in *Drosophila paramelancia*. *Genetics* 46: 177-202.
- Taylor, P. D. 1981. Intra-sex and inter-sex interactions as sex ratio determinants. *Nature* 291: 64-66.
- , and M. G. Bulmer. 1980. Local mate competition and the sex ratio. *J. Theor. Biol.* 86: 409-19.
- Tomlinson, P. B. 1980. *The Biology of Trees Native to Tropical Florida*. Harvard Univ. Printing Office, Allston, Massachusetts.
- Trivers, R. L., and H. Hare. 1976. Haplodiploidy and the evolution of social insects. *Science* 191: 249-63.
- , and D. E. Willard. 1973. Natural selection of parental ability to vary the sex ratio of offspring. *Science* 179: 90-92.
- Velthuis, H. H. W., F. M. Velthuis-Kluppell, and G. A. H. Bossink. 1965. Some aspects of the biology and dynamics of *Nasonia vitripennis* (Walker) (Hymenoptera:Pteromalidae). *Ent. Exp. Appl.* 8: 205-27.
- Verner, J. 1965. Selection for the sex ratio. *Amer. Nat.* 99: 419-21.
- Wade, M. J. 1978. A critical review of the models of group selection. *Quart. Rev. Biol.* 53: 101-14.
- , 1982. Group selection: migration and the differentiation of small populations. *Evolution* 36: 949-61.
- Walker, I. 1967. Effect of population density on the viability and fecundity in *Nasonia vitripennis* Walker (Hymenoptera, Pteromalidae). *Ecology* 48: 294-301.
- Werren, J. H. 1980a. Sex ratio adaptations to local mate competition in a parasitic wasp. *Science* 208: 1157-59.
- , 1980b. Studies in the evolution of sex ratios. Ph.D. Thesis, University of Utah, Salt Lake City.
- , 1983. Sex ratio evolution under local mate competition. *Evolution* 37: 116-24.
- White, M. J. D. 1973. *Animal Cytology and Evolution*, 3rd ed. Cambridge Univ. Press, Cambridge.

- Wiebes, J. T. 1979. Co-evolution of figs and their insect pollinators. *Annu. Rev. Ecol. Syst.* 10: 1-12.
- . 1982. The phylogeny of the Agaonidae (Hymenoptera, Chalcidoidea). *Nether. J. Zool.* 32: 395-411.
- . in press. Records and descriptions of Pegoscapus Cameron (Hymenoptera; Chalcidoidea, Agaonidae). *Proc. Kon. Ned. Akad. Wetensch. (C)*, Vol. 86.
- Wilson, D. S. 1980. *The Natural Selection of Populations and Communities*. Benjamin/Cummings Publishing Co., Menlo Park, California.
- , and R. K. Colwell. 1981. Evolution of sex ratio in structured demes. *Evolution* 35: 882-97.
- Wrensh, D. L., and S. S. Y. Young. 1978. Effects of density and host quality on rate of development, survivorship and sex ratio in the carmine spider mite. *Environ. Ent.* 7: 499-501.
- Wright, S. 1968. *Evolution and the Genetics of Populations, Vol. 1. Genetic and Biometric Foundations*. Univ. of Chicago Press, Chicago.
- . 1969. *Evolution and the Genetics of Populations, Vol. 2. The Theory of Gene Frequencies*. Univ. of Chicago Press, Chicago.
- . 1977. *Evolution and the Genetics of Populations, Vol. 3. Experimental Results and Evolutionary Deductions*. Univ. of Chicago Press, Chicago.
- . 1978. *Evolution and the Genetics of Populations, Vol. 4. Variability within and among Natural Populations*. Univ. of Chicago Press, Chicago.
- Wylie, H. G. 1965. Discrimination between parasitized and unparasitized house fly pupae by females of Nasonia vitripennis (Walker)(Hymenoptera: Pteromalidae). *Canadian Ent.* 97: 279-86.
- Zaher, M. A., K. K. Shehata, and H. El-Khatib. 1979. Population density effects on biology of Tetranychus arabicus, the common spider mite in Egypt. *Rec. Adv. Acarology* 1: 507-509.


BIOGRAPHICAL SKETCH

Steven Alan Frank was born on 8 November, 1957, in Rochester, New York. He received a Bachelor of Science degree from the University of Michigan in 1979, and entered graduate school at the University of Florida in 1980. He received a Master of Statistics degree from the University of Florida in April, 1983. He will return to Ann Arbor in the Fall of 1983 to continue his studies in biology.

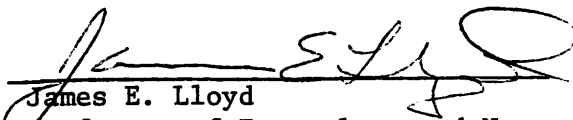
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.


H. Jane Brockmann, Chairperson
Associate Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.


Pauline O. Lawrence
Associate Professor of Zoology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Science.


James E. Lloyd
Professor of Entomology and Nematology

This thesis was submitted to the Graduate Faculty of the Department of Zoology in the College of Liberal Arts and Sciences and to the Graduate School, and was accepted as partial fulfillment of the requirements for the degree of Master of Science.

August, 1983

Dean for Graduate Studies and
Research