Sex Ratio Evolution and Resource Allocation in the Multiple-Queen Ant *Leptothorax acervorum*

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THE ANT

These are a species of four-winged insects, that have been famous from all antiquity for their social and industrious habits; that are offered as a pattern of parsimony to the profuse, and unremitting diligence to the sluggard.

Georges Louis Leclerc, 1707-1788,
Comte de Buffon,
Buffon's Natural History.

Abstract

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Current evolutionary theories have attempted to explain the greater than random variation in allocation of resources to new queens and new males (sex allocation), as well as allocation of resources to new sexuals versus new workers (resource allocation) in social Hymenoptera. Because of relatedness asymmetries within social insect colonies, queens and workers are in conflict over the optimal allocation of resources to brood. This thesis examines three populations of the facultatively polygynous ant species *Leptothorax acervorum* over several years. Data on colony size, number of colony queens, production of new queens, new males and new workers are examined for each colony sampled. I investigate the factors affecting how resources are divided amongst brood in colonies, and consider which party controls allocation of these resources.

Monogynous and polygynous colonies differ in their allocation strategies. While there are considerable differences between populations, monogynous colonies tend to invest more resources into new sexuals with polygynous colonies investing in more new workers to stock daughter buds. Evidence of local resource competition also supports colony budding as a reproductive strategy by some colonies. Sex allocation decisions appear to reflect within-colony relatedness in two study populations. Environmental influences such as local nest site availability and resource abundance also appear to influence allocation decisions at the colony level. Small colonies tend to concentrate on colony growth with larger colonies producing more new sexuals. There is no evidence of partial queen control over sex or resource allocation. Instead, workers seem to respond to information about within-colony relatedness as well as environmental factors when making sex and resource allocation decisions.

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George Chan,
Institute of Zoology,
London,
November 1996.

Chapter 1

INTRODUCTION

§1.1 Kin selection and Hamilton's Rule

William Hamilton's (1964) theory of kin selection ushered in a scientific revolution arguably unseen in evolutionary biology since Darwin's (1859) "Origin of Species". The intrinsic importance of his theory was the promise of a means for testing evolutionary hypotheses using quantitative data: the stuff of scientific research. Hamilton's insight was to realise that a gene for altruistic behaviour could spread through a population by influencing its bearers to direct their altruism towards other individuals bearing copies of itself. The likelihood of finding copies of a gene in other individuals is dependent on the relatedness between those individuals, and on the frequency of the focal gene in the population as a whole. Relatedness is a measure of genetic similarity between individuals. When a gene's average frequency in a group of potential recipients of altruistic behaviour is regressed, across all groups in a population, against the average frequency in a group containing a potential actor, the sign and slope of the regression line equals the regression relatedness for a given locus (e.g. Pamilo and Crozier 1982). In evolutionary terms, fitness is gained from the passing on of multiple copies of genes, with the obvious route for gene transmission being from parent to offspring. Hamilton realised that an individual could gain fitness benefits by helping any related individuals (not just offspring) to pass on copies of genes which they share in common.

In its simplest form, kin selection is based on Hamilton's Rule which states that

$$rb - c > 0$$

must be satisfied for a gene for altruism to spread, where b is the benefit to an individual for receiving an act of altruism devalued by the relatedness (r) of the actor to the recipient of the behaviour, and c is the cost of performing the act. The currency in which the cost and benefit are measured is the change in expected offspring number. Thus, a gene for altruism can be favoured by natural selection if its bearers have a higher than average inclusive fitness. That is, if the sum total of an individual's personal fitness (number of offspring), as well as it's influence on the fitness of others, weighted by the relatedness between them, is greater on average than the personal fitness of individuals lacking a gene for altruism.

§1.2 Fisher's sex ratio theory

The field of sex ratio evolution considers how resources should be divided between males and females. Because investment in these fractions can be estimated, the predictions made by the theory can often be tested. Fisher (1930) originally showed that the sex investment ratio will be at equilibrium for the party controlling allocation of resources when investment in females and males brings equal fitness returns per unit of energy expended. At this point,

$$(r_F \,.\, V_F \,.\, MS_F) \,/\, c = r_M \,.\, V_M \,.\, MS_M,$$

where r = regression relatedness of the party controlling allocation to females and males, V = sex-specific reproductive value of the sexes, MS = mating success of the sexes, and c = the cost ratio of producing females : males (taken from Bourke and Franks 1995, box 4.1). In terms of kin selection, the males and females of diploid species are equally efficient at passing on genes for altruism, and therefore have equal reproductive values. Mating success refers to the relative abundance of potential mates in a population. For diploid species, a parent investing in offspring is equally related to both sexes. Assuming no sexual dimorphism (c = 1), the stable sex investment ratio for diploid parents is:

$$(0.5 \text{ x } 1 \text{ x } MS_F) \,/\, 1 = (0.5 \text{ x } 1 \text{ x } MS_M)$$

$$MS_F = MS_M,$$

that is when there are equal numbers of males and females in the population.

§1.3 *The importance of social insects for the study of kin selection and sex allocation*

The Hymenoptera (ants, bees and wasps) represent an enigma in the study of social evolution. Darwin (1859) considered the greatest challenge to his theory of evolution by natural selection to be the existence of neuter workers in social insect colonies. Because of the unusual method of sex determination in the Hymenoptera, unfertilised, haploid eggs become males, and fertilised, diploid eggs develop into females. As a consequence, coefficients of relatedness between colony members differ from those found in diploid species. Specifically, females (including workers) are related to sisters by $r = 0.75$, and to males by $r = 0.25$. (These values are 'life-for-life' relatedness coefficients. They differ from regression relatedness measures in that they implicitly control for the sex-specific reproductive value of the sexes. Thus, females, being diploid, have twice the reproductive value of haploid males, and are therefore twice as efficient at passing on copies of genes, including those for altruism). Hamilton realised that the extraordinarily high degree of relatedness between females in social insect colonies meant that workers could receive inclusive fitness benefits by rearing highly related sisters instead of their own offspring.

Trivers and Hare (1976) combined Fisher's (1930) sex ratio theory with Hamilton's kin selection theory to explain the unusual female-biased population sex ratios in the Hymenoptera. Because of workers' relatedness asymmetry (life-for-life relatedness with females : life-for-life relatedness with males), the stable population sex ratio at equilibrium will differ from 1 : 1. In fact, the stable population sex ratio for the party controlling sex allocation is predicted to equal its relatedness asymmetry. This is because, rearranging the equation for Fisher's sex ratio equilibrium:

$$(r_F \,.\, V_F \,.\, MS_F) \,/\, c = r_M \,.\, V_M \,.\, MS_M$$

$$cMS_M \,/\, MS_F = r_F \,.\, V_F \,/\, r_M \,.\, V_M.$$

Recalling that 'life-for-life relatedness' = regression relatedness x sex specific reproductive value, and that mating success = 1 / frequency of a sex in the population, we get:

$$cF \,/\, M = \text{relatedness asymmetry.}$$

In fact, the sex-specific cost ratio drops out of the equation when measuring sex allocation in terms of investment in sexuals, rather than numbers of sexuals. Thus, when there is one singly-mated queen per colony, and workers are sterile, the stable sex investment ratio for workers is:

$$r_F \,.\, V_F \,.\, MS_F = r_M \,.\, V_M \,.\, MS_M$$
$$0.75 \text{ x } 1 \text{ x } MS_F = 0.5 \text{ x } 0.5 \text{ x } MS_M$$
$$3MS_F = MS_M.$$

Workers prefer three times as much investment in new female sexuals as in new male sexuals. The stable population sex investment ratio for queens under the same conditions is:

$$r_F \,.\, V_F \,.\, MS_F = r_M \,.\, V_M \,.\, MS_M$$
$$0.5 \text{ x } 1 \text{ x } MS_F = 1.0 \text{ x } 0.5 \text{ x } MS_M$$
$$MS_F = MS_M.$$

Therefore, at sex ratio equilibrium, queens prefer equal investment in the sexes, while workers prefer a female-biased sex investment ratio. This results in a conflict of interest between queens and workers over the optimal population sex investment ratio to produce. Trivers and Hare (1976) argued that the strong female bias seen across many populations of social Hymenoptera was evidence of worker control of sex allocation, and field studies have generally supported this (Trivers and Hare 1976; Nonacs 1986a; Boomsma 1989; Bourke and Franks 1995; Crozier and Pamilo 1996).

§1.4 Split sex ratios and resource allocation in the application of kin selection and sex ratio theory to social insects

Trivers and Hare's (1976) theory was based on a population of monogynous (single-queened) colonies, with each queen being singly-mated, and workers being completely sterile. Furthermore, random mating was also assumed. Few field studies have reported these exact conditions for a population of social Hymenoptera. Notably, Hasegawa (1994) found a population sex investment ratio of 0.75 (proportion of females) for the ant species *Colobopsis nipponicus*, which satisfied Trivers and Hare's basic conditions (also see Pearson *et al.* 1995; Banschbach and Herbers 1996a, b). In natural populations of social insects, these conditions are rarely found. Indeed, it is often the deviations from normality, or in this instance, the hypothetical situation that allows for theories to be tested. Multiple mating by queens, polygyny (multiple queens) and worker reproduction all cause changes in within-colony relatedness asymmetry with the result that the preferred sex investment ratios of workers also change. The relatedness asymmetry of queens remains unaffected by these changes, and their preferred sex investment ratio also remains unaffected. The power of Trivers and Hare's theory is that these changes in sex allocation patterns can be predicted, and evidence for the predictions can be sought in natural or laboratory conditions. A greater understanding of the biology of Hymenopteran species, and more importantly of the genetic composition of colonies and populations using recently-developed molecular techniques such as the analysis of microsatellite DNA, has allowed testing of these theoretical predictions.

Split sex ratios

Boomsma and Grafen (1990, 1991) extended the predictive power of sex allocation theory for the social Hymenoptera by considering between-colony variation in workers' relatedness asymmetry in a population. This variation may occur when colonies differ in the numbers of queens they contain, the mating frequencies of queens, or if there is a degree of worker male-production. If workers can assess the relative relatedness asymmetry of their colony, they should be selected to skew their colony's brood sex ratio towards the sex to which they are more closely related than the average in the population. If discrete classes in a population systematically contribute different sex ratios to the same generation of brood, split sex ratios (Grafen 1986) will occur.

Boomsma and Grafen (1990, 1991) predicted that, under worker control of sex allocation, colonies with a relatedness asymmetry that is high relative to the population average should raise entirely or strongly female-biased broods. Colonies with lower than average relative relatedness asymmetry are expected to raise entirely or strongly male-biased broods. Queens' relatedness asymmetry is not affected by queen number or frequency of mating, so split sex ratios are not expected under queen control of sex allocation. The strength of Boomsma and Grafen's split sex ratio theory is that it allows colony-level sex allocation patterns to be explicitly predicted. Furthermore, these patterns are predicted to be associated with measurable parameters of individual colonies: namely number of queen matings, or number of colony queens.

Split sex ratio theory (Boomsma and Grafen 1990, 1991) is explicit about how workers are expected to respond to relative relatedness asymmetry in a frequency-dependent manner. From Fisher's (1930) sex ratio theory, it was seen that the stable sex ratio equals a party's relatedness asymmetry because then there will be equal fitness payoffs for producing either sex. However, for a party with high relative relatedness asymmetry compared with the population average, females will have higher than average mating success and there will be greater fitness returns to that party for the production of females. The inverse argument holds true for a class with lower than average relatedness asymmetry favouring male production. When the population sex investment ratio reaches the relatedness asymmetry of one of the classes, it stabilises because then there is no more incentive for that class to bias the sex ratio any further as it receives equal fitness from both sexes. The other class responds by producing single sex broods. The actual sex investment ratio predicted for a class depends on the relative frequency of classes in the population, and on the relative relatedness asymmetries of those classes. Thus, neither relatedness asymmetry class may be able to return the population sex investment ratio to its own relatedness asymmetry. Then, both classes may produce single sex broods, with the population sex investment ratio resting between the specific relatedness asymmetries of the classes.

That colonies tend to produce single-sex broods more frequently than by chance alone has been known for some time (e.g. Nonacs 1986a). Split sex ratios were first shown to be correlated with worker relatedness asymmetry in a species of ant with multiple mating by Sundström (1994a). She showed that colonies of the ant *Formica truncorum* headed by a singly-mated queen produced strongly female-biased broods, while colonies of ants from the same population headed by a multiply-mated queen produced male-biased broods as predicted by Boomsma and Grafen (1990, 1991) (also see Bourke and Chan 1994, Appendix D). Several studies have shown that sex ratios change with queen number in ants (e.g. Ward 1983; Elmes 1987a, b; Herbers 1984, 1990; reviewed by Boomsma 1993). Split sex ratios arising from polygyny and associated with the number of queens in a colony were reported in a paper from this study. Chan and Bourke (1994, Appendix C) described a population of the species *Leptothorax acervorum* in which colonies with a single queen produced significantly female biased-broods, while polygynous colonies from the same population produced male-biased broods. Evans (1995) found that split sex ratios in the ant *Myrmica tahoensis* were linked to the degree of within-colony relatedness. In an elegant brood-manipulation experiment, he showed that the degree of genetic diversity amongst developing brood directly affected the sex allocation decisions of workers. Split sex ratios have also been demonstrated in eusocial wasps by Queller *et al.* (1993), and in eusocial bees by Mueller (1991) and Mueller *et al.* (1994), and Packer and Owen (1994).

Resource allocation

Studies of social insect evolution have concentrated largely on the field of sex allocation. Recent extensions to the theory have made predictions about how resources should be divided between the production of sexuals and colony maintenance in the form of new workers (Pamilo 1991a). Again, a conflict of interest is predicted to arise between queens and workers over the proportion of resources to be allocated to the various fractions, and again, kin selection theory allows these predictions to be explicit and quantifiable.

Pamilo (1991a) argued that a queen's preference for herself remaining her colony's source of fitness is greater than her workers' preference for whom should be the colony's main source of fitness. A queen is related to herself by $r = 1$, whereas workers are related to her by $r = 0.5$. Thus, queens prefer to invest more resources into colony maintenance to remain the colony's reproductive than do workers, who prefer more investment in new sexuals. Another way of looking at this conflict over resource allocation stems from the basic sex allocation conflict. Since new workers and new queens both develop from diploid brood, a queen prefers more diploid brood to develop into new workers, whereas workers prefer more diploid brood to develop into new queens. Several factors are predicted to affect the conflict over resource allocation. First, if workers' relatedness asymmetry is relatively low, their preferred sex investment ratio will be close to that of queens', and the conflict over what proportion of diploid brood should develop into new queens and new workers will be reduced. Second, if dispersing queens have little chance of founding new colonies independently, the natal colony becomes a more important source of fitness to both queens and workers. Thus, both queens and workers prefer investing in colony maintenance in the form of new workers. Finally, if independent colony founding is likely, and colony survival prospects are low, workers are predicted to favour a decreasing proportion of sexuals as colony survival prospects increase. Queens are predicted to favour an increasing proportion of sexuals as survival prospects increase under any conditions.

Sundström (1995) demonstrated worker control over resource allocation between sexuals and colony maintenance in the ant species *Formica truncorum*, as predicted by Pamilo's (1991a) model. She found that a monogynous population of the species reduced allocation to sexuals with increasing colony size (taken as an indicator of survival prospects) as predicted under worker control of resource allocation. A polygynous population of the same species failed to show any significant trend. Backus (1995) examined division of resources between sexual and worker production in two separate populations of the ant species *Leptothorax longispinosus*. Inter-population differences in resource allocation patterns appeared to stem from differences in the degree of ecological constraints, especially nest site limitation. Banschbach and Herbers (1996a, b) also examined division of resources between colony growth and sexual output in two separate populations of the ant *Myrmica punctiventris*. Considerable differences were found between populations in terms of resource allocation strategies which were put down to inter-population differences in ecological constraints, social structures and queen-worker conflict, as well as colony size.

This new field of social insect research is likely to gain increasing attention, but has largely been neglected because accurate data on the number of new workers produced by colonies have been difficult to collect for many reasons. Furthermore, the division of resources between colony growth and sexual output may be strongly affected by the strength of ecological constraints, which may be difficult to measure.

§1.5 Facultatively polygynous ants as tools for split sex ratio and resource allocation studies

Facultatively polygynous ant species have monogynous and polygynous colonies in the same population. Queens within colonies are usually related because of the readoption of some daughter queens into the natal colony. Because relatedness asymmetry is expected to vary with queen number, and sex and resource allocation are expected to be affected by variable relatedness asymmetry, facultatively polygynous ants are useful tools for studying these theories. Specifically, as the number of queens per colony rises, workers relatedness asymmetry will fall to levels approaching the queens' relatedness asymmetry. In colonies with a single queen, workers' relatedness asymmetry should be high (around 0.75). Assuming that queen number is a reliable predictor of within-colony relatedness asymmetry, monogynous colonies are predicted to favour female-biased broods, and polygynous colonies to favour male-biased broods. Overall, facultatively polygynous ant populations are strong candidates for finding sex ratios split by queen number, and by association, relatedness asymmetry.

Monogynous and polygynous colonies within the same population are also likely to adopt different strategies for the allocation of resources to sexuals versus colony maintenance. When queen and workers have very different relatedness asymmetries, their conflict over resource allocation should be highest. According to Pamilo's (1991a) model, a decreasing proportion of sexual brood with increasing colony survivorship (linked with increasing colony size) is evidence of worker control of resource allocation. This is more likely to occur in monogynous, but not polygynous colonies.

§1.6 The study species: **Leptothorax acervorum**

It is becoming clear that patterns of sex and resource allocation in social insects are interesting at the colony as well as population level (e.g. Pamilo 1991a; Boomsma 1993). As noted by Crozier and Pamilo (1996), studies of colony-level sex allocation patterns within populations, and comparisons between populations of the same species which differ in aspects of sex allocation can reveal the processes underlying allocation decisions in social insects. This thesis attempts such an in-depth study.

Leptothorax acervorum is a facultatively polygynous ant found throughout Northern Europe, and is an ideal species for the study of sex and resource allocation. First, many aspects of the life history of the species have been detailed (Buschinger 1968, 1981; Heinze and Buschinger 1988; Bourke 1991, 1994; Bourke and Heinze 1994; Chan and Bourke 1994; Heinze *et al.* 1995a, b). In addition, allozyme and microsatellite studies provide information about the genetic composition of colonies and populations of this species (Douwes *et al.* 1987; Stille *et al.* 1991; Stille and Stille 1992, 1993; Heinze *et al.* 1995a, b; Heinze 1995). Queens appear to be singly-mated, and workers non-reproductive (Heinze *et al.* 1995a). Queen number usually varies between one and around ten, and nest-mate queens are related (Stille *et al.* 1991; Heinze *et al.* 1995b). As a result, intra-colony relatedness varies between colonies according to queen number (Heinze *et al.* 1995b). Sexuals almost certainly mate away from the natal colony in mating swarms (Bourke and Heinze 1994), and lack of inbreeding in this species suggests that mating is random (Heinze *et al.* 1995b). Finally, *L. acervorum* colonies are small and large numbers of whole colonies can be collected from a single population. Each colony can easily be censused for queen and worker numbers, as well as numbers of new queens, new males and new workers produced. This allows patterns of allocation to be examined at both the colony and the population levels.

In the seminal 'Structure of Scientific Revolutions', Thomas Kuhn (1962) considered that, "Mopping-up operations are what engage most scientists throughout their careers. They constitute what I am ... calling normal science. ... normal-scientific research is directed to the articulation of those phenomena and theories that the paradigm already supplies." Sex and resource allocation studies in the social insects are the most direct means available for testing the predictions of kin selection theory, which currently underpins much of modern evolutionary thought. The study of social evolution in Hymenoptera as 'normal science' is exciting because it lies so close to the heart of the modern evolutionary paradigm: kin selection.

§1.7 Aims and outline of this thesis

This thesis was directed at examining the processes underlying sex allocation and resource allocation in social Hymenoptera. A central question upon which most of the studies were based is who actually controls the allocation of these resources.

Associated with this was the question of how the number of queens in a colony, which is assumed to be linked with the degree of relatedness within colonies, affects the allocation decisions of the parties in those colonies with single, or multiple queens. Non-genetic factors were also considered for their effects on allocation decision-making processes. Samples from populations of *L. acervorum* were collected from the field and examined to see if their patterns of allocation matched those predicted by theory.

This study examined colony-level sex and resource allocation patterns across three consecutive years in one population. Two other populations were also sampled and examined for inter-population differences in sex and resource allocation. Importantly, entire colonies were sampled for new sexual and new worker production. Previous field studies have either sampled only part of the total brood production of colonies, or have not confidently censused entire colonies, because they may occupy several nest-sites. In addition, new worker production has rarely been estimated alongside sexual production. This thesis represents one of the most detailed studies of sex and resource allocation at the colony and population levels yet attempted.

This thesis is organised as follows. The following chapter is a detailed examination of sex allocation in a single population of *L. acervorum* over three consecutive years. Chapter three compares the sex allocation patterns from three separate populations of the same species, and considers what factors underlie different sex ratio patterns within and between them. The fourth chapter examines the allocation of resources between sexual production and colony maintenance in the form of new workers in the three study populations. I conclude with a summary of allocation patterns in the study species, and discuss whether kin-selection based theories of social insect evolution are supported in this study.

Chapter 2

SPLIT SEX RATIOS IN *LEPTOTHORAX ACERVORUM*

§2.1 Introduction

Sex allocation theory considers how resources should optimally be allocated to male and female offspring (Fisher 1930). In social Hymenopteran societies (ants, bees and wasps), workers and queens invest resources into raising brood. Because of the unusual haplodiploid system of sex determination, Hymenopteran queens and workers are not equally related to the brood they raise, and a parent-offspring conflict arises over the optimal allocation of resources to male and female fractions in the brood (Hamilton 1964; Trivers 1974). Trivers and Hare (1976) first considered the possibility that workers control the sex ratio in social insect colonies by favouring the production of female offspring to which they are more closely related than to males.

Queens are predicted to favour an equal investment in sons and daughters as they contribute an equal genetic fraction to both sexes. The sex ratio will be stable for the party controlling sex allocation when it equals the ratio 'life-for-life relatedness with females' : 'life-for-life relatedness with males'. Boomsma and Grafen (1990, 1991) termed this ratio the relatedness asymmetry.

Trivers and Hare's (1976) prediction of a 3:1 female to male population sex investment ratio as favoured by workers has generally been supported in the literature (Trivers and Hare 1976; Nonacs 1986a; Boomsma 1989; Pamilo 1990; Bourke and Franks 1995; Crozier and Pamilo 1996). However, many populations of ants produce sex ratios that are considerably different from the predicted 3:1 ratio, and individual colonies have been shown to produce single-sex broods more frequently than would be expected by chance alone (Nonacs 1986a).

Split sex ratio theory (Boomsma and Grafen 1990, 1991) is a more recent extension of sex allocation theory which accounts for single-sex broods at the colony level. The degree of intracolony relatedness within a population will vary if there are between-colony differences in either number of colony queens, or frequency of matings by queens. These factors affect the workers' relatedness asymmetry with the effect that they should bias their colony's brood sex ratio towards the sex to which they are more closely related than the population average. Thus, under worker control, polygynous (multiply-queened) colonies should produce relatively male-biased broods compared with monogynous (single queen) colonies which should produce female-biased broods. This is because in polygynous colonies, many queens contributing to the brood lowers the workers' relative relatedness with females compared with males, and the advantage of producing female-biased broods is reduced. The queens' relative relatedness to males and females does not change with increasing queen number, so their preferred sex investment ratio remains equal.

There is rapidly mounting evidence in support of split sex ratio theory in the literature. In the ant species *Formica truncorum*, Sundström (1994a) found that singly-mated queen colonies produced female-biased broods, while colonies containing multiply-mated queens in the same population produced male-biased broods (see also Bourke and Chan 1994; Sherman and Shellman-Reeve 1994; Sundström 1994b). Chan and Bourke (1994) reported that sex investment ratio was associated with whether colonies were monogynous or polygynous in the ant *Leptothorax acervorum.* Evans (1995) found that sex ratio was directly linked to colony relatedness asymmetry and not queen number in the polygynous ant *Myrmica tahoensis.* Boomsma (1993) reported that male-bias in the brood tended to rise with colony-queen number in other polygynous ant species (studies include Ward 1983; Elmes 1987a, b; and Herbers 1984, 1990 in *Leptothorax* species). Furthermore, evidence of sex ratio biasing associated with relative relatedness asymmetry has been reported in social bees and wasps (Boomsma 1991; Mueller 1991; Mueller *et al.* 1994; Queller *et al.* 1993; Packer and Owen 1994).

Workers' relative relatedness asymmetry is not the only factor predicted to contribute towards sex ratio bias in social insects, and some authors deny the importance of worker control (e.g. Alexander and Sherman 1977; Sherman and Shellman-Reeve 1994). Polygynous ant colonies are frequently known to inhabit several nest-sites (polydomy), and reproduction can occur dependently if a queen, or groups of queens leave the natal colony with a fraction of workers and establish a new colony nearby the old one (colony budding). Male biased broods are expected to stem from both polydomy (Pamilo and Rosengren 1983) and colony budding (Pamilo 1990) because related queens will compete for local resources (local resource competition: Clark 1978; Frank 1987; Pamilo 1991a). Thus there will be diminishing returns on increasing production of new queens while the production of new males will bring linear fitness returns. Alternatively, if an increase in queen number within a colony means that queens can usurp more control of the sex ratio in their conflict of interest with workers, polygyny is expected to be correlated with male-biased broods (Herbers 1984, 1990). On the other hand, Nonacs (1986a, b) predicted that productive colonies will increase female bias when local resources are abundant. Finally, if related males are competing for matings with females in a population, local mate competition (Frank 1987) causes diminishing returns on the increased production of males but not females, so female bias in brood sex ratios is expected to increase with increasing sexual productivity.

An ideal species for the study of sex allocation is the ant *Leptothorax acervorum*. Chan and Bourke (1994) previously found sex ratios to be split by colony class (monogynous versus polygynous) in this species and for this study the same population was examined over three consecutive years. There is a growing literature about the life history of this species (Buschinger 1968, 1981; Heinze and Buschinger 1988; Bourke 1991, 1994; Bourke and Heinze 1994; Chan and Bourke 1994; Heinze *et al.* 1995a, b), including its genetic profile (Douwes *et al.* 1987; Stille *et al.* 1991; Stille and Stille 1992, 1993; Heinze *et al.* 1995a, b; Heinze 1995). Colonies are small, abundant and easily censused (Bourke 1991) allowing sex allocation to be examined at both colony and population levels (Boomsma 1993; Chan and Bourke 1994; Sundström 1994; Bourke and Franks 1995; Crozier and Pamilo 1996). Colonies vary in queen number, and nest-mate queens are known to be related (Stille *et al.* 1991; Heinze *et al.* 1995a, b). Within-colony relatedness is known to be associated with queen number in this species (Heinze *et al.* 1995a, b). Since split sex ratio theory predicts that sex allocation decisions should be based on variable within-colony relatedness (Boomsma and Grafen 1990), sex ratios were compared between colonies with different numbers of queens. Furthermore, within polygynous colonies, the effect of queen number on sex allocation was examined to see whether queens gained partial control over sex ratios at high queen to sexual brood ratios (Herbers 1984, 1990). If there is non-random mating or dispersal of sexuals in this population, colonies should change their sex investment ratios according to their sexual productivity. Thus, if there is local mate competition, colonies should produce more female biased sex ratios at higher levels of sexual productivity as there will be diminishing returns on increasing investment in males (Frank 1987). Similarly, colonies with high levels of sexual productivity are expected to produce increasingly male-biased broods if there is local resource competition, due to diminishing returns on new queen production (Frank 1987; Pamilo 1991a). Finally, the effects of resource abundance on sex allocation were examined by seeing if colonies with low productivity levels produced more male-biased broods (Nonacs 1986a, b).

This study found split sex ratios in the ant species *Leptothorax acervorum* over three consecutive years. Monogynous colonies produced strongly female-biased broods while polygynous colonies produced significantly more male-biased broods as predicted by split sex ratio theory (Boomsma and Grafen 1990, 1991). These sex investment ratios were consistent with worker control over sex allocation based on workers' variable relatedness asymmetry to males and females and not partial queen control over sex allocation. There was no evidence of local mate competition or resource abundance effects but diminishing production of males in more productive polygynous colonies suggested that local resource competition may occur, stemming from a degree of colony budding in this population. These results support the previous study on this population (Chan and Bourke 1994) and show that this population is consistent in its sex allocation strategies across years.

§2.2 Materials and methods

Study species and population

Leptothorax acervorum is a facultatively polygynous ant species commonly found throughout temperate coniferous forests, boreal and alpine regions of the British Isles and Northern and Central Eurasia (Collingwood 1979, Heinze *et al.* 1995b). Polygyny occurs secondarily through readoption of related nestmate queens (Douwes *et al.* 1987; Stille *et al.* 1991; Heinze *et al.* 1995b). Each colony typically contains between several dozen and hundreds of workers, and there are rarely more than ten mated queens per colony. Whole, single colonies are found inside cavities in rotting twigs, under bark, or occasionally in the roots of dead trees and under stones. Collection of whole colonies (minus foraging workers) from a single site (monodomy) allows an accurate census of all adults and brood in every colony collected.

The study population of *L. acervorum* was collected from Santon Warren, Thetford Forest, Norfolk, U.K., and was sampled over three consecutive years (see also Bourke 1991, 1993; Chan and Bourke 1994).

Collection and culture of colonies

From Santon Warren, 47 colonies were collected on 17 June 1993, 49 colonies were collected on 8 June 1994 and 102 colonies were collected over two days on June 13 and 20, 1995. Sampling of populations was carried out before July so that no brood had yet eclosed into adults (Chan and Bourke 1994).

Collection of ants in the field involved breaking open dead twigs to find whole colonies. Random sampling of the population was assumed as all colonies found were collected, and all collection within the population took place in the same general region (including across-year sampling). Twigs containing colonies were then placed inside plastic bags and returned to the laboratory. Colonies were removed from twigs, aspirated and placed inside standard nests, and maintained in the laboratory based on the methods of Bourke (1991). Standard nests were made of two rectangular glass slides separated by a cardboard border with an opening in one end. Nests were kept inside clear plastic dishes (10 x 10 x 2 cm) under natural lighting and at room temperature. Colonies were fed on a regime of water (damp cotton wool), sugar solution and mealworm larvae, renewed every 2-3 days.

Census of colonies

Immediately after collection, colonies were censused for the first time. All adults and pupae were counted from each colony by transferring individuals using fine forceps, allowing an accurate measure of each colony's composition. Colonies were maintained in the laboratory until all pupae had eclosed into adults before being censused for a second time for all individuals present. Comparison of the first with the second census gave each colony's annual production of new workers, new queens and new males on top of its original composition.

All mated colony queens had shed their wings (dealate). Some alate (winged) virgins may have shed their wings before the first census, but after collection from the field. During the second census, all dealate queens were removed for ovarian dissection according to the methods of Bourke (1991) to establish reproductive status. Mated queens have spermathecæ (sperm receptacles) visibly full of sperm (under a compound microscope), while virgin queens have clear spermathecæ. Other dissection data collected included number of yolky eggs present out of total number of eggs present, and approximate ovariole length was recorded (length categories ranged from very small through small, small-medium, medium, medium-large, large, very large and very-very-large). Corpora lutea (residual ovarian structures, by-products of egg production) were also counted. In cases where ovarian dissections were only partially complete, but the spermatheca could not be found, these other data could be used to establish whether a queen had been mated or not. Mated queens were counted as colony queens while non-mated queens were taken to be newly eclosed and thus were counted as part of the new queen production. This was justified because A.F.G. Bourke (unpublished data) found that of 72 queens collected from Santon Warren between April and May (1990-1991), all were mated (reported in Chan and Bourke 1994). Furthermore, A.F.G. Bourke (unpublished data) found that *L. acervorum* sexuals from Santon Warren do not mate in the natal colony (reported in Chan and Bourke 1994).

Over three consecutive years, 198 colonies were collected from Santon Warren containing 14 464 workers and 516 queens, the latter of which were all dissected. These colonies produced 24 521 new brood including workers, queens and males (Appendix A).

Genetic composition of the populations

The Santon Warren population was studied genetically by Heinze *et al.* (1995b) using allozyme analysis of two polymorphic loci on samples collected in 1991 and

1992. As in other polygynous *L. acervorum* populations (Stille *et al.* 1991; Heinze *et al.* 1995b), queens within colonies were related on average (queen-queen relatedness was 0.26), indicating that many queens are readopted by their natal colony. Worker-worker relatedness (± s.e.) was significantly higher in monogynous colonies (0.50 ± 0.06; n = 34 colonies) than in polygynous ones (0.28 ± 0.09; n = 19 colonies) (Heinze *et al.* 1995b). I therefore infer that workers' relatedness asymmetry was truly lower in polygynous colonies than in monogynous ones. Worker-worker relatedness presumably fell below 0.75 in monogynous colonies despite the likelihood that queens mate singly (Stille *et al.* 1991; Heinze *et al.* 1995b) because some had previously been polygynous (Chan and Bourke 1994; Heinze *et al.* 1995a). The genetic data also suggested that inbreeding was absent from the Santon Warren population (Heinze *et al.* 1995b). Finally, low worker egg-laying rates imply that worker male production was negligible (Bourke 1991).

L. acervorum males almost certainly mate both in large swarms and near nests (Bourke and Heinze 1994). The lack of evidence for inbreeding suggests that males disperse widely enough to render local mate competition among them unlikely. However, there is strong evidence that polygynous *L. acervorum* colonies reproduce by budding (see, for example, Stille and Stille 1993; Bourke and Heinze 1994; Chan and Bourke 1994), suggesting that local resource competition due to budding is probable (see Chan and Bourke 1994).

Estimation of mean dry weights

Samples of callow workers, alate queens and males were taken from several monogynous and polygynous colonies from Santon Warren in 1993 and 1994 [Appendix B, Table B.1]. These were dried in an oven for 24 h, at 60 °C, and each ant individually weighed on an electronic microbalance. Thorax length was measured using a dissection microscope attached to a computer-based image analysis system (NIH Image). Using a basic thorax length-dry weight comparison, the dry weight for a larger sample of ants could be estimated where only the thorax was available for measurements [Appendix B]. Samples of all three castes of ant from several monogynous and polygynous colonies in 1994 and 1995 were measured for thorax length using this technique (except in polygynous colonies in 1995 where males and queens were unavailable for measurement).

§2.3 Results

Population composition

This facultatively polygynous population produced 53% polygynous colonies in 1993, 24% in 1994 and 20% in 1995; the remaining colonies were monogynous or queenless (Table 2.1). Queenless colonies were classified as monogynous for all analyses. This was justified because queenless colonies were more likely to have been previously monogynous than polygynous. Furthermore, there was no difference in mean sex investment ratios between queenless and monogynous colonies from 1994 (t-test: $t = 0.67$, d.f. = 29 $p > 0.05$) or 1995 (t-test: $t = 1.45$, d.f. = 65, $p > 0.05$), when the number of queenless colonies exceeded one. In polygynous colonies, queen number had harmonic mean values of 5.5 in 1993, 4.3 in 1994 and 3.8 in 1995. Harmonic mean queen number is assumed to be a more reliable predictor of within colony relatedness because the change in relatedness of adding successive queens is weighted by the total number of queens already present (Wade 1985; Queller 1993). The overall change in relatedness within a colony is greater when adding a second queen to a monogynous colony than when adding a tenth queen to a nine-queen colony, and the harmonic mean reflects this. Mean log queen numbers were 0.846 in 1993, 0.736 in 1994 and 0.749 in 1995 (Appendix A) and these means were found not to differ significantly (ANOVA: $F_{2,48} = 2.72$, $p > 0.05$). The overall mean log queen number was 0.749 with overall harmonic mean for this population being 4.5 queens over three years.

Caste dry weights and investment ratios

The average dry weights of callow workers and alate (winged) queens were 0.353 mg and 0.506 mg respectively. A significant difference in mean log dry weight of males was found between monogynous and polygynous colonies (Appendix B). Male dry weight averaged 0.510 mg in monogynous colonies and 0.444 mg in polygynous colonies. Boomsma (1989) suggested that investment ratios should not be calculated from dry weight ratios, but from a cost ratio calculated as (female : male dry weight ratio)$^{0.7}$. Trivers and Hare (1976) noted that using dry weights to estimate investment parameters may underestimate the production costs of males relative to females. Queens contain metabolically inactive stores of fat reserves lacking in males, and sexual size dimorphism is likely to result in different metabolic rates between the sexes (Boomsma and Isaaks 1985; Passera and Keller 1987; Bourke and Franks 1995). Boomsma *et al.* (1995) justified this adjustment of investment ratios, although they noted that several authors recommend that 'considerable caution should be exercised in using such conversions' (Crozier and Pamilo 1992), and that it may be inappropriate in studies involving bees and wasps (Helms 1994). This study found queens and males to have almost identical dry weight ratios in monogynous colonies, so dry weight ratios were confidently used as investment ratios. Polygynous colonies found some sexual dimorphism to exist between queens and males. In this class of colony, the queen : male dry weight ratio was 0.506 / 0.444 = 1.14, and this ratio became 1.10 using Boomsma's (1989) 0.7 power-conversion factor. This difference of 3.5% was considered insignificant so that using dry weight ratios or cost ratios should provide similar sex investment estimates. A comparison between sex investment ratios within the polygynous colony class using both dry weight and Boomsma's (1989) cost ratio to calculate investment ratios confirmed that the two techniques give very similar results (see below and Table 2.5). For the remainder of the study, it was decided that dry weight ratios would be used. Later chapters in this thesis consider investment to new workers as well as new sexuals. As there is no appropriate estimate of a cost ratio power conversion between new workers : new queens : new males, dry weight ratios were considered to be a more consistent method for estimating all investment ratios.

Sex investment ratios

Sex investment ratios were calculated from mean dry weight values for each caste [Appendix B]. These values were multiplied by numbers of individuals of each respective caste to give dry weight investment for males, females and workers, from which investment ratios were calculated (Boomsma 1989).

Two colonies out of 47 produced no sexuals in 1993, while six out 49 in 1994, and 13 out of 102 in 1995 produced no sexuals (Appendix A). The population sex investment ratios for the three years 1993-1995 were 0.52, 0.58 and 0.68 respectively (Table 2.2) (all sex investment ratios are given as proportion of females). These values all lay between the queen's favoured 0.5 sex investment ratio and workers' 0.75 ratio. Overall these population sex investment ratios did not differ significantly from 0.5, and differed significantly from 0.75 in 1993, and when population sex investment ratios were pooled over all three years (Table 2.4). There was no effect of year on population mean sex ratio (ANOVA: $F_{2,127} = 2.175$, $p > 0.05$), so sex ratio data were pooled over the three years. The overall mean weighted sex investment ratio (95% confidence limits) for this population was 0.60 (0.56-0.64) (n = 170 colonies). As colonies varied in sexual output, each colony was weighted by its total sexual productivity divided by the population mean sexual productivity. These weighted values adjust for each colony's relative contribution to the overall population sex ratio in a frequency-structured manner (J.J. Boomsma, personal communication; Chan and Bourke 1994; Bourke and Franks 1995, Box 5.1).

Split sex ratios

In all 3 years, monogynous colonies produced female-biased broods while polygynous colonies produced male-biased broods (Table 2.3; Figure 2.1). Within each year, the mean weighted sex investment ratios for monogynous and polygynous colony classes were found to be different (Table 2.3). This difference was significant in two out of three years and nearly significant in 1994 when the sex investment ratios produced by the different colony classes were clearly in the predicted direction (Table 2.3). Monogynous colonies always produced sex investment ratios that did not differ significantly from 0.75, whereas those produced by polygynous colonies were always significantly less than 0.75 (Table 2.4).

A comparison of mean sex ratios in both colony classes (monogynous versus polygynous) across all three years found a strong effect of colony class on sex investment ratio (ANOVA, class: $F_{1,164} = 7.51$, $p = 0.007$) but no effect of year (year: $F_{2,164} = 0.07$, $p > 0.05$; year * class: $F_{2,164} = 0.06$, $p > 0.05$). Within each colony class, mean sex ratio data were pooled for all three years. Over three consecutive years, monogynous and polygynous colonies produced mean sex ratios (95% confidence limits) of 0.67 (0.63-0.71) ($n = 119$) and 0.32 (0.25-0.40) ($n = 51$) respectively. These pooled means were found to be significantly different (t-test: $t = 4.17$, d.f. = 164, $p < 0.0001$). Overall, monogynous colonies were found to produce sex investment ratios that differed significantly from 0.5, but not from 0.75, whereas polygynous colonies produced sex investment ratios that were significantly lower than both 0.5 and 0.75 (Table 2.4). Thus, in this population of facultatively polygynous ants, sex ratio was split by colony class; a function of queen number.

Split sex ratios were also examined using Boomsma's (1989) 0.7 power-conversion factor (Boomsma *et al.* 1995). Mean dry weight values for males and alate queens in monogynous colonies were nearly identical so the 0.7 power-conversion factor had no effect on queen : male investment ratios, and thus on the calculation of sex investment ratios in monogynous colonies. Following the methods of Bourke and Franks (1995: box 5.1), the estimated energetic female : male cost ratio (c) = (female : male dry weight cost ratio)$^{0.7}$. In polygynous colonies this gives $c = (0.506 / 0.444)^{0.7} = 1.10$. Using this new cost ratio to calculate sex investment ratios in polygynous colonies gave slightly more male bias than using dry weight ratios (Table 2.5). Using Boomsma's (1989) 0.7 power-conversion factor did not alter the findings that sex ratio splitting occurred between monogynous and polygynous colony classes. All subsequent analyses used dry weight ratios.

Queen number and sex investment ratios

Polygynous colonies produced a sex ratio closer to that favoured by queens than did monogynous colonies. Either multiple queens are more capable of turning the sex ratio in their favour than single queens are (partial queen control: Herbers 1984, 1990), or these split sex ratios arise from a combination of factors including workers' response to relative relatedness asymmetry (Boomsma and Grafen (1990, 1991), and ecological factors. The effects of queen number on sex allocation were examined. Within the polygynous class, sex allocation did not change with increasing queen number (multiple regression: $F_{1,35} = 1.98$, $p > 0.05$; Table 2.6). (This and all following analyses omitted colonies producing fewer than 6 sexuals, and colony SD 93 32 as it produced an exceptionally high queen number (43 queens). In 1994 and 1995, no colony contained more than 13 queens (see Appendix A). Queen number was transformed by taking logarithms).

Partial queen control is predicted to depend on the relative ability of queens to manipulate brood; a function of the size of the sexual brood mass and the colony (Herbers 1984, 1990). Thus, sex investment ratios were also regressed on residual queen number taken from the regression of total sexual productivity on log queen number. Year was included in the multiple regression. Again, no relationship was found to exist between sex investment ratio and residual log queen number (multiple regression: $F_{1,35} = 0.58$, $p > 0.05$; Table 2.7; Figure 2.2).

This relationship was also investigated non-parametrically by comparing Kendall rank-order partial correlation coefficients (Siegel and Castellan 1988). Again, within polygynous colonies, there was no correlation between sex ratio and queen number when colony size (number of old workers) and log total sexual productivity were controlled for separately (Table 2.8). Within the monogynous colony class, the partial correlation coefficient between colony size and sex investment ratio (controlling for log total sexual productivity) was non-significant in all three years (1993: $r = 0.02$, d.f. = 14, $p > 0.05$; 1994: $r = 0.29$, d.f. = 22, $p > 0.05$; 1995: $r = -0.18$, d.f. = 54, $p > 0.05$). This suggests that there was no difference in brood sex ratios produced by monogynous colonies according to number of old workers in the colony. Queens in small monogynous colonies of this species clearly have no more control over sex allocation than queens in large colonies, or cohorts of queens in polygynous colonies (cf. Nonacs 1986b).

Resource abundance, local resource competition and local mate competition

If resources in a population are limited, female larvae destined to become new queens may be diverted to become less energetically costly workers (Nonacs 1986a). Large colonies are assumed to be able to gather resources more effectively and should concentrate on queen production. Small unproductive colonies should then produce a higher percentage of male offspring (Nonacs 1986a). The effects of colony productivity on sex allocation were investigated to see if sex investment ratios became more female biased as sexual productivity increased. Monogynous colonies showed no significant partial correlation between sex investment ratio and log total sexual productivity with colony size (number of old workers) held constant (Table 2.9). In 1993, polygynous colonies showed a significant negative partial correlation (Table 2.9). This trend occurred in all three years, but proved non-significant in the second and third years. This most likely stemmed from very small sample sizes, as the corresponding partial correlation coefficients are all negative and of similar value. Thus, monogynous colonies do not alter their sex allocation strategy with increasing sexual productivity, but polygynous colonies appear to invest more in males as colonies produce more sexuals.

To investigate this pattern further, multiple regression models of log new queen and log new male production were examined for the effects of increasing log total sexual productivity as well as colony class (monogyny or polygyny), and year (Table 2.10). There was no effect of year or any of its interaction components on production of either sexual, while there was an effect of colony class, suggesting that monogynous and polygynous colonies have different sex allocation strategies at different levels of total sexual productivity. Specifically, as total sexual production increased, monogynous colonies significantly increased investment of resources into males and new queens ($b = 0.89$ and $b = 1.01$, respectively, $F_{1,87} = 101.19$ and $F_{1,97} = 504.85$, respectively, both $p < 0.0001$) (Figure 2.3), and there was no difference between the gradients of the regression lines ($F_{1,184} = 1.70$, $p > 0.05$). Within polygynous colonies, investment in both males and new queens rose significantly with increasing total sexual production ($b = 1.16$ and $b = 0.67$, respectively, $F_{1,32} = 66.23$ and $F_{1,35} = 30.80$, respectively, both $p < 0.0001$) (Figure 2.3), but the gradient of the regression line was steeper for new male production ($F_{1,67} = 7.03$, $p < 0.05$) suggesting that at high levels of sexual output, polygynous colonies invest relatively fewer resources into new queen production compared with new male production.

Resource abundance and local mate competition appear not to affect sex allocation in this population. There was no increase in female-biased sex ratios with increasing productivity as predicted by Nonacs (1986a, b) under resource abundance. Local mate competition predicts that colonies should produce a fixed number of males and thereafter produce only females (Frank 1987), whereas male production rose constantly with increasing sexual productivity in both colony classes. On the other hand, local resource competition predicts that female production should tail off at high levels of sexual productivity because related females may be competing for resources, thereby devaluing the fitness returns of increasing queen production. This pattern was seen in polygynous colonies in this population which would be expected if there was some reproduction through colony budding. *L. acervorum* is thought to divide reproduction between emission of sexuals and a degree of colony budding in polygynous colonies (Stille and Stille 1993; Heinze *et al.* 1995a, b), and the sex allocation schedules of this population support this. Colony budding in this population is considered further in Chapter 4.

§2.4 *Discussion*

The pattern of sex allocation in this population of the facultatively polygynous ant *L. acervorum* was clearly split between monogynous and polygynous classes of colony. Ideally, the precise within-colony relatedness asymmetry values of workers to brood for each colony would be needed to test the prediction that workers actually respond to relative relatedness asymmetry when making brood sex allocation decisions. This level of genetic profiling was not available, but Heinze *et al.* (1995b) showed that within-colony relatedness is sensitive to queen number in this species, and that average worker-worker relatedness was higher in monogynous colonies than in polygynous colonies. Therefore, in this population workers did appear to be biasing brood sex ratios according to their relative relatedness to male and female brood. The overall population sex investment ratio gradually became more female-biased which was likely to have stemmed from a decrease in polygyny in later years. This population appeared to be unaffected by resource abundance effects (Nonacs 1986a, b) and local mate competition (Frank 1987). Polygynous colonies reduced their investment in new queens at higher levels of sexual productivity suggesting that they may experience local resource competition (Frank 1987) stemming from colony budding (Pamilo 1991b). It is highly likely that this species of ant employs budding as reproductive strategy (Stille and Stille 1993; Chan and Bourke 1994; Heinze *et al.* 1995b) and this is further examined in Chapter 4. Monogynous colonies are expected to respond to the male-biased sexual production in polygynous colonies with sex ratio compensation by producing excess female sexuals. Both queens and workers are expected to respond in this way but the apparent lack of queen number-effects over sex allocation in this study suggests that workers control sex allocation.

Within the polygynous class, there was no effect of increasing queen number on the sex ratio, nor was there relative male bias in monogynous colonies with low sexual productivity. Under the predictions of partial queen control over sex allocation, the higher the queen to sexual brood ratio the more likely queens are to produce their favoured relative male bias (Herbers 1984, 1990). In addition, workers appear not to assess the exact number of queens when making their sex allocation decisions (Ratnieks 1990; Boomsma 1993; Evans 1995). Ratnieks and Boomsma (1995) showed that in Hymenoptera species with multiple mating, if workers can assess the number of patrilines within a colony more accurately than by random guessing, worker-controlled split sex ratios can evolve to be an evolutionarily stable strategy (Maynard Smith 1982). This argument presumably extends to workers assessment of queen number in polygynous species. The difference in sex ratio favoured by colonies of different queen number within the polygynous fraction of the population is likely to be very small. The cues by which workers assess colony class must be sensitive enough to detect differences between single and doubly-queened colonies. If the cues are based on odour or genetic diversity (Ratnieks 1990), the increasing differences encountered with each successive addition of *n* queens to the colony diminish by 1/*n*. With queen recruitment and queens dying every year (Heinze *et al.* 1995a), the cues upon which workers count queen number may reflect an average queen number per colony across the population, but not an accurate one for queen number greater than two. Thus, annual fluctuations in genetic profiles within polygynous colonies may not allow an accurate assessment of queen number above two queens.

How do the emerging patterns of sex allocation at both the colony and whole population levels meet with the predicted outcome? Boomsma and Grafen (1990, 1991) offer a precise method of calculating the expected sex ratios of colony classes in a population based on relatedness asymmetries of workers to siblings. Relatedness asymmetry values were not known for this population and so colonies were assigned to two discrete classes based on genetic and life history information for this and similar populations (Heinze *et al.* 1995b). A further complication for the prediction of the exact population sex investment ratio arises under polygyny with readoption of related queens, as in *L. acervorum* (Stille *et al.* 1991; Heinze *et al.* 1995a, b). Since fitness is being considered for all dispersing sexuals, the fitness derived from females must be devalued by the parameter 'q' (Pamilo 1990) which is the proportion of fitness derived from dispersing females only. There is no known measure for 'q' in this or any known population, but Pamilo has shown that, under queen control, a population sex investment ratio of $q/(q+1)$ is expected, although it may be more male-biased than this if local resource competition comes into effect. Workers in polygynous colonies are still expected to favour a male-biased brood affected both by local resource competition and their relatedness asymmetry devalued by 'q'. Male bias in polygynous colonies is expected under both worker and queen control though queens are still expected to favour stronger male bias, with monogynous colonies balancing the population sex ratio by concentrating on female-production.

In this population, between 20 and 50 percent of colonies were polygynous. Thus, unless reproductive skew was almost complete as it is in functionally monogynous, non-European populations of *Leptothorax acervorum*, 'q' would be considerably less than one. Using the predictions of Bourke and Heinze (1994), reproductive skew in the Santon Warren population should be low, and as polygynous colonies had significantly lower average worker-worker relatedness values than monogynous colonies, queens must share reproduction. Thus, under queen control, the population sex investment ratio is not predicted to rise above 0.5 [i.e. $q/(q+1)$ when $q = 1$] (Pamilo 1990); in this population, the mean sex investment ratio was found to be 0.60. Alongside the absence of a relationship between queen number and sex ratio (controlling for colony size) this further suggests that queen control of sex allocation was negligible. Instead, it seems that workers produced individual class-specific sex ratios reflecting relative relatedness asymmetry to females and males. The likelihood that polygynous colonies in this population of *L. acervorum* reproduce partly by budding suggests that male bias in the polygynous colony-class stemmed from a degree of local resource competition between related queens as well as a response to relatedness asymmetry. Monogynous colonies would then be compensating for male-bias by over-producing females on top of their own relatedness asymmetry-induced female preference.

§2.5 Summary

1. In a population of the facultatively polygynous ant species, *Leptothorax acervorum*, between 20% and 53% of colonies were polygynous. Queen number varied from between one queen per colony and 43, though 84% of polygynous colonies contained ten or fewer queens. The overall harmonic mean queen number was 4.5.

2. Monogynous colonies produced female-biased broods and polygynous colonies concentrated on male production. Over three consecutive years, the brood sex investment ratio (95% confidence limits) in monogynous colonies was 0.67 (0.63-0.71) and in polygynous colonies was 0.32 (0.25-0.40). The overall population sex investment ratio over the three years was 0.60 (0.56-0.64).

3. Within polygynous colonies, there was no effect of queen number on sex investment ratios. High queen : worker ratios or queen : sexual brood ratios did not result in stronger male-biased broods as favoured by queens. Within monogynous colonies, single queens in smaller colonies had no more success in produce male-biased broods than single queens in large colonies. Thus, partial queen control over sex allocation appears unlikely in this population.

4. Both local mate competition and the resource abundance hypotheses predict that sex ratio should become more female biased with increasing sexual productivity. Within monogynous colonies, male and new queen production increased together. Polygynous colonies produced relatively fewer new queens at high levels of sexual productivity. Thus, local mate competition and resource abundance appear not to affect this population.

5. Local resource competition is predicted to cause diminishing returns on new queen

production at high levels of sexual productivity. This pattern was seen in polygynous colonies suggesting that they reproduce at least partially through colony budding.

6. Sex allocation in this population is consistent with workers responding to their relative relatedness asymmetry to females and males, and not with partial queen control. Polygynous colonies are likely to reproduce through a combination of budding and emission of sexuals resulting in male biased sex ratios. Monogynous colonies should then respond by producing strongly female biased broods to balance the overall population sex investment ratio.

7. Precise calculation of predicted sex ratios cannot be made because several parameters were not, or could not be measured. Further data on actual relatedness asymmetries of workers to brood, the extent of colony budding and local resource competition, fitness derived from dispersing females and the degree of error in workers' assessment of within-colony relatedness are needed before predicted sex investment ratios can be calculated for any Hymenopteran species.

Table 2.1. Numbers of monogynous, polygynous, and queenless colonies collected in each year from Santon Warren. Unclassifiable colonies could not be assigned to either colony class as they contained queens whose spermathecæ could not be found when dissected.

year	1993	1994	1995
monogynous	21	25	65
polygynous	25	11	20
queenless	1	10	14
unclassifiable	-	3	3
total	47	49	102

Table 2.2. Population sex investment ratios for Santon Warren in all three years sampled with 95% confidence intervals. All sex investment ratios are expressed as proportion of investment in female sexuals. For each year, the total number of individual new males and new queens produced is given. Sex investment ratios were calculated from mean dry mass estimates of the sexes according to the methods of Bourke and Franks (1995, box 5.1).

year	1993	1994	1995
sex investment ratio	0.52	0.58	0.68
95% confidence limits	0.45-0.59	0.49-0.66	0.62-0.73
number of queens	1641	1126	2693
number of males	1590	863	1300
number of colonies	45	40	85

Table 2.3. Sex investment ratios for monogynous and polygynous colony class fractions in all three years with 95% confidence intervals. All sex investment ratios are expressed as proportion of investment in female sexuals. For each colony class in each year, the total number of individual new males and new queens produced is given. Sex investment ratios were calculated from mean dry mass estimates of the sexes according to the methods of Bourke and Franks (1995, box 5.1). *t*-tests compare the differences between mean sex investment ratios of monogynous and polygynous colonies in the same year.

year	1993		1994		1995	
class	M	P	M	P	M	P
sex investment ratio	0.62	0.31	0.66	0.36	0.70	0.32
95% confidence limits	0.54-0.70	0.21-0.41	0.58-0.73	0.14-0.57	0.65-0.76	0.16-0.48
number of queens	1340	301	947	179	2607	86
number of males	828	762	493	370	1092	208
number of colonies	21	24	31	9	67	18

year	1993	1994	1995
t-test between M & P sex investment ratios	$t = 2.04$, d.f. = 43, $p < 0.05$	$t = 1.86$, d.f. = 26, $0.05 < p < 0.10$	$t = 2.97$, d.f. = 68, $p < 0.05$

Table 2.4. Comparison of sex allocation patterns in Santon Warren with 0.5 (queen's preferred) and 0.75 (workers' preferred) proportional investment in females. One-sample *t*-tests are for deviation of sample sex investment ratio (SIR) from 0.5 and 0.75. *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, NS = $p > 0.05$. The comparison is made for the population sex investment ratio for each year separately, as well as pooled for all three years, and also for class specific (monogynous and polygynous) sex investment ratios within years, and pooled for all three years.

Population sex investment ratios

year	SIR	df	0.5 *t*	*p*	df	0.75 *t*	*p*
1993	0.52	44	0.20	NS	44	2.13	*
1994	0.58	39	0.86	NS	39	1.90	NS
1995	0.68	84	1.79	NS	84	0.73	NS
pooled	0.60	169	1.70	NS	169	2.46	*

Class-specific sex investment ratios

year by class		SIR	df	0.5 *t*	*p*	df	0.75 *t*	*p*
1993	M	0.62	20	0.90	NS	20	1.03	NS
	P	0.31	23	2.29	*	23	5.32	***
1994	M	0.66	30	1.36	NS	30	0.82	NS
	P	0.36	8	1.27	NS	8	3.46	**
1995	M	0.70	66	2.03	*	66	0.47	NS
	P	0.32	17	2.21	*	17	5.28	***
pooled	M	0.67	118	2.6	*	118	1.10	NS
	P	0.32	50	3.46	**	50	8.32	***

Table 2.5. Comparison of sex investment ratios in polygynous colonies from Santon Warren using dry weight investment ratios (M / F) and Boomsma's (1989) power-conversion factor cost ratio, (M / F)$^{0.7}$. All sex investment ratios are expressed as mean proportion of investment in female sexuals with 95% confidence intervals.

year	1993		1994		1995	
analysis	M / F	(M / F)$^{0.7}$	M / F	(M / F)$^{0.7}$	M / F	(M / F)$^{0.7}$
sex investment ratio	0.31	0.30	0.36	0.34	0.32	0.30
95% confidence limits	0.21-0.41	0.20-0.40	0.14-0.57	0.13-0.55	0.16-0.48	0.15-0.46
number of females	301		179		86	
number of males	762		370		208	
number of colonies	24		9		18	

Table 2.6. Multiple regression model of sex investment ratio testing for the effects of log queen number and year. * denotes the variables in the model being tested. Factors 1 through 3 in the model are: 1. log queen number; 2. year; 3. log queen number.year (interaction). % Var gives the percentage of variance explained by the model. N/A= residual variance exceeds variance of Y variate. d.f. lost = the numbers of degrees of freedom lost by dropping the variable being tested. The subsequent *F* and *p* values are given for the result of dropping the variable. *p*-values above 0.05 are left out of the final model. The final model proved to be non-significant.

model	1	2	3	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	N/A	5	0.70	0.625				
b	*	*		N/A	3	0.75	0.528	log queen number.year	2	0.67	0.520
c	*			2.6	1	1.98	0.169	year	2	0.20	0.821

Table 2.7. Multiple regression model of sex investment ratio testing for the effects of residual log queen number and year. Residuals were calculated from the regression of total sexual productivity on log queen number. The table layout for the multiple regression model is described in Table 2.6. Factors 1 through 3 in the model are: 1. residual log queen number; 2. year; 3. residual log queen number.year (interaction). The final model proved to be non-significant.

model	1	2	3	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	N/A	5	0.47	0.798				
b	*	*		N/A	3	0.39	0.763	residual log queen number.year	2	0.62	0.546
c	*			N/A	1	0.58	0.450	year	2	0.31	0.734

Table 2.8. Kendall rank-order partial correlation coefficients for polygynous colonies between sex investment ratio and log queen number, controlling for colony size (number of old workers) and total sexual productivity separately.

year	1993	1994	1995
controlling for colony size	$T = -0.28$, $n = 18$, $p > 0.05$	$T = 0.02$, $n = 8$, $p > 0.05$	$T = 0.15$, $n = 11$, $p > 0.05$
controlling for log total sexual productivity	$T = -0.17$, $n = 18$, $p > 0.05$	$T = 0.02$, $n = 8$, $p > 0.05$	$T = 0.14$, $n = 11$, $p > 0.05$

Table 2.9. Partial correlation coefficients between sex investment ratio and log total sexual productivity with colony size (number of old workers) held constant. Monogynous and polygynous colonies were considered separately.

year	1993	1994	1995
monogynous	$r = -0.18$, d.f. = 14, $p > 0.05$	$r = -0.12$, d.f. = 22, $p > 0.05$	$r = 0.18$, d.f. = 54, $p > 0.05$
polygynous	$r = -0.53$, d.f. = 15, $p < 0.05$	$r = -0.64$, d.f. = 5, $p > 0.05$	$r = -0.40$, d.f. = 8, $p > 0.05$

Table 2.10. Multiple regression model of log new queen and log new male production, testing for the effects of log total sexual productivity, colony class and year. The table layout for the multiple regression model is described in Table 2.6. Factors 1 through 7 in the model are: 1. tsp (log total sexual production); 2. class (monogynous or polygynous); 3. year; 4. tsp.class (interaction); 5. class.year (interaction); 6. tsp.year (interaction); 7. tsp.class.year (interaction).

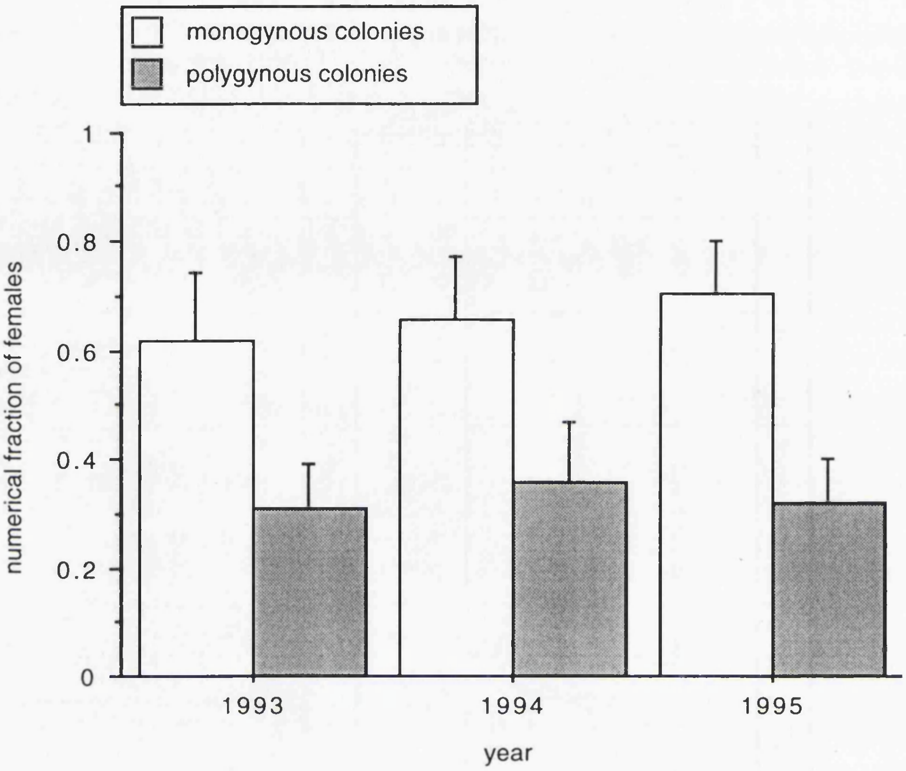
log new queen production

model	1	2	3	4	5	6	7	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	*	*	*	*	79.0	11	47.05	<.001				
b	*	*	*	*	*	*		79.2	9	58.10	<.001	tsp.class.year	2	0.29	0.751
c	*	*	*	*	*			79.5	7	75.88	<.001	tsp.year	2	0.01	0.991
d	*	*	*	*				79.3	5	104.51	<.001	class.year	2	1.64	0.198
e	*	*	*					77.7	4	118.68	<.001	tsp.class	1	10.33	0.002
f	*	*		*				79.5	3	175.69	<.001	year	2	0.35	0.703

log new male production

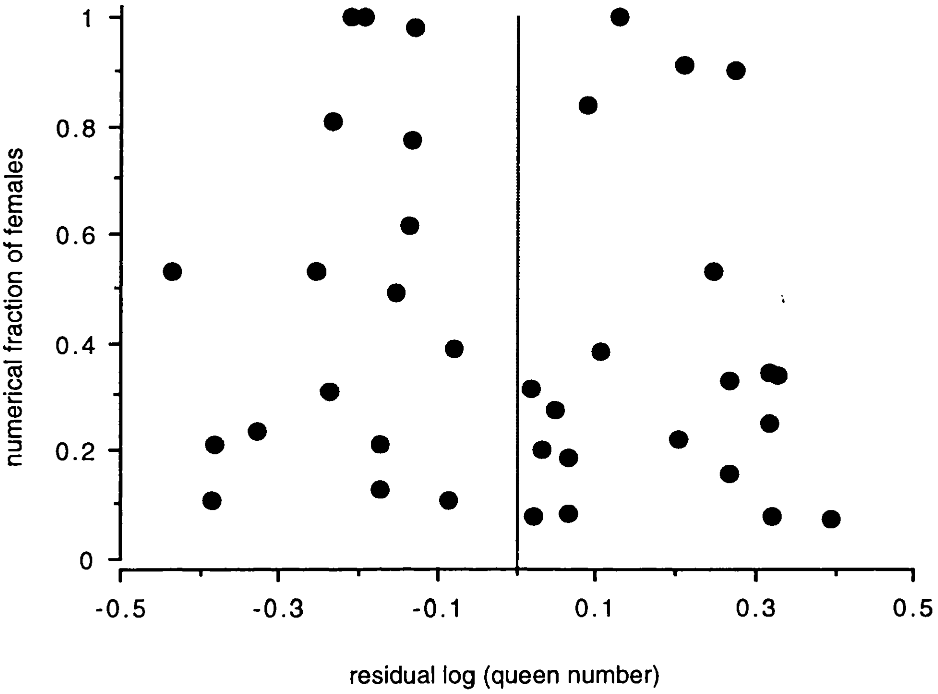
model	1	2	3	4	5	6	7	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	*	*	*	*	57.4	11	15.91	<.001				
b	*	*	*	*	*	*		58	9	19.75	<.001	tsp.class.year	2	0.09	0.918
c	*	*	*	*	*			58.4	7	25.51	<.001	tsp.year	2	0.46	0.634
d	*	*	*	*				58.2	5	34.95	<.001	class.year	2	1.36	0.261
e	*	*	*					57.6	4	42.49	<.001	tsp.class	1	2.52	0.115
f	*	*						57.0	2	81.88	<.001	year	2	1.86	0.160
g	*							53.8	1	143.35	<.001	class	1	9.21	0.003

Figure 2.1. Split sex ratios in three consecutive years from one population of the facultatively polygynous ant *Leptothorax acervorum*.



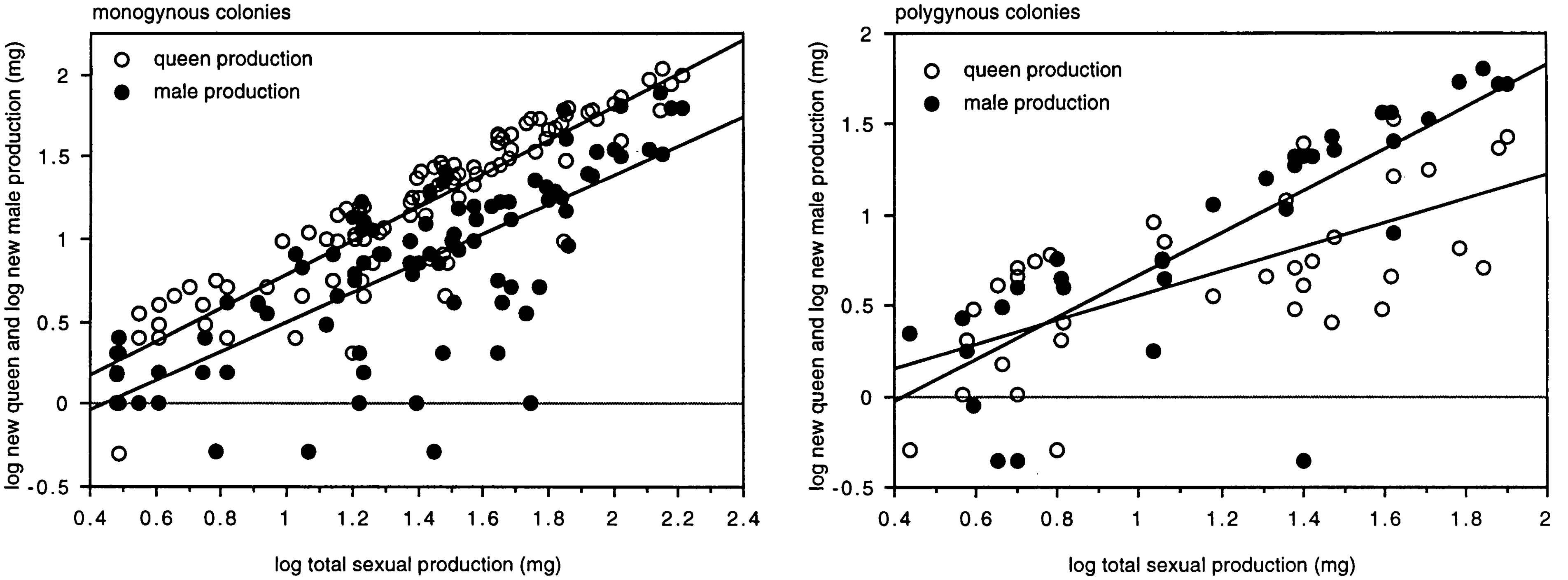
Population sex investment ratios are weighted means with standard error bars for three consecutive years split by colony class (monogynous versus polygynous). In all three years, monogynous colonies produced more female-biased brood than polygynous colonies, although this difference was not significant in 1994 (*t*-test: $t = 1.86$, d.f. = 26, $0.05 < p < 0.10$; 1993: $t = 2.04$, d.f. = 43, $p < 0.05$; 1995: $t = 2.97$, d.f. = 68, $p < 0.05$). Pooled over three consecutive years, monogynous colonies produced a mean sex ratio (95% confidence limits) of 0.67 (0.63-0.71) ($n = 119$) and polygynous colonies 0.32 (0.25-0.40) ($n = 51$). These means were found to be significantly different ($t = 4.17$, d.f. = 164, $p < 0.0001$).

Figure 2.2. Relation between sex investment ratio and residual log queen number in polygynous colonies.



Residuals were taken from the regression of log queen number on total sexual production. The regression proved non-significant ($F_{1,35} = 0.58$, $p = 0.45$). There was also no significant regression of sex investment ratio on log queen number ($F_{1,35} = 1.98$, $p = 0.17$). This demonstrates that queen number, and thus partial queen control, did not affect sex allocation. These analyses omitted colonies producing five or fewer sexuals, and one colony with an exceptionally high queen number (Appendix A).

Figure 2.3. Relations between new male and new queen production and total sexual production in monogynous and polygynous colonies of *Leptothorax acervorum*, in terms of biomass produced.



log new male production (mg) and log new queen production (mg) were regressed on log total sexual production (mg) for monogynous and polygynous colonies separately. Data were pooled for all three years. In monogynous colonies, the regressions were both significant (males: $b = 0.89$, $F_{1,87} = 101.19$, $p < 0.0001$; queens: $b = 1.01$, $F_{1,97} = 504.85$, $p < 0.0001$) and there was no difference between the gradients of the regression lines ($F_{1,184} = 1.70$, $p > 0.05$). In polygynous colonies the regressions were both significant (males: $b = 1.16$, $F_{1,32} = 66.23$, $p < 0.0001$; queens: $b = 0.67$, $F_{1,35} = 30.80$, $p < 0.0001$), but the gradient for new male production was significantly steeper than for new queen production ($F_{1,67} = 7.03$, $p < 0.05$).

Chapter 3

AN INTER-POPULATION COMPARISON OF SEX ALLOCATION STRATEGIES IN *LEPTOTHORAX ACERVORUM*

§3.1 Introduction

The study of evolution in social insects is strongly grounded in Hamilton's (1964) kin selection theory. Trivers and Hare (1976) combined kin selection theory with Fisher's (1930) sex ratio theory to explain the unusual sex investment ratios seen in social Hymenopteran (ants, bees and wasps) populations. They showed that the population will be at sex ratio equilibrium for the party controlling allocation of resources to sexual offspring, when the sex investment ratio (ratio of investment in females : males) equals its relatedness asymmetry (life-for-life relatedness with females : life-for-life relatedness with males) (Boomsma and Grafen 1990, 1991). In the simplest case of a single, once-mated queen with sterile workers, the queen should favour equal numbers of sons and daughters (sexuals) because she is equally related to both. Workers are predicted to favour a female-biased sex investment ratio of 3:1 because their relatedness to females is 0.75 and to males is 0.25. In general, a large number of studies have supported Trivers and Hares (1976) prediction of worker-controlled sex ratios (Trivers and Hare 1976; Nonacs 1986a; Boomsma 1989; Pamilo 1990; Bourke and Franks 1995, Crozier and Pamilo 1996).

Rarely in Hymenopteran populations are the basic assumptions of one singly-mated queen per colony, with worker sterility, met. Colonies with multiply-inseminated queens, multiple queens and/or worker reproduction all have the effect of lowering the average workers' relatedness asymmetry within colonies (Boomsma and Grafen 1990, 1991; Bourke and Franks 1995; Crozier and Pamilo 1996), with the result that population sex ratios are less female-biased than the 3/4 proportionate investment in females predicted by Trivers and Hare (1976). If there is between-colony variation in the extent of any of these traits, some colonies will have a higher workers' relatedness asymmetry than others in the same population. Boomsma and Grafen (1990, 1991) showed that in this case, workers are expected to capitalise on this relative relatedness asymmetry by biasing their colony's brood toward the sex to which they are more closely related than the average for the population. In the case of variable queen number per colony, single-queen colonies (monogyny) are expected to produce mostly females, while multiple-queen colonies (polygyny) should favour a male bias (Boomsma 1993). Trivers and Hare's theory also assumed random mating and dispersal of sexuals. If these assumptions are not met, the relative sex-specific reproductive value and mating success of male and female sexuals are affected (Oster *et al.* 1977; Grafen 1986; Taylor 1988; Boomsma and Grafen 1991; Pamilo 1991a, b; Ratnieks and Reeve 1992; Bourke and Franks 1995; Crozier and Pamilo 1996). Again, the predicted change in sex allocation expected under both worker and queen control can be calculated using the theories of kin selection, sex allocation and parent-offspring conflict (Trivers 1974). However, if several factors are contributing towards a departure from the predicted Trivers and Hare sex ratio, it may become increasingly difficult to calculate the expected pattern of sex allocation in a population, especially if the relative strengths of these factors are unknown.

An increasing number of field studies are finding evidence that split sex ratios (Grafen 1986) match the theory predicted by Boomsma and Grafen (1990, 1991). Studies in Hymenoptera other than ants in which split sex ratios have been found include those by Boomsma (1991), Mueller (1991), Mueller *et al.* (1994), Queller *et al.* (1993) and Packer and Owen (1994). Sundström (1994a) found split sex ratios in ants with partial multiple-mating. Colonies of *Formica truncorum* with singly-mated queens were found to have significantly more female-biased broods than colonies from the same population with multiply-inseminated queens, which had male-biased broods (see also Bourke and Chan 1994; Sherman and Shellman-Reeve 1994; Sundström 1994b). Split sex ratios have also been demonstrated in ants with variable queen numbers. Chan and Bourke (1994) found that monogynous colonies of the ant *Leptothorax acervorum* produced a higher degree of female bias than polygynous colonies in the same population, which opted for more male-production (also see Chapter 2). Evans (1995) studied relatedness and sex allocation differences in polygynous ants using microsatellites. He found that *Myrmica tahoensis* workers in female-producing colonies had higher average relative relatedness asymmetry than workers in male-producing colonies. Other examples of male-bias being associated with increasing queen number were found by Ward (1983), Elmes (1987a, b), and Herbers (1984, 1990) in *Leptothorax* species (reviewed by Boomsma 1993).

Given the large number of factors that may potentially affect sex ratios in ants, can we gain some insight into the decision making policies of the party (or parties) controlling allocation of resources to sexuals in real populations? Crozier and Pamilo (1996) offer several suggestions on how to approach this problem. First, an examination of the sex ratios from many different species has already given the general impression that observed populations match predicted theory. On a finer scale, studies of populations within species that differ in one or several particular factors known to affect sex allocation can give an idea of how strongly those factors affect allocation decisions. Within those populations, colony-level sex ratios offer insight into decision making at the level of the individual(s) involved in allocating resources to brood. Further still, behavioural observations of individuals actually implementing decisions should be studied at the colony level to understand how the sex ratio is established. Finally, a thorough understanding of the processes at all levels of sex allocation should allow an accurate prediction of expected sex ratios at both colony and population levels under a given set of known parameters. An ideal study will contain detailed information for several populations about relatedness structure at the level of the colony and the whole population, reproductive values of the sexes, energetic cost ratio of the sexes, mating and dispersal systems along with modes of reproduction, social structures within colonies and resource availability (based on Bourke and Franks 1995: Table 4.5).

Leptothorax acervorum is a model species for this sort of hierarchical study. *L. acervorum* has been studied by several authors, and many aspects of the species' life history traits are becoming characterised (Buschinger 1968, 1981; Heinze and Buschinger 1988; Bourke 1991, 1994; Bourke and Heinze 1994; Chan and Bourke 1994; Heinze 1995; Heinze *et al.* 1995a, b). This study focuses on facultatively polygynous populations of the species, found in boreal habitats of Northern Europe. Sociogenetic data on populations have largely been drawn from censuses, and allozyme and microsatellite studies (Douwes *et al.* 1987; Stille *et al.* 1991; Stille and Stille 1992, 1993; Heinze 1995; Heinze *et al.* 1995a, b). Queens appear to be singly-mated, and workers non-reproductive (Heinze *et al.* 1995a). Queen number usually varies from one queen per colony to around ten, though many more queens have been reported (Chan and Bourke 1994), and nest-mate queens are related (Stille *et al.* 1991; Heinze *et al.* 1995b). As a result, intra-colony relatedness varies between colonies according to queen number (Heinze *et al.* 1995b). Furthermore, queens within polygynous colonies from Northern European habitats are predicted to contribute to sexual brood in roughly equal quantities (i.e. reproductive skew is low) (cf. Bourke and Heinze 1994). Sexuals almost certainly mate away from the natal colony in mating swarms (Bourke and Heinze 1994), and the lack of inbreeding in this species suggests that mating is random (Heinze *et al.* 1995b). Random mating means that local mate competition (diminishing returns on new male production because related males compete for matings with females) (Alexander and Sherman 1977; Frank 1987) is unlikely to affect sex allocation. Newly mated queens either establish new colonies independently, or are readopted into their natal colonies as reproductive queens (Douwes *et al.* 1987; Bourke and Heinze 1994; Heinze *et al.* 1995a, b). Genetic surveys (Douwes *et al.* 1987; Stille *et al.* 1991; Stille and Stille 1992, 1993) suggest that colony budding is another mode of reproduction in this species. As a consequence, queens in daughter buds may compete with related queens from the original nest for resources (local resource competition) resulting in diminishing fitness returns on the production of new females (Frank 1987; Pamilo 1991a). Since both local mate competition and local resource competition predict a reduction in the production of one of the sexes at high levels of sexual productivity, the effect of either factor on sex allocation can be examined by looking at changes in sex investment ratios as total sexual productivity varies. Finally, Nonacs (1986a) found that a large number of ant species (including members of the genus *Leptothorax*) increased investment in new queens as total sexual productivity increased. He argued that this was because larger colonies had more resource abundance available to raise more costly sexuals (Nonacs 1986b). Resource abundance was examined in this study by looking for positive partial correlations between sex investment ratio (numerical fraction of females) and total sexual productivity, with colony size held constant.

This chapter investigated colony-level sex allocation in three populations of *L. acervorum*. The effects of queen number on sex allocation were examined. Under worker control, sex ratios are predicted to correlate with workers' relatedness asymmetry, but not under queen control. If queens may gain partial control of sex allocation, a relationship between the ratio of number of queens to number of workers is expected. Information about the genetic profile of two of these populations supports the sex allocation data as queen number and intra-colony relatedness are not always expected to covary in a simple fashion. The findings suggest that workers controlled sex allocation by responding to genetic information about their own colonies and the population as a whole. A previous study found evidence of local resource competition in this species (Chan and Bourke 1994), and its effects were examined here by looking for a decrease in production of female sexuals at high levels of total sexual productivity within colonies. In two populations, local resource competition appeared to be present, though its effects were stronger in one of them. The detailed life history data for this species, along with precise colony production schedules allowed a more in-depth view of the processes affecting sex allocation decision-making in ants than had previously been possible.

§3.2 Materials and methods

Study species and populations

Leptothorax acervorum is a facultatively polygynous ant species commonly found throughout temperate coniferous forests, boreal and alpine regions of the British Isles and Northern and Central Eurasia (Collingwood 1979, Heinze *et al.* 1995b). Polygyny occurs secondarily through readoption of related nestmate queens (Douwes *et al.* 1987; Stille *et al.* 1991; Heinze *et al.* 1995b). Each colony typically contains between several dozen and hundreds of workers, and there are rarely more than ten mated queens per colony. Whole, single colonies are found inside cavities in rotting twigs, under bark, or occasionally in the roots of dead trees and under stones. Collection of whole colonies (minus foraging workers) from a single site (monodomy) allows an accurate census of all adults and brood in every colony collected.

Three separate populations of *L. acervorum* were sampled between 1993 and 1995. One study population from Santon Warren, Thetford Forest, Norfolk, U.K. was sampled over three consecutive years (see also Bourke 1991, 1993; Chan and Bourke 1994). Another population collected from Aberfoyle in Strathclyde, Scotland was sampled in 1994 and 1995. A population from Roydon Wood in the New Forest, Hampshire, U.K. was sampled in 1995.

Collection and culture of colonies

From Santon Warren, 47 colonies were collected on 17 June 1993, 49 colonies were collected on 8 June 1994 and 102 colonies were collected over two days on June 13 and 20, 1995 (Appendix A). The Aberfoyle population was sampled on 25 May, 1994 and 23 May, 1995; respectively, 34 and 19 colonies were collected (Appendix A). From Roydon Wood, 36 colonies were collected on 4 June, 1995 (Appendix A). Sampling of populations was carried out before July so that no brood had yet eclosed into adults (Chan and Bourke 1994).

Collection of ants in the field involved breaking open dead twigs to find whole colonies. Random sampling of populations was assumed as all colonies found were collected, and all collection within a population took place in the same general region (including across-year sampling). Twigs containing colonies were then placed inside plastic bags and returned to the laboratory. Colonies were removed from twigs, aspirated and placed inside standard nests, and maintained in the laboratory based on the methods of Bourke (1991). Standard nests were made of two rectangular glass slides separated by a cardboard border with an opening in one end. Nests were kept inside clear plastic dishes (10 x 10 x 2 cm) under natural lighting and at room temperature. Colonies were fed on a regime of water (damp cotton wool), sugar solution and mealworm larvae, renewed every 2-3 days.

Census of colonies

Immediately after collection, colonies were censused for the first time. All adults and pupae were counted from each colony by transferring individuals using fine forceps allowing an accurate measure of each colony's composition. Colonies were maintained in the laboratory until all pupae had eclosed into adults before being censused for a second time for all individuals present. Comparison of the first with the second census gave each colony's annual production of new workers, new queens and new males on top of its original composition (Appendix A).

All mated colony queens had shed their wings (dealate). Some alate (winged) virgins may have shed their wings before the first census, but after collection from the field. During the second census, all dealate queens were removed for ovarian dissection according to the methods of Bourke (1991) to establish reproductive status. Mated queens have spermathecæ (sperm receptacles) visibly full of sperm (under a compound microscope), while virgin queens have clear spermathecæ. Other dissection data collected included number of yolky eggs present out of total number of eggs present, and approximate ovariole length was recorded (length categories ranged from very small through small, small-medium, medium, medium-large, large, very large and very-very-large). Corpora lutea (residual ovarian structures, by-products of egg production) were also counted. In cases where ovarian dissections were only partially complete, but the spermatheca could not be found, these other data could be used to establish whether a queen had been mated or not. Mated queens were counted as colony queens while non-mated queens were taken to be newly eclosed and thus were counted as part of the new queen fraction. This was justified because A.F.G. Bourke (unpublished data) found that of 72 queens collected from Santon Warren between April and May (1990-1991), all were mated (reported in Chan and Bourke 1994). Furthermore, A.F.G. Bourke (unpublished data) found that *L. acervorum* sexuals from Santon Warren do not mate in the natal colony (reported in Chan and Bourke 1994).

In total, from three different populations, 287 colonies were collected containing 22 322 workers and 1 064 queens, the latter of which were all dissected. These colonies produced 33 955 new brood including workers, queens and males (Appendix A).

Genetic composition of the populations

The Santon Warren population was studied genetically by Heinze *et al.* (1995b) using allozyme analysis of two polymorphic loci on samples collected in 1991 and 1992. The Roydon Wood population was studied genetically by A. Hingle (unpublished). Within-colony relatedness was examined in 20 colonies using five polymorphic allozyme loci. The results of these studies are given in Tables 3.5 and 3.6, and are discussed in the Results section. The genetic data suggested that inbreeding was absent from the Santon Warren population (Heinze *et al.* 1995b), and low worker egg-laying rates imply that worker male production was negligible (Bourke 1991).

L. acervorum males almost certainly mate both in large swarms and near nests (Bourke and Heinze 1994). The lack of evidence for inbreeding suggests that males disperse widely enough to render local mate competition among them unlikely. However, there is strong evidence that polygynous *L. acervorum* colonies reproduce by budding (see, for example, Stille and Stille 1993; Bourke and Heinze 1994; Chan and Bourke 1994), suggesting that local resource competition due to budding is probable (see Chan and Bourke 1994).

Estimation of mean dry weights

Samples of callow workers, alate queens and males were taken from several monogynous and polygynous colonies from Santon Warren in all three years. [Appendix B, Table B.1]. These were dried in an oven for 24 h, at 60 °C, and each ant individually weighed on an electronic microbalance. Thorax length was measured using a dissection microscope attached to a computer-based image analysis system (NIH Image). Using a basic thorax length-dry weight comparison, the dry weight for a larger sample of ants could be estimated where only the thorax was available for measurements. Samples of all three castes of ant from several monogynous and polygynous colonies in 1994 and 1995 were measured for thorax length using this technique (except in polygynous colonies in 1995 where sexuals were unavailable for measurement).

§3.3 Results

§3.3.1 Population composition

Santon Warren

The overall mean sex investment ratio (95% confidence limits) pooled over three years was 0.60 (Table 3.1; also see Chapter 2, Tables 2.1 and 2.2). There was no significant difference in population sex investment ratios between years (ANOVA: $F_{2,127} = 2.18, p > 0.05$). Over the three years that this population was censused it produced an average of 28% polygynous colonies, though it became increasingly monogynous (Table 3.2). The harmonic mean queen number in polygynous colonies was 4.5 overall (Table 3.2). Harmonic mean queen number is thought to be an accurate estimator of within colony relatedness because changes in relatedness with queen number are weighted by the number of queens already present (Wade 1985; Queller 1993). Thus, the addition of a second queen to a monogynous colony produces a greater change in within-colony relatedness (assuming queens share reproduction equally) than the addition of successive queens to a highly polygynous colony.

Aberfoyle

The Aberfoyle population of *L. acervorum* produced strongly female-biased brood. The mean population sex investment ratio (95% confidence limits) was 0.71 (0.64-0.77) ($n = 47$ colonies) (Table 3.1). There was no significant difference in population sex investment ratios over the two years that this population was censused (t-test: $t = 0.26$, d.f. $= 45, p > 0.05$). Over two years, the population was found to contain 25% polygynous colonies (Table 3.2). The overall harmonic mean queen number for this population was 6.5, although when two colonies producing over 100 queens each (Appendix A) were excluded, this fell to 5.4 (Table 3.2).

Roydon Wood

This population produced an extremely male biased mean sex ratio (95% confidence limits) of 0.13 (0.06-0.20) (n = 33 colonies). Out of 36 colonies collected, 25 (69%) were polygynous. This is the highest fraction of polygynous colonies of the three populations studied, and higher than almost any reported previously for this species (Stille *et al.* 1991: 27%, 29%, 35% and 50%; Heinze *et al.* 1995b report five populations producing over 50% polygynous colonies, including one of 79%, though in all of these fewer than 15 colonies were sampled). The harmonic mean queen number in polygynous colonies was 3.5 (Table 3.2).

§3.3.2 Mean population sex investment ratios

Population mean sex investment ratios for the three populations were 0.60 for Santon Warren, 0.71 for Aberfoyle, and 0.13 for Roydon Wood (Table 3.1; Figure 3.1). Overall, there was a significant difference between the means of the three populations (ANOVA: $F_{2,247} = 5.92$, $p < 0.005$). However, Fisher's protected least significant difference test showed that the mean population sex investment ratio of Roydon Wood differed from the means of the other two populations (Roydon Wood, Santon Warren: critical difference = 0.299, $p < 0.005$; Roydon Wood, Aberfoyle: critical difference = 0.357, $p < 0.005$), which showed no difference between themselves (Santon Warren, Aberfoyle: critical difference = 0.259, $p > 0.05$).

Overall, as the degree of polygyny in a population increased (Table 3.2), the population sex investment ratio decreased (Figure 3.2), although the trend was non-significant (Spearman rank correlation: $r_S = -0.66$, $n = 6$, $p > 0.05$).

§3.3.3 Split sex ratios

Out of the three study populations, only Santon Warren had split sex ratios stemming from queen number. Aberfoyle and Roydon Wood showed no significant within-population difference between monogynous and polygynous colonies sex investment ratios (Figure 3.3).

Santon Warren

Over three consecutive years, monogynous colonies produced more female-biased sex investment ratios than polygynous colonies in the same year (Chapter 2, Table 2.3). Within both colony classes, there was no difference in sex investment ratios between years (ANOVA: $F_{2,164} = 0.07, p > 0.05$), so data within classes were pooled across years. Overall, monogynous colony broods were significantly more female biased than polygynous colony broods (t-test: $t = 4.17$, d.f. = 164, $p < 0.0001$). Mean sex investment ratios (95% confidence limits) for monogynous and polygynous colonies were 0.67 (0.63-0.71; n = 119 colonies) and 0.32 (0.25-0.40; n = 51 colonies). This population had sex investment ratios split by colony class.

Aberfoyle

Within years, monogynous and polygynous colonies did not produce significantly different sex ratios in either year (Table 3.3). There was no difference in mean sex investment ratios between monogynous colonies from 1994 and 1995 (t-test: $t = 0.11$, d.f. = 33, $p > 0.05$), nor did polygynous colonies show a difference in mean sex investment ratio between years (t-test: $t = 0.28$, d.f. = 10, $p > 0.05$). Pooled for both years, monogynous colonies produced a mean sex investment ratio of 0.66, while in polygynous colonies it was 0.75 (Table 3.3). Overall, there was no significant difference between these means (Table 3.3). Thus, this population did not show sex ratios split by gyny class.

Roydon Wood

Monogynous and polygynous colonies produced mean sex investment ratios (95% confidence limits) of 0.14 (-0.08-0.35) (n = 10 colonies) and 0.12 (0.06-0.19) (n = 23 colonies) respectively. There was no significant difference between these sex investment ratios (t-test: $t = 0.20$, d.f. = 31, $p > 0.05$). Thus, in this population, sex investment ratios were not split by colony gyny class.

§3.3.4 Queen number and sex investment ratios

All three populations were examined for an effect of queen number on sex allocation in polygynous colonies. A multiple regression model tested sex investment ratio for the effects of log queen number and population. The effect of population was found to be significant to the model (multiple regression, 'population': $F_{2,60} = 8.31$, $p < 0.001$; Table 3.4). This is because the three populations varied in their sex allocation schedules. Log queen number and its interaction term were not significant in the model (multiple regression, 'log queen number': $F_{1,61} = 0.51$, $p > 0.05$; 'log queen number.population': $F_{2,60} = 1.73$, $p > 0.05$; Table 3.4). Sex ratio was similarly unaffected by residual log queen number (generated from the regression of log queen number on total sexual productivity) over the three populations (multiple regression, 'residual log queen number': $F_{1,61} = 1.74$, $p > 0.05$; 'residual log queen number.population': $F_{2,60} = 0.10$, $p > 0.05$; Table 3.5). Thus, in all the three populations examined in this species, sex allocation in polygynous colonies was unaffected by queen number, even when total sexual productivity was taken into account (Figure 3.4).

§3.3.5 Relatedness and sex investment ratios

Santon Warren

Heinze *et al.* (1995b) report from allozyme data that in years 1991-1992, the Santon Warren population had average worker-worker relatedness in monogynous colonies of 0.50, and in polygynous colonies of 0.28 (Table 3.6). They found these means to differ significantly (*t*-test: $t = 2.95$, d.f. = 51, $p < 0.01$; Heinze *et al.* 1995b). Overall, the mean weighted average population value was 0.42 (Table 3.6). Within the polygynous colony class, queen-queen relatedness was 0.26 (Table 3.6).

Roydon Wood

A. Hingle (unpublished results) analysed within-colony relatedness in 20 colonies from the Roydon Wood population. Up to 12 workers, and all available queens were examined at five polymorphic loci (glucose-6-phosphate isomerase, phosphoglucomutase-1, glucose-6-phosphate dehydrogenase, aconatase-1 and aconatase-2) and average relatedness was calculated from the allozyme data. Over the whole population, average worker-worker relatedness was 0.44 and average queen-queen relatedness within polygynous colonies was 0.11 (Table 3.6). Average worker-worker relatedness was slightly higher in monogynous colonies (0.55) than in polygynous colonies (0.40), but the difference was not significant (*t*-test: $t = 1.03$, d.f. = 18, $p > 0.05$; Table 3.6). He also examined average worker-worker relatedness in colonies producing males only, and colonies producing both male and female sexuals. There was no significant difference in average worker-worker relatedness between male-only producing colonies (0.57) and colonies producing mixed brood (0.37) (*t*-test: $t = 0.85$, d.f. = 18, $p > 0.05$; Table 3.7). The two colony types also did not differ in average queen-queen relatedness (*t*-test: $t = 1.11$, d.f. = 13, $p > 0.05$; Table 3.7).

§3.3.6 *Sexual productivity and sex investment ratios*

Local mate competition and local resource competition are both predicted to affect a colony's sex allocation according to its sexual productivity. The relationships between increasing log total sexual production and log new male and log new female production were investigated using multiple regression with colony class and population in the models. (In this and all subsequent analyses involving log total sexual production, log new male production, and log new queen production, production values are in milligrams). The model for 'log new queen production' found several interaction terms to be significant to the model ('pop.class': $F_{2,181} = 8.95$, $p < 0.001$; 'log total sexual productivity.class': $F_{1,182} = 9.02$, $p < 0.005$; Table 3.8). The model for 'log new male production' found both 'population' and 'class' to be significant to the model ('population': $F_{2,175} = 5.58$, $p = 0.004$; 'class': $F_{1,176} = 7.99$, $p = 0.005$; Table 3.8). Because population and/or its interaction terms were significant factors in both models, separate regressions were considered for each population. Furthermore, for each population, the partial correlation between sex investment ratio and log total sexual production were examined, holding colony size (old worker number) constant, to look for effects of relative productivity on sex allocation (cf. Nonacs 1986b).

Santon Warren

The multiple regression model of log new queen production found a significant effect of class which suggested that monogynous and polygynous colonies have different schedules for the production of new queens (multiple regression for new queens: 'log total sexual production.class': $F_{1,134} = 10.33$, $p = 0.002$; Table 3.9). Year was not significant to the model which means that colonies were consistent in their patterns of sex allocation with levels of total sexual production from year to year (Table 3.9). Data for new queen production were pooled for year.

Both classes of colony increased investment in new queens with rising total sexual productivity (regression, monogynous colonies: $b = 1.01$, $F_{1,97} = 504.85$, $p < 0.0001$; polygynous colonies: $b = 0.67$, $F_{1,35} = 30.80$, $p < 0.0001$) but the regression line was significantly steeper for monogynous than polygynous colonies ($F_{1,132} = 10.95$, $p < 0.005$) suggesting that at higher levels of investment in sexuals, polygynous colonies divert resources away from new queen production (Figure 3.5).

The multiple regression model of log new male production found an effect of class on new male production (multiple regression for new males: 'class': $F_{1,121} = 9.21$, $p = 0.003$; Table 3.9). There was no effect of year, again suggesting that colonies are consistent in new male production, as in new queen production, from year to year (Table 3.9).

Data for log new male production were pooled across years. Both colony classes increased investment in new males with rising total sexual productivity (regression, monogynous colonies: $b = 0.89$, $F_{1,87} = 101.19$, $p < 0.0001$; polygynous colonies: $b = 1.16$, $F_{1,32} = 66.23$, $p < 0.0001$). There was no difference between the regression gradients for the two colony classes ($F_{1,119} = 2.72$, $p > 0.05$; Figure 3.5).

Within the polygynous colony class, the regression gradient for log new male production ($b = 1.16$) was found to be significantly steeper than that for log new queen production ($b = 0.67$) ($F_{1,67} = 7.03$, $p < 0.05$; Figure 3.5). Again, this confirms that at higher levels of investment in sexuals, polygynous colonies invested more resources in raising new males. The corresponding comparison of gradients for monogynous colonies found no significant difference between increasing new male ($b = 0.89$) and new female production ($b = 1.01$) ($F_{1,184} = 1.70$, $p > 0.05$; Figure 3.5).

Within the monogynous colony class pooled across all three years, there was no significant partial correlation between sex investment ratio and log total sexual productivity with colony size (old worker number) held constant ($r = 0.03$, d.f. = 97, $p > 0.05$). The corresponding partial correlation for polygynous colonies pooled across years showed a strongly significant negative trend ($r = -0.44$, d.f. = 35, $p < 0.01$), confirming that in polygynous colonies, the sex ratio grew more male biased as sexual productivity increased, whereas in monogynous colonies it remained constant.

Aberfoyle

Log new queen production rose with increasing log total sexual productivity (b = 1.14, $F_{1,30}$ = 81.43, $p < 0.001$; Table 3.10). Log new male production also increased with log total sexual productivity (b = 0.66, $F_{1,29}$ = 11.00, $p = 0.002$; Table 3.10), but the regression line for new queens was steeper than for new males ($F_{1,59}$ = 13.13, $p < 0.001$: Figure 3.6). There was no effect of year or class or their interaction terms in either of the models (Table 3.10).

There was a significant positive partial correlation between sex investment ratio and log total sexual productivity with old worker number held constant ($r = 0.40$, d.f. = 44, $p < 0.01$) when both years and classes were pooled. This suggested that highly productive colonies of both classes produced a stronger female bias than less productive ones. Increasing female investment with increasing resources (total sexual production) has been found in many other species (reviewed in Nonacs 1986a, b). Diminishing returns on male production at high levels of sexual productivity is predicted to stem from local mate competition (Frank 1987). This study did not differentiate between resource abundance and local mate competition in this population.

Roydon Wood

Log new male production increased with log total sexual production (b = 1.16, $F_{1,22}$ = 107.67, $p < 0.001$; Figure 3.7). There was no effect of class on new male production (multiple regression: Table 3.11). There was no relationship between log new queen production and log total sexual productivity ($F_{1,14}$ = 2.84, $p > 0.05$; Figure 3.7), and class was not a significant factor in the model (multiple regression: Table 3.11).

Under local resource competition, Frank (1987) predicted a constant level of investment in females up to a threshold, with male-only investment afterwards. The pattern of investment in new sexuals in this population suggested that related queens may have experienced strong local resource competition.

The partial correlation between log total sexual productivity and sex investment ratio with old worker number held constant gave a significant negative relationship ($r = -0.50$, d.f. = 21, $p < 0.02$). As in the Santon Warren population, this suggested that at high levels of sexual investment, allocation to new queens was reduced.

§3.4 Discussion

The three populations compared in this study adopted very different sex allocation strategies. Santon Warren and Aberfoyle contained 28% and 25% polygynous colonies respectively and produced female-biased population sex investment ratios while the Roydon Wood population contained 69% polygynous colonies and was extremely male-biased (Table 3.12). Within populations, only Santon Warren had sex investment ratios split by colony class, where monogynous colonies produced strongly female-biased broods and the broods produced by polygynous colonies were significantly more male-biased. In both the Aberfoyle and Roydon Wood populations, monogynous and polygynous colonies did not differ in their sex allocation strategies (Table 3.12). Worker-worker relatedness within colonies from Santon Warren in 1991-1992 was higher in monogynous than in polygynous colonies (Heinze *et al.* 1995b). A similar study found no difference in average within-colony worker-worker relatedness between monogynous and polygynous colonies from Roydon Wood (A. Hingle, unpublished) (Table 3.12). Assuming that worker-worker relatedness reflects relatedness asymmetry within colonies, this supports the prediction that workers bias brood sex investment ratios according to information about the genetic composition of their colony and the population. Colonies from Santon Warren always increased new male and new queen production with increasing sexual productivity, but in polygynous colonies, new queen production was reduced at higher levels of total sexual production (Table 3.12). This suggested that local resource competition between related queens in neighbouring colonies may have stemmed from colony budding. All colonies from Roydon Wood increased investment into new male production with rising total sexual production, while new queen production remained unaffected (Table 3.12). Again, this suggested that local resource competition may have contributed to strong male bias in this population's sex investment ratio. Sex investment ratios became increasingly female-biased in the Aberfoyle population with increasing total sexual productivity in both colony classes (Table 3.12). This was likely to have stemmed from resource abundance effects, as the mating system of most ant species excludes the likelihood of local mate competition (Bourke and Franks 1995). There was no evidence of partial queen control over sex allocation in any of the population studied. Instead, workers appeared to control sex allocation by responding to information about the genetic composition of their colonies. Population sex investment ratios reflected the degree of polygyny in each population, while colony sex investment ratios in more productive colonies seemed to be affected by local resource competition in two out of three populations, and resource abundance in the third.

Colony-level sex allocation

There was no evidence from any population that queens gained even partial control over sex allocation. This is in agreement with a growing number of studies that support worker control of sex allocation in ants (reviews in Bourke and Franks 1995; Crozier and Pamilo 1996; although see Herbers 1984, 1990; Backus 1995). Instead, workers appeared to control sex allocation by biasing brood sex investment ratios towards the sex to which they were most closely related, if there was any difference in relatedness asymmetry at all. Although no study has yet managed to associate split sex ratios with actual workers' relatedness asymmetry to brood, Sundström (1994a) found that sex ratios in the ant *Formica truncorum* were split according to differences in worker-worker relatedness induced by multiple mating. Evans (1995) found split sex ratios in the facultatively polygynous ant *Myrmica tahoensis* to be related to the degree of nestmate relatedness in the direction predicted by split sex ratio theory

(Boomsma and Grafen 1990, 1991).

Sex allocation decisions in two of the three study populations appeared to be influenced by local resource competition, likely to have stemmed from colony budding (Table 3.12). Male bias associated with colony budding or polydomy (also predicted to cause local resource competition) has also been reported in *Rhytidoponera* species (Ward 1983), *Formica exsecta* (Pamilo and Rosengren 1983), and *F. truncorum* (Sundström 1995). The Aberfoyle population increased proportional investment in new queens with increasing total sexual productivity (Table 3.12). This trend is known from several studies (Nonacs 1986a, b; Boomsma 1993) including other *Leptothorax* species (Herbers 1984, 1990; Nonacs 1986a, b). Whereas this trend is predicted under local mate competition (Frank 1987), Bourke and Franks (1995) point out that the mating biology of most ant species excludes the likelihood of this factor (however, see Hasegawa and Yamaguchi 1995). Poorly resourced colonies may be diverting resources away from more costly queens into new workers, although Crozier and Pamilo (1996) point out that it is not clear whether an association between sex investment ratios and resource abundance reflects a naturally selected decision-making process, or whether it is a mechanistic reflex of female caste determination. Finally, sex allocation decisions made at the colony level may depend on sex allocation trends occurring in the population as a whole. If one class of colony is specialising in the production of one sex, the other class of colony will be selected to specialise in the other sex in a process known as sex ratio compensation (Taylor 1981). Sex ratio compensation is discussed under population-level sex allocation below.

There are several reasons why polygynous species may not show relatedness asymmetry split according to colony gyny class or queen number in a simple fashion. Polygynous colonies characteristically have relatively low within colony genetic relatedness (Keller 1995). Furthermore, Nonacs (1988) showed that polygyny should be associated with low queen survivorship and/or high queen turnover (also see Keller 1995). Heinze *et al.* (1995b) showed that in several, but not all populations of facultatively polygynous *Leptothorax acervorum* studied, worker-worker relatedness in monogynous colonies was lower than the 0.75 predicted by Trivers and Hare (1976), but higher than the average worker-worker relatedness in polygynous colonies from the same population. They considered that seasonal changes in queen number through recruitment or departure (i.e. colony budding or queen death) may frequently cause colonies to change between monogyny and polygyny. Thus, a colony that is currently monogynous may have a worker force that was established under polygyny, and the genetic relatedness between those workers will be lower than predicted under monogyny (also see Heinze 1995).

High levels of colony budding should have a strong effect on worker-worker relatedness within colonies across a population. Consider a polygynous colony with several reproductive queens. If one queen leaves the colony with a group of workers, and establishes a new colony, the average worker-worker relatedness within the new monogynous daughter bud will be equal to that of the parent colony in the first year, and will increase thereafter as the single queen produces all new offspring. If workers base their sex allocation decisions on average within-colony relatedness to new queen-produced brood, the brood's sex ratio is expected to reflect the colony queen number. If their decisions are based on overall genetic diversity within a colony including worker-worker relatedness, their estimation of queen number will be incorrect. Extensive colony budding in a population may mean that monogynous and polygynous colonies show no difference in colony relatedness structure.

Bourke *et al.* (unpublished) found that, in some polygynous colonies of *L. acervorum* from Santon Warren, none of the recently eclosed brood could be assigned to a queen present in the colony (also see Seppä 1994). This may have stemmed from the death or departure of the queen(s) that laid those brood. Thus, the number of queens in a colony may not be an accurate reflection of the genetic composition of the colony. As a consequence, workers biasing brood sex investment ratios according to within-colony genetic cues may appear to be making non-adaptive sex allocation decisions. Extensive colony budding may have been the cause of Roydon Wood's absence of split sex ratios. This explanation does not extend to the Aberfoyle population given that there was no apparent effect of local resource competition. Some other factor, possibly resource abundance but not relatedness asymmetry, seems to be driving sex allocation at the colony level in this population.

Population-level sex allocation

Taylor (1981) predicted that when one class of colony specialises in the production of one sex, the remaining colonies in the population should return the population sex investment ratio to the workers' equilibrium by over-producing the other sex. He termed this process sex ratio compensation. Colonies within the three study populations tended to specialise in the production of biased brood sex ratios for different reasons. In Santon Warren, monogynous colonies appeared to respond to a high relative relatedness asymmetry by producing mostly females, while the polygynous colonies produced mostly males in response to lower relatedness asymmetry (Table 3.12). On top of this, the more productive of the polygynous colonies seemed to reduce female bias even further because of local resource competition. Thus, the overall female bias in monogynous colonies may have been partially in response to strong male bias in polygynous colonies by sex ratio compensation, and partially due to workers response to relatedness asymmetry. A population sex investment ratio of less than 3/4 female investment would be expected under partial polygyny, and the fact that female bias in the population sex investment ratio increased as the degree of polygyny in the population fell is in agreement with this (Table 3.12).

The Aberfoyle and Roydon Wood populations did not appear to produce sex ratios influenced by colony class (Table 3.12). In both populations, sex investment ratios changed according to total sexual productivity (Table 3.12). The Aberfoyle population appears to have responded to resource abundance effects which predicts increasing female bias with increasing total sexual productivity. However, it is not clear whether small colonies are producing a relative male bias, with larger, more productive colonies compensating by over producing female-biased sex ratios or vice versa with large colonies investing in female biased broods, and smaller colonies balancing the population sex investment ratio by concentrating on male production. The overall population sex investment ratio was more female-biased than that of Santon Warren, although not to a significant degree, and this is almost certainly a result of Aberfoyle's polygynous colonies not producing a male bias, as well as an apparent absence of local resource competition (Table 3.12). The population sex investment ratio for Roydon Wood appeared to be strongly influenced by local resource competition in both monogynous and polygynous colonies. While male production increased in both colony classes with increased total sexual production (Table 3.12), sex ratio compensation was unlikely to have been involved. Instead, all colonies appeared to invest in a certain number of new queens, and investment in new males thereafter with the result that more productive colonies produced stronger male-biased broods. The relative absence of new queens suggested that independent nest founding is unlikely, and that all colonies reproduce by budding. The evidence of strong local resource competition alongside the high percentage of polygynous colonies in this population are the likely causes of the strong male bias in the population sex investment ratio.

General discussion

Sex ratio theory, based on kin selection theory predicts that the party controlling sex allocation can maximise its own fitness by basing sex allocation decisions on relatedness information available to them at both colony and population levels. The results of this study suggest that this is happening. Under facultative polygyny, workers in monogynous colonies are expected to have higher average relatedness asymmetry than those in polygynous colonies. Furthermore, workers are then expected to act upon this information by producing sex ratios biased towards the sex to which they are most closely related, which they appear to be doing. However, intra-colony relatedness appears not to vary with queen number in a simple fashion, and inter-population variation may be considerable. If queen turnover and colony budding are present they may cause variation in intra-colony relatedness, and as a result, there may not be a clear division in relatedness asymmetry classes upon which workers can act. In such a situation, workers are no longer expected to produce split sex ratios.

The mode of colony founding may have profound effects on sex allocation decisions in facultatively polygynous ants. If colony founding by single, dispersing queens is extremely risky, colony budding and high levels of polygyny through queen readoption may predominate over independent colony founding. Habitat saturation and nest site availability are considered to be factors affecting degree of polygyny (Herbers 1986; Keller 1995). If budding is the dominant mode of colony reproduction in a population, and single queens emigrate from their natal colonies with a group of workers, a reasonably high level of monogyny may be encountered without there being any relatedness asymmetry in the population. In this scenario, a high degree of male bias is expected to result from local resource competition, and this is seen in the Roydon Wood population.

It may be reasonable to assume that the Aberfoyle population had no split sex ratios because there were no clearly defined relatedness asymmetry classes. It appears that local resource competition and local mate competition do not significantly affect this population. Being largely monogynous, a population sex ratio that is female-biased, but that is below 0.75 as predicted by Trivers and Hare is expected, and this is seen. Resource abundance effects may be causing the preference for female sexuals in larger, well resourced colonies. Why colony classes were not split is unclear, but may stem from rapid turnover of queens with colonies frequently changing gyny class. Heinze *et al.* (1995a) found that alate queens from polygynous colonies in the Nürnberger Reichswald in Germany were closely related enough to be full sisters. Since queens may take up to two years to develop, he suggested that monogynous colonies may produce female-biased broods under monogyny, and then adopt daughter queens so that newly polygynous colonies appear to be producing non-adaptive sex ratios. A similar scenario as this may have occurred in the Aberfoyle population, though it is purely speculative to say so. In Santon Warren, gyny-class appears to be a predictor of relatedness. Since a colony's sex investment ratio appears to reflect its gyny class and female sexual brood may take up to two years to develop (Buschinger 1967; Heinze *et al.* 1995a), colony class may be consistently more stable across years than in the other two populations. It may be that split sex ratios are a reflection of colony stability with long-lived queens and mixed modes of colony founding.

§3.5 Summary

1. Three populations of the ant *Leptothorax acervorum* produced very different population sex investment ratios. In Santon Warren, the numerical fraction of females in the population was 0.60 over three years, in Aberfoyle it was 0.71 over two years, and in Roydon Wood it was 0.13.

2. There was a strong (but insignificant) trend between the frequency of polygynous colonies in a population and the sex investment ratio of that population. Highly polygynous populations tended to invest more resources into new male-production.

3. Only one population had sex investment ratios split by colony gyny class. In Santon Warren, monogynous colonies produced female-biased sex investment ratios (0.67), while polygynous colonies produced male-biased sex investment ratios (0.32). In Aberfoyle and Roydon Wood, the colony classes did not differ in their mean sex investment ratios.

4. There was no effect of queen number on sex investment ratios in polygynous colonies. Sex investment ratios in small monogynous colonies were no closer to the queens preferred level than in larger monogynous colonies. These findings did not vary between populations. Overall, there was no evidence for partial queen control

over sex allocation in any of the populations studied.

5. There were differences in genetic structures of two populations for which relatedness data were available. In Santon Warren, monogynous colonies had higher average worker-worker relatedness than polygynous colonies (Heinze *et al.* 1995b). In Roydon Wood, there was no difference in average worker-worker relatedness between the colony classes (Hingle, unpublished). In conjunction with the sex investment ratios data, there appears to be a relationship between sex allocation and degree of relatedness within colonies. However, it is not possible to know whether workers are responding to this genetic information, because it isn't known if the degree of worker-worker relatedness is an accurate predictor of relatedness asymmetry.

6. Patterns of investment in new males and new queens with increasing investment in total sexual productivity varied between populations. In monogynous colonies from Santon Warren, new male and new queen production increased together with total sexual productivity. In polygynous colonies from Santon Warren, new queen production reduced at high levels of total sexual productivity. This suggested some degree of local resource competition stemming from reproduction through colony budding. A similar but more extreme pattern was found in Roydon Wood, where male production increased with increasing total sexual production, while only a few new queens were produced in any colony.

7. Aberfoyle's colonies increased new queen production at high levels of total sexual productivity relative to new male production. This was more likely to have stemmed from resource abundance effects than from local mate competition.

8. Sex allocation in this species appears to be consistent with worker, and not queen, control. Allocation decisions appear to be affected by colony relatedness values,

frequency of polygyny in populations, and environmental factors which vary between populations.

9. Detailed information about environmental factors such as nest site availability, habitat saturation and resource availability would allow a better understanding of how these factors affected sex allocation at the colony- as well as population-level.

Table 3.1. Population sex investment ratios (SIR) for all three populations sampled with 95% confidence limits. All sex ratios are expressed as proportion of females. Sex investment ratios were calculated from mean dry mass estimates of the sexes according to the methods of Bourke and Franks (1995, box 5.1). Colonies producing no sexuals and colonies that couldn't be classified as monogynous or polygynous were excluded (Appendix A).

population	Santon Warren	Aberfoyle	Roydon Wood
1993	0.52 (0.45-0.59) $n = 45$	*	*
1994	0.58 (0.49-0.66) $n = 40$	0.66 (0.56-0.76) $n = 30$	*
1995	0.68 (0.62-0.73) $n = 85$	0.75 (0.68-0.83) $n = 17$	0.13 (0.06-0.20) $n = 33$
pooled	0.60 (0.56-0.64) $n = 170$	0.71 (0.64-0.77) $n = 47$	0.13 (0.06-0.20) $n = 33$

Table 3.2. Percentage of polygynous colonies in each population, with harmonic mean queen numbers. n = number of colonies.

population	Santon Warren	Aberfoyle	Roydon Wood
1993	45%, 5.5 $n = 47$	*	*
1994	22%, 4.3 $n = 49$	23%, 5.8 $n = 34$	*
1995	20%, 3.8 $n = 102$	26%, 5.0 $n = 19$	69%, 3.5 $n = 36$
pooled	28%, 4.5 $n = 198$	25% 5.4 $n = 53$	69%, 3.5 $n = 36$

Table 3.3. Sex investment ratios (95% confidence limits) for monogynous and polygynous colonies in the Aberfoyle population. All sex investment ratios are expressed as proportion of investment in female sexuals. For each colony class in each year, the total number of individual new males and new queens produced is given. Sex investment ratios were calculated from mean dry mass estimates of the sexes according to the methods of Bourke and Franks (1995, box 5.1). *t*-tests compare the differences between mean sex investment ratios of monogynous and polygynous colonies in the same year.

year	1994		1995		pooled	
class	M	P	M	P	M	P
sex investment ratio	0.65	0.68	0.68	0.79	0.66	0.75
95% confidence limits	0.52-0.78	0.46-0.89	0.55-0.81	0.66-0.92	0.57-0.75	0.65-0.86
number of queens	461	272	276	624	737	896
number of males	245	148	129	187	374	335
number of colonies	23	7	12	5	35	12

year	1994	1995	pooled
t-test between M & P sex investment ratios	$t = 0.08$, d.f. = 28, $p > 0.05$	$t = 0.32$, d.f. = 15, $p > 0.05$	$t = 0.37$, d.f. = 45, $p > 0.05$

Table 3.4. Multiple regression model of sex investment ratio testing for the effects of log queen number and population (Santon Warren, Aberfoyle and Roydon Wood). * denotes the variables in the model being tested. Factors 1 through 3 in the model are: 1. log queen number (lqn), 2. population, and 3. log queen number. population (interaction). % Var gives the percentage of variance explained by the model. N/A= residual variance exceeds variance of Y variate. d.f. lost = the numbers of degrees of freedom lost by dropping the variable being tested. The subsequent *F* and *p* values are given for the result of dropping the variable. *p*-values above 0.05 are left out of the final model.

model	1	2	3	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	27.4	5	5.69	<.001				
b	*	*		25.5	3	8.08	<.001	lqn.population	2	1.73	0.185
c	*			1.0	1	1.59	0.211	population	2	8.31	<.001
d		*		26.1	2	11.96	<.001	lqn	1	0.51	0.477

Table 3.5. Multiple regression model of sex investment ratio testing for the effects of residual log queen number (rlqn) and population (pop) (Santon Warren, Aberfoyle and Roydon Wood). Residuals were calculated from the regression of log queen number on sexual productivity. The table layout for the multiple regression model is described in Table 3.4. Factors 1 through 3 in the model are: 1. residual log queen number, 2. population, and 3. residual log queen number. population (interaction).

model	1	2	3	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	24.7	5	5.07	<.001				
b	*	*		27.0	3	8.66	<.001	rlqn.pop	2	0.10	0.908
c	*			0.6	1	1.36	0.248	pop	2	8.85	<.001
d		*		26.1	2	11.96	<.001	rlqn	1	1.74	0.193

Table 3.6. A comparison of average intra-colony relatedness (±SE, where available) and sex investment ratios (95% confidence limits). *n* = number of colonies. Relatedness estimates for Santon Warren (SD) come from Heinze *et al.* (1995b), and those for Roydon Wood (RW) come from A. Hingle (unpublished).

comparison	queen-queen relatedness	worker-worker relatedness	sex investment ratio
SD monogynous	-	0.50 (±0.06) *n* = 34	0.67 (0.63-0.71) *n* = 119
SD polygynous	0.26 (±0.09) *n* = 154	0.28 (±0.09) *n* = 19	0.32 (0.25-0.40) *n* = 51
SD population	-	0.42, *n* = 53	0.60 (0.56-0.64) *n* = 170
RW monogynous	-	0.55 (±0.18) *n* = 5	0.14 (-0.08-0.35) *n* = 10
RW polygynous	0.11 (±0.10) *n* = 15	0.40 (±0.06) *n* = 15	0.12 (0.06-0.19) *n* = 23
RW population	-	0.44 (±0.06) *n* = 20	0.13 (0.06-0.20) *n* = 33

Table 3.7. Relatedness values (r) with 95% confidence limits (95%CL) in different classes of colony in the Roydon Wood population. The class 'males only' refers to colonies that produced only male sexuals, the class 'mixed brood' refers to colonies producing male and female sexuals. n = number of colonies.

colony type	males only colonies r (95%CL)	mixed brood colonies r (95%CL)	monogynous colonies r (95%CL)	polygynous colonies r (95%CL)
worker-worker relatedness	0.570 (0.259-0.881) $n = 7$	0.368 (0.235-0.501) $n = 13$	0.548 (0.040-1.056) $n = 5$	0.402 (0.280-0.524) $n = 15$
queen-queen relatedness	-0.172 (-0.633-0.289) $n = 4$	0.207 (-0.031-0.445) $n = 11$	n/a	0.106 (-0.100-0.312) $n = 15$

Table 3.8. Multiple regression models of log new queen and log new male production, testing for the effects of log total sexual productivity, colony class and population. Production data from all three populations (Santon Warren, Aberfoyle and Roydon Wood) are included. The table layout for the multiple regression models is described in Table 3.4. Factors 1 through 7 in the model are: 1. tsp (log total sexual production); 2. class (monogynous or polygynous); 3. pop (population); 4. tsp.class (interaction); 5. pop.class (interaction); 6. tsp.pop (interaction); 7. tsp.class.pop (interaction).

log new queen production

model	1	2	3	4	5	6	7	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	*	*	*	*	80.1	11	67.84	<.001				
b	*	*	*	*	*	*		79.9	9	81.60	<.001	tsp.pop.class	2	1.93	0.149
c	*	*	*	*	*			79.8	7	103.97	<.001	tsp.pop	2	1.44	0.240
d	*	*	*	*				77.7	5	128.82	<.001	pop.class	2	8.95	<.001
e	*	*	*		*			77.7	6	114.27	<.001	tsp.class	1	9.02	0.003

log new male production

model	1	2	3	4	5	6	7	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	*	*	*	*	54.5	11	20.30	<.001				
b	*	*	*	*	*	*		54.6	9	24.64	<.001	tsp.class.pop	2	0.91	0.406
c	*	*	*	*	*			54.0	7	30.73	<.001	tsp.pop	2	2.01	0.138
d	*	*	*	*				53.8	5	42.16	<.001	pop.class	2	1.51	0.224
e	*	*	*					53.8	4	52.47	<.001	tsp.class	1	0.97	0.326
f	*	*						51.2	2	93.79	<.001	pop	2	5.58	0.004
g	*		*					51.8	3	64.46	<.001	class	1	7.99	0.005

Table 3.9. Multiple regression models of log new queen and log new male production in Santon Warren, testing for the effects of log total sexual productivity, colony class and year. The table layout for the multiple regression models is described in Table 3.4. Factors 1 through 7 in the model are: 1. tsp (log total sexual production); 2. class (monogynous or polygynous); 3. year; 4. tsp.class (interaction); 5. class.year (interaction); 6. tsp.year (interaction); 7. tsp.class.year (interaction).

log new queen production

model	1	2	3	4	5	6	7	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	*	*	*	*	79.0	11	47.05	<.001				
b	*	*	*	*	*	*		79.2	9	58.10	<.001	tsp.class.year	2	0.29	0.751
c	*	*	*	*	*			79.5	7	75.88	<.001	tsp.year	2	0.01	0.991
d	*	*	*	*				79.3	5	104.51	<.001	class.year	2	1.64	0.198
e	*	*	*					77.7	4	118.68	<.001	tsp.class	1	10.33	0.002
f	*	*		*				79.5	3	175.69	<.001	year	2	0.35	0.703

log new male production

model	1	2	3	4	5	6	7	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	*	*	*	*	57.4	11	15.91	<.001				
b	*	*	*	*	*	*		58	9	19.75	<.001	tsp.class.year	2	0.09	0.918
c	*	*	*	*	*			58.4	7	25.51	<.001	tsp.year	2	0.46	0.634
d	*	*	*	*				58.2	5	34.95	<.001	class.year	2	1.36	0.261
e	*	*	*					57.6	4	42.49	<.001	tsp.class	1	2.52	0.115
f	*	*						57.0	2	81.88	<.001	year	2	1.86	0.160
g	*							53.8	1	143.35	<.001	class	1	9.21	0.003

Table 3.10. Multiple regression models of log new queen and log new male production in Aberfoyle, testing for the effects of log total sexual productivity, colony class and year. The table layout for the multiple regression models is described in Table 3.4. Factors 1 through 7 in the model are: 1. tsp (log total sexual production); 2. class (monogynous or polygynous); 3. year; 4. tsp.class (interaction); 5. class.year (interaction); 6. tsp.year (interaction); 7. tsp.class.year (interaction).

log new queen production

model	1	2	3	4	5	6	7	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	*	*	*	*	67.2	7	10.07	<.001				
b	*	*	*	*	*	*		68.4	6	12.20	<.001	tsp.class.year	1	0.06	0.802
c	*	*	*	*	*			68.7	5	14.61	<.001	tsp.year	1	0.78	0.385
d	*	*	*	*				69.4	4	18.60	<.001	class.year	1	0.39	0.538
e	*	*	*					70.5	3	25.69	<.001	tsp.class	1	0.03	0.875
f	*	*						71.4	2	39.64	<.001	year	1	0.14	0.708
g	*							72.2	1	81.43	<.001	class	1	0.15	0.699

log new male production

model	1	2	3	4	5	6	7	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	*	*	*	*	8.9	7	1.42	0.245				
b	*	*	*	*	*	*		12.7	6	1.73	0.157	tsp.class.year	1	0.00	0.979
c	*	*	*	*	*			16.2	5	2.16	0.092	tsp.year	1	0.02	0.889
d	*	*	*	*				19.1	4	2.77	0.049	class.year	1	0.10	0.749
e	*	*	*					22.0	3	3.82	0.021	tsp.class	1	0.02	0.895
f	*	*						23.4	2	5.59	0.009	year	1	0.50	0.486
g	*							25.0	1	11.00	0.002	class	1	0.41	0.527

Table 3.11. Multiple regression model of log new queen and log new male production in Roydon Wood, testing for the effects of log total sexual productivity and colony class. The table layout for the multiple regression models is described in Table 3.4. Factors in the model are: 1. tsp (log total sexual production); 2. class (monogynous or polygynous); 3. tsp.class (interaction).

log new queen production

model	1	2	3	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	3.9	3	1.20	0.351				
b	*	*		5.9	2	1.47	0.267	tsp. class	1	0.75	0.403
c	*			10.9	1	2.84	0.114	class	1	0.26	0.617

log new male production

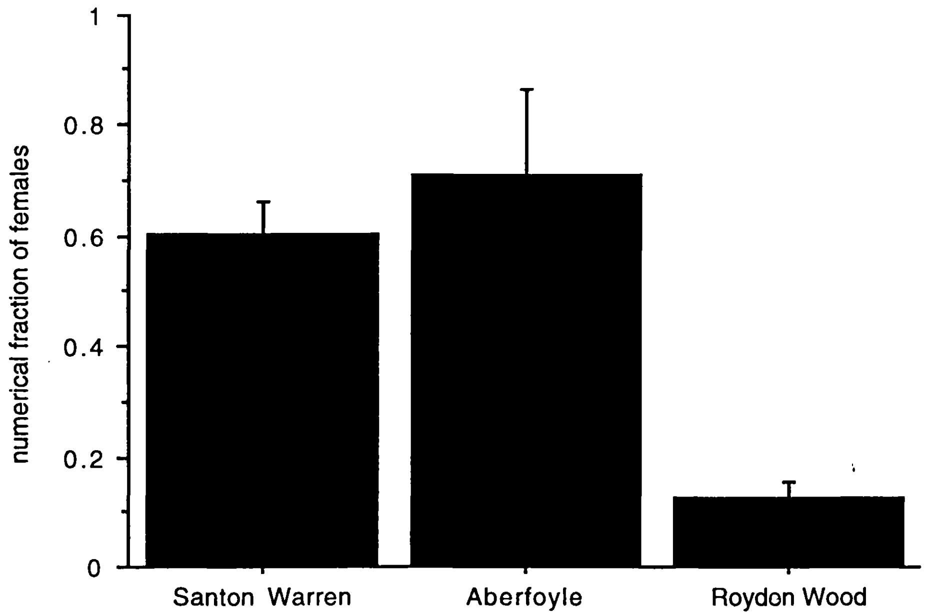
model	1	2	3	% Var	d.f.	*F*	*p*	Variable tested	d.f. lost	*F*	*p*
a	*	*	*	83.9	3	40.96	<.001				
b	*	*		82.9	2	56.78	<.001	tsp. class	1	2.17	0.156
c	*			82.3	1	107.67	<.001	class	1	1.76	0.198

Table 3.12. Summary of the main parameters relating to sex allocation in each study population.

population	Santon Warren	Aberfoyle	Roydon Wood
% polygynous colonies	28	25	69
harmonic mean queen number	4.5	6.5 (5.4 excluding two very large colonies)	3.5
population sex investment ratio (SIR) measured as proportion of females	0.60	0.71	0.13
class-specific sex investment ratio	M = 0.67, P = 0.32 significant diff.	M = 0.66, P = 0.75 non-significant diff.	M = 0.14, P = 0.12 non-significant diff.
effect of queen number on SIR	none	none	none
within-colony relatedness	W-W = 0.42 Q-Q = 0.26	n/a	W-W = 0.44 Q-Q = 0.11
class-specific relatedness between workers	M = 0.50, P = 0.28 significant diff.	n/a	M = 0.55, P = 0.40 non-significant diff.
SIR and total sexual production	Mono: NQ⬀ = NM⬀ Poly: NM⬀ > NQ⬀	M & P: NQ⬀ > NM⬀	M & P: NM⬀, NQ (non-signif)

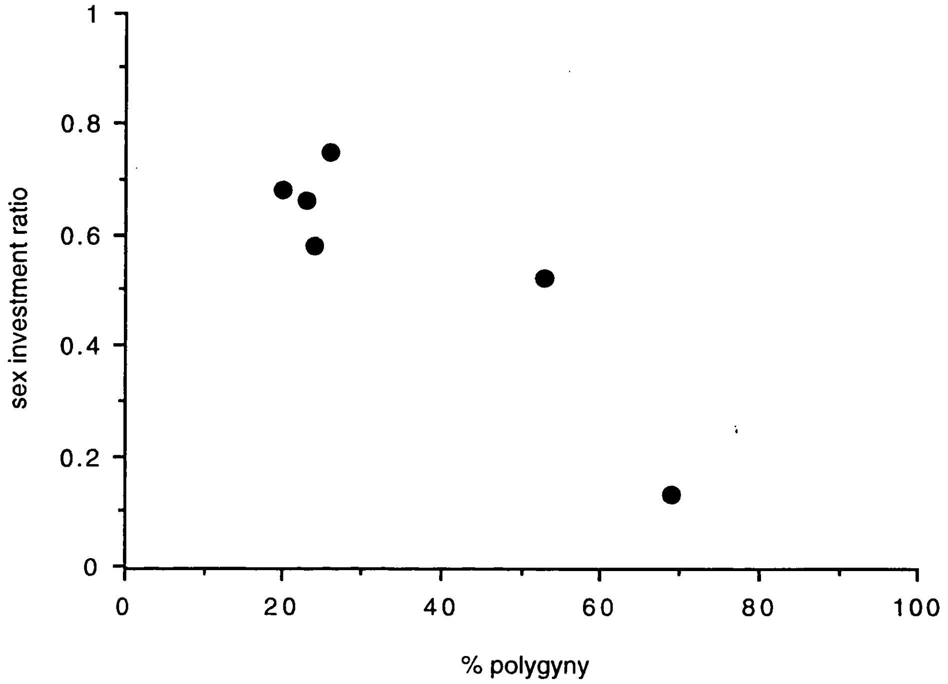
M = monogynous colonies, P = polygynous colonies, W = worker, Q = dealate queen, NQ = new queens, NM = new males,
⬀ = increased significantly as SIR increased, > indicates a significantly greater rate of increase, = indicates do difference.

Figure 3.1. Population mean sex investment ratios for the three study populations.



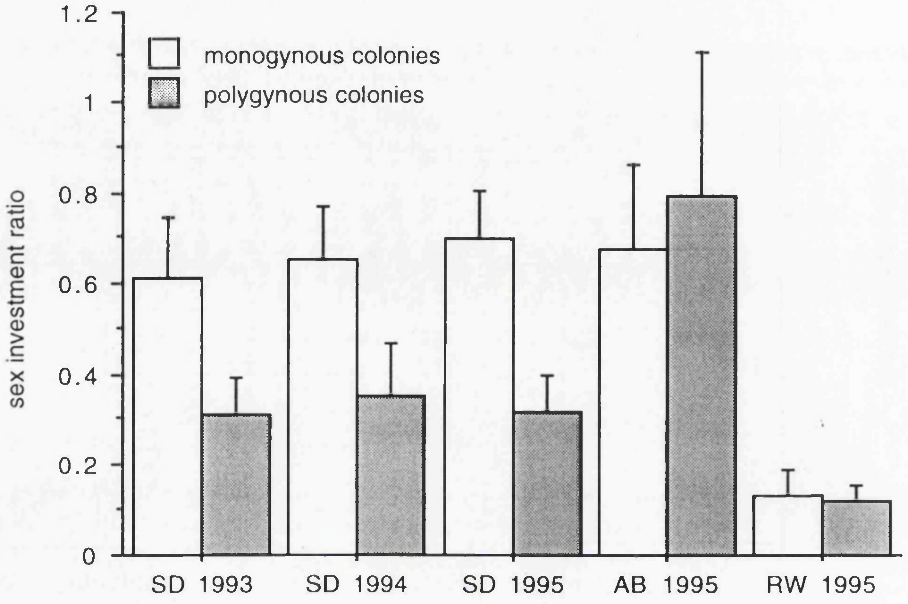
Mean sex investment ratios for each population pooled across years. Bars indicate one standard error. Sex investment ratios are measured as numerical fraction of females. Sample sizes for each population were 170 colonies (Santon Warren), 47 colonies (Aberfoyle), 33 colonies (Roydon Wood) (Appendix A).

Figure 3.2. Change in population sex investment ratio (numerical fraction of females) with the degree of polygyny in all three populations.



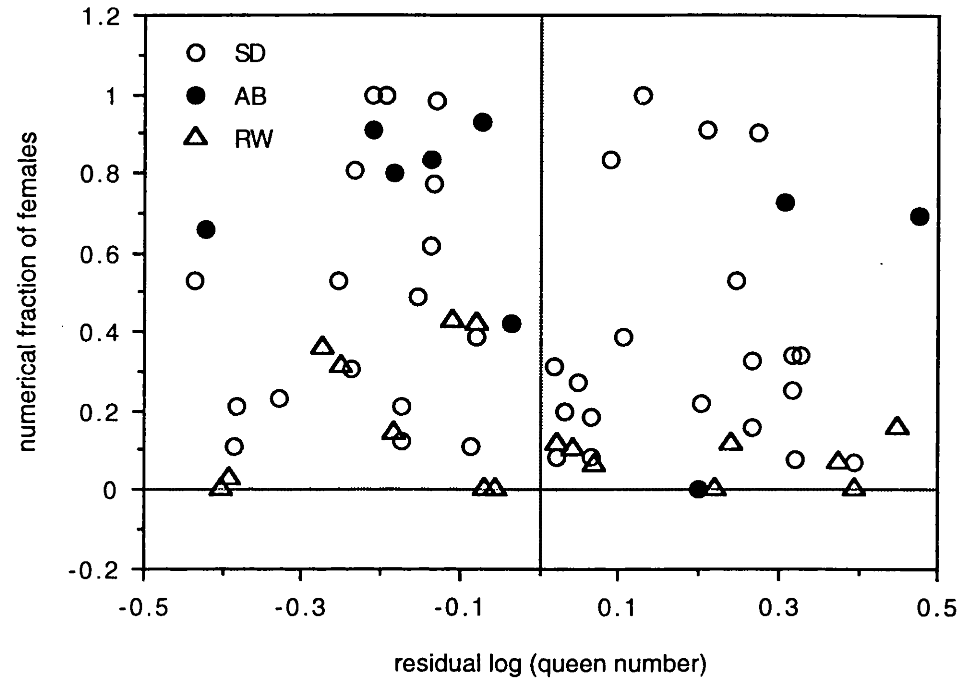
Plot of population sex investment ratio (numerical fraction of females) with the degree of polygyny for each year within populations. % polygyny refers to the percentage of polygynous colonies out of all colonies within a population. The trend proved non-significant (Spearman rank correlation: $r_S = -0.66$, $n = 6$, $p > 0.05$).

Figure 3.3. Sex investment ratios divided by class for each year and population sampled.



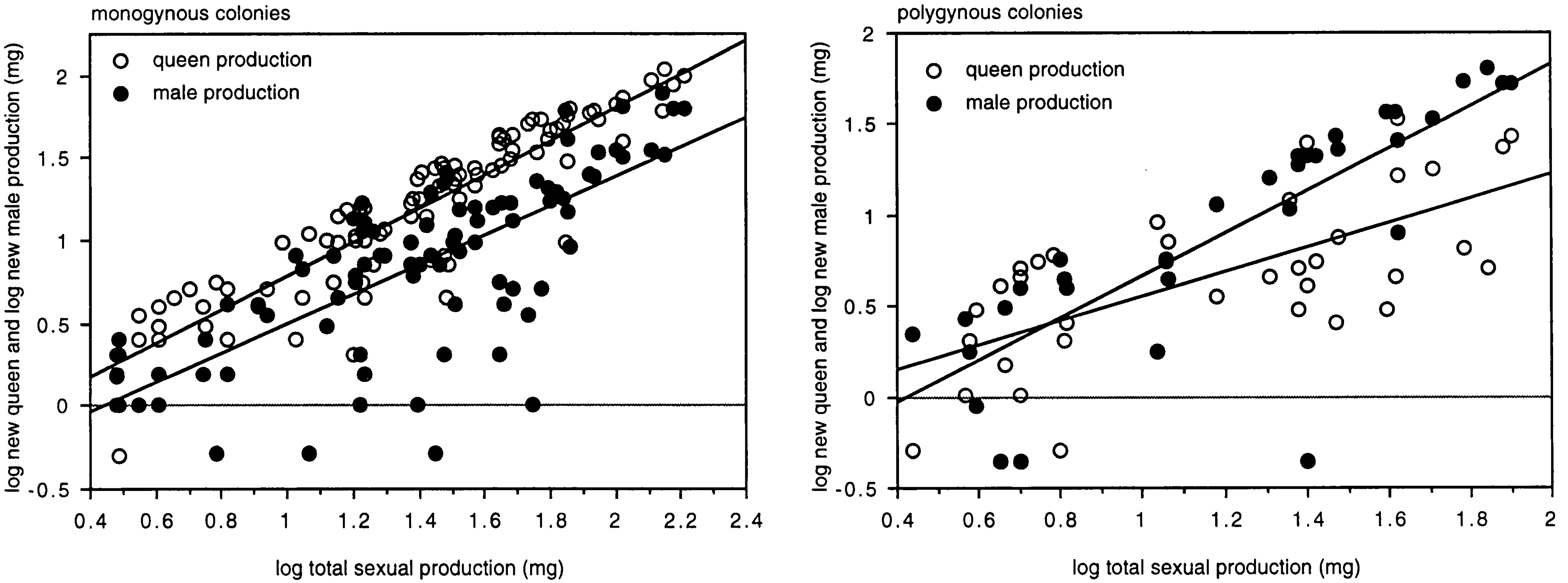
Sex investment ratios measured as numerical fraction of females. SD = Santon Warren, AB = Aberfoyle, RW = Roydon Wood. Number of colonies in each sample group were: SD 1993 M = 21, P = 24; SD 1994 M = 31, P = 9; SD 1995 M = 67, P = 18; AB 1994 M = 23, P = 7; AB 1995 M = 12, P = 5; RW 1995 M = 10, P = 23.

Figure 3.4. Plot of sex investment ratio against residual log queen number in polygynous colonies.



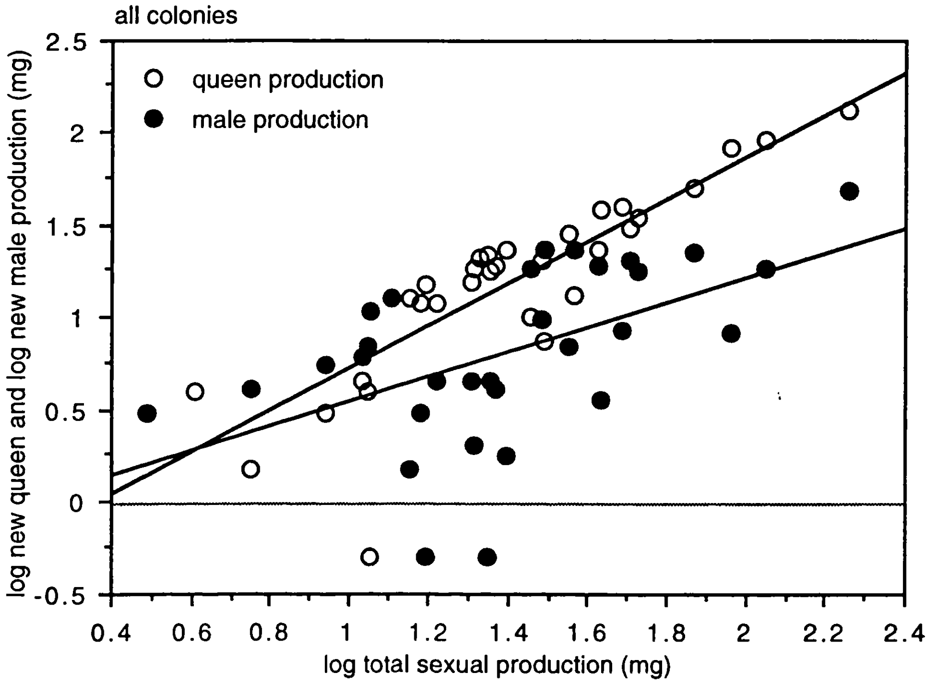
Residuals were taken from the regression of log queen number on log total sexual production. The regression proved non-significant (Table 3.5), and there was no effect of population or year. There was also no significant regression of sex investment ratio on log queen number (Table 3.4) with no effect of population or year. This analysis omitted colonies producing five or fewer sexuals (Appendix A).

Figure 3.5. Relations between new male and new queen production and total sexual production in monogynous and polygynous colonies of *Leptothorax acervorum* from Santon Warren, in terms of biomass produced.



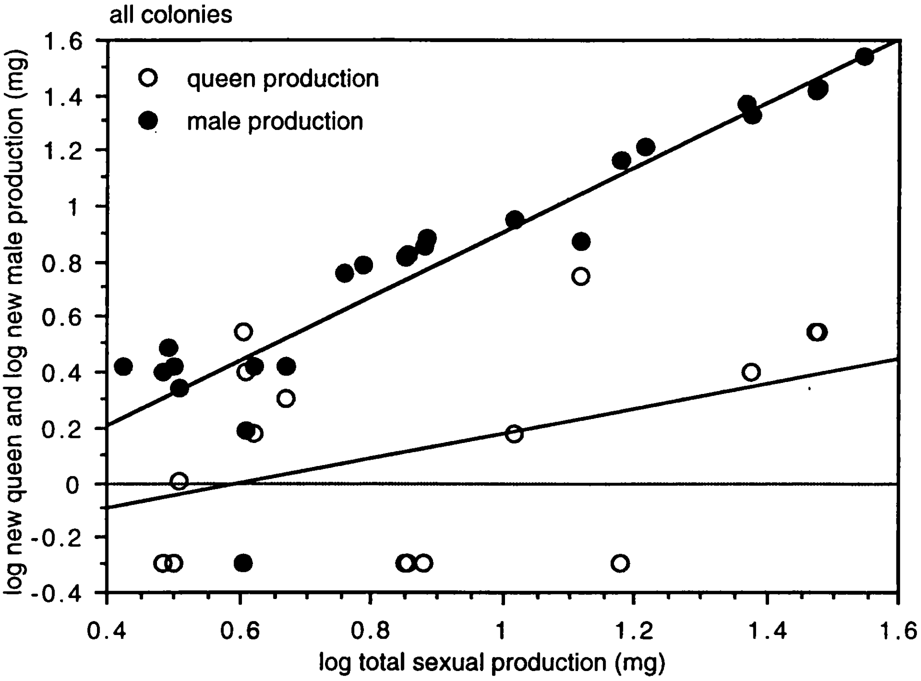
log new male production (mg) and log new queen production (mg) were regressed on log total sexual production (mg) for monogynous and polygynous colonies separately. Data were pooled for all three years. In monogynous colonies, the regressions were both significant (males: $b = 0.89$, $F_{1,87} = 101.19$, $p < 0.0001$; queens: $b = 1.01$, $F_{1,97} = 504.85$, $p < 0.0001$) and there was no difference between the gradients of the regression lines ($F_{1,184} = 1.70$, $p > 0.05$). In polygynous colonies the regressions were both significant (males: $b = 1.16$, $F_{1,32} = 66.23$, $p < 0.0001$; queens: $b = 0.67$, $F_{1,35} = 30.80$, $p < 0.0001$), but the gradient for new male production was significantly steeper than for new queen production ($F_{1,67} = 7.03$, $p < 0.05$).

Figure 3.6. Relations between new male and new queen production and total sexual production in monogynous and polygynous colonies from Aberfoyle, in terms of biomass produced.



log new male production (mg) and log new queen production (mg) were regressed on log total sexual production (mg). Data were pooled across both years and classes (Table 3.10). Both regressions were significant (log new male production: $b = 0.66$, $F_{1,29} = 11.00$, $p = 0.002$; log new queen production: $b = 1.14$, $F_{1,30} = 81.43$, $p < 0.001$), and there was a significant difference between the gradients of the regression lines ($F_{1,59} = 13.13$, $p < 0.001$).

Figure 3.7. Relations between new male and new queen production and total sexual production in monogynous and polygynous colonies from Roydon Wood, in terms of biomass produced.



log new male production (mg) and log new queen production (mg) were regressed on log total sexual production (mg), pooled across monogynous and polygynous colonies (Table 3.11). The regression of log new male production was significant ($b = 1.16$, $F_{1,22} = 107.67$, $p < 0.001$), and the regression of log new queen production was non-significant ($F_{1,14} = 2.84$, $p > 0.05$)

Chapter 4

RESOURCE ALLOCATION IN *LEPTOTHORAX ACERVORUM*

§4.1 Introduction

An important life history trade-off that all organisms must consider is between 'present' and 'expectation of future' reproduction (Stearns 1992); Williams (1966) termed this 'the cost of reproduction'. Over-investment in an individual's current reproductive effort may reduce its own fitness by decreasing its potential for future reproduction. Social insect colonies must divide their resources between current investment in sexual males and females, and new workers that will ensure the colony survives to produce sexuals in future broods.

Based on Hamilton's (1964) inclusive fitness theory, Trivers (1974) considered the conflict of interests between parents and offspring over how resources should be optimally allocated to brood. Specifically, an individual controlling distribution of resources can maximise its own fitness by allocating resources to individuals according to its relatedness to them. Resource allocation in social insects is particularly interesting because of the unusual patterns of relatedness stemming from haplodiploid sex-determination (Trivers and Hare 1976). The study of parent-offspring conflict over resource allocation in social Hymenoptera (ants, bees and wasps) has extensively covered how resources are allocated to male and female sexuals, and theoretical predictions have generally been supported by numerous field studies (Trivers and Hare 1976; Bourke and Franks 1995; Crozier and Pamilo 1996). However, few studies have considered how resources are allocated between colony maintenance (production of new workers) and sexual output.

In a perennial colony headed by one singly-mated queen where workers are sterile, Pamilo (1991a) predicted that there should be queen-worker conflict over allocation of resources to new worker and sexual fractions. Queens are related to both male and female grand-offspring equally (relatedness = 1/4), whereas workers are related to their nephews by $r = 1/8$ and to nieces by $r = 3/8$. Thus, he argued that workers value female reproductives more than queens do, and should prefer to have diploid brood develop into new queens, whereas queens prefer a higher proportion of diploid brood to develop into new workers. Another way of looking at this is that a queen wants herself to remain a reproductive individual as long as possible to maximise her fitness returns because she is more closely related to herself ($r = 1$) than to her reproductive offspring ($r = 0.5$). Workers are less related to their queen ($r = 0.5$) than the queen is to herself, so their priority to maintain her as their source of fitness is less than hers because of another source of fitness, namely sexual brood. Pamilo (1991a: p. 92) argued that "workers prefer investing in highly related sisters." While it is true that workers are more closely related to sisters ($r = 0.75$) than to their mother ($r = 0.5$), from a sterile worker's point of view the queen, who carries the worker's paternal genes as sperm, is the progenitor of all the worker's genes as well as all her sibs' genes (Bourke and Franks 1995). Thus, workers should be indifferent to mothers and sibs as a source of inclusive fitness, while the queen should prefer to invest in colony maintenance more strongly than workers do.

Pamilo's (1991a) model also predicts that in species which readopt daughter queens into the natal colony, the degree of queen-worker conflict over resource allocation varies according to two parameters besides genetic relatedness: colony survivorship and the probability of independent nest-founding success. Large colonies are considered more likely than small colonies to survive to breed again in the future, and have lower maintenance costs (Pamilo 1991a; Sundström 1995). Therefore, colony size is taken as a measure of probability of survival. Both workers and queens are predicted to invest more resources into new sexuals in large colonies because their natal colony already represents a secure source of future fitness. If dispersing alate queens are unlikely to establish new colonies independently, the nest becomes a more important source of inclusive fitness to workers. Under these conditions, a more robust strategy for both workers and queens is to produce a small number of new queens for readoption (and possibly some for dispersal depending on environmental conditions), and to concentrate on worker production, both to ensure colony survival, and to stock daughter colonies through budding, or fission (Pamilo 1990; Crozier and Pamilo 1996). If dispersing queens are likely to found new colonies independently, queen-worker conflict over resource allocation is expected to be strong in smaller colonies. A queen in a small colony will increase investment into new sexuals only as colony size (and therefore survivorship) increases. Workers in small colonies will prefer a heavy investment in new sexuals because they see the natal colony as a relatively unimportant source of fitness (compared with the queen). The natal colony becomes a less risky form of fitness-investment in larger colonies, and workers are expected to increase their investment in new workers with increasing colony size. When colony size becomes large enough that its survival is relatively certain, workers are again expected to increase investment in new sexuals. Thus, a falling percentage of sexual brood with increasing colony size is expected only under worker control of resource allocation, and this should occur in small colonies when dispersing solitary queens are likely to found new nests. This would result in a negative partial correlation of percentage of sexual brood and number of workers, with total sexual productivity controlled for. A rising percentage of sexual brood (seen as a corresponding positive partial correlation) does not differentiate between worker or queen control of resource allocation.

Several studies have previously attempted to test Pamilo's (1991a) model of resource allocation to workers and sexuals. Sundström (1995) examined resource allocation in separate monogynous (single-queened colonies) and polygynous (multiply-queened colonies) populations of the ant *Formica truncorum*. Her results were strongly supportive of worker control of resource allocation as predicted by Pamilo's model. Specifically, the percentage of sexual brood was found to decrease with increasing colony size in monogynous but not polygynous colonies. Polygynous colonies not only have a higher average survival probability due to their being larger than monogynous colonies, but queen-worker conflict over allocation of resources to brood is reduced because of lower intra-colony relatedness between individuals (Boomsma and Grafen 1990; Pamilo 1991a; Sundström 1995). Sexual and worker production were estimated using mark-recapture of brood, and may not represent true estimates of the mass of brood produced. This would be true especially if there were temporal differences in the development of different castes.

Backus (1995) examined patterns of resource allocation in the ant *Leptothorax longispinosus*. Nests were collected and fully censused for queens, workers and new brood production. However, this species is known to be polydomous (single colonies occupying multiple nest-sites) and this study was limited to examining resource ratios for individual nests only. Thus, census figures did not accurately reflect a cohesive unit of brood production. Instead, individual nests may only have reflected colony-level resource allocation patterns if they represented the majority of a colony's queens, brood and worker force, and if the ratio of male : female : new worker brood was equal in all nests within a colony. Backus' (1995: p. 777) justification for examining nest-level allocation was that "Herbers (1990) shows that this is an appropriate level for questions of sex allocation in this species" (i.e. *L. longispinosus*). However, Herbers (1990: p. 182) claimed that nests often represent colony fractions, and could "draw no conclusions about colony-level sex allocation". Banschbach and Herbers (1996a, b) examined resource allocation in *Myrmica punctiventris*. Using contextual analysis, they found a significant effect of the number of workers in a nest on the proportion of resources allocated to new workers. The effect of changing colony size on percentage of sexual / new workers in the brood was not examined. Furthermore, *M. punctiventris* is also a polydomous species, so resource allocation patterns at the colony level may have been difficult to assess accurately.

Leptothorax acervorum is an ideal species for the study of resource allocation. First, colonies are monodomous (single colonies occupy single nests: Chan and Bourke 1994), so whole colonies (minus a fraction of foraging workers) can be collected and examined. Second, colonies are small enough so that a large number of colonies can be entirely censused, giving an idea of population-wide patterns of resource allocation. This species is facultatively polygynous; thus, there is variation in queen-number between colonies. Resource allocation is expected to be affected by variation in intra-colony relatedness (stemming from differing numbers of reproductive queens: Pamilo 1991a; Sundström 1995), and the effects of colony class (monogynous and polygynous) on resource allocation are examined here. Sex allocation field studies have typically supported sex allocation theory at the population level. Recent studies have shown that patterns are interesting and variable at both population and colony levels (e.g. Sundström 1994a; Chan and Bourke 1994; Evans 1995). Thus, accurate censusing of entire colonies is necessary to understand resource allocation at both the colony and population levels. Pamilo's (1991a) model specifically predicts resource allocation within colonies to be sensitive to both within-colony relatedness and colony size. These predictions are easily testable in *L. acervorum*.

This study found that three populations of the ant *Leptothorax acervorum* allocated resources to sexuals and workers in different ways. Colony size was important in the ratio of sexuals to workers produced by colonies, and there was also likely to be an effect of intra-colony relatedness. In this species, colony-budding appears to be an important mode of reproduction in polygynous colonies with dispersal of sexuals in both classes of colony (Chan and Bourke 1994). Inter-population differences in the likelihood of independent nest-founding success appears to affect the proportion of resources allocated to new workers to stock daughter colony buds. Local resource competition appears to cause sex ratios to tend towards male-bias.

§4.2 Materials and methods

Study species and populations

Leptothorax acervorum is a facultatively polygynous ant species commonly found throughout temperate coniferous forests, boreal and alpine regions of the British Isles and Northern and Central Eurasia (Collingwood 1979, Heinze *et al.* 1995b). Polygyny occurs secondarily through readoption of related nestmate queens (Douwes *et al.* 1987; Stille *et al.* 1991; Heinze *et al.* 1995b). Each colony typically contains between several dozen and hundreds of workers, and there are rarely more than ten mated queens per colony. Whole, single colonies are found inside cavities in rotting twigs, under bark, or occasionally in the roots of dead trees and under stones. Collection of whole colonies (minus foraging workers) from a single site (monodomy) allows an accurate census of all adults and brood in every colony collected.

Three separate populations of *L. acervorum* were sampled between 1993 and 1995. One study population from Santon Warren, Thetford Forest, Norfolk, U.K. was sampled over three consecutive years (see also Bourke 1991, 1993; Chan and Bourke 1994). Another population collected from Aberfoyle in Strathclyde, Scotland was sampled in 1994 and 1995. A population from Roydon Wood in the New Forest, Hampshire, U.K. was sampled in 1995.

Collection and culture of colonies

From Santon Warren, 47 colonies were collected on 17 June 1993, 49 colonies were collected on 8 June 1994 and 102 colonies were collected over two days on June 13 and 20, 1995 (Appendix A). The Aberfoyle population was sampled on 25 May, 1994 and 23 May, 1995; respectively, 34 and 19 colonies were collected (Appendix A). From Roydon Wood, 36 colonies were collected on 4 June, 1995 (Appendix A). Sampling of populations was carried out before July so that no brood had yet eclosed into adults (Chan and Bourke 1994).

Collection of ants in the field involved breaking open dead twigs to find whole colonies. Random sampling of populations was assumed as all colonies found were collected, and all collection within a population took place in the same general region (including across-year sampling). Twigs containing colonies were then placed inside plastic bags and returned to the laboratory. Colonies were removed from twigs, aspirated and placed inside standard nests, and maintained in the laboratory based on the methods of Bourke (1991). Standard nests were made of two rectangular glass slides separated by a cardboard border with an opening in one end. Nests were kept inside clear plastic dishes (10 x 10 x 2 cm) under natural lighting and at room temperature. Colonies were fed on a regime of water (damp cotton wool), sugar solution and mealworm larvae, renewed every 2-3 days.

Census of colonies

Immediately after collection, colonies were censused for the first time. All adults and pupae were counted from each colony by transferring individuals using fine forceps allowing an accurate measure of each colony's composition. Colonies were maintained in the laboratory until all pupae had eclosed into adults before being censused for a second time for all individuals present. Comparison of the first with the second census gave each colony's annual production of new workers, new queens and new males on top of its original composition (Appendix A).

All mated colony queens had shed their wings (dealate). Some alate (winged) virgins may have shed their wings before the first census, but after collection from the field. During the second census, all dealate queens were removed for ovarian dissection according to the methods of Bourke (1991) to establish reproductive status. Mated queens have spermathecæ (sperm receptacles) visibly full of sperm (under a compound microscope), while virgin queens have clear spermathecæ. Other dissection data collected included number of yolky eggs present out of total number of eggs present, and approximate ovariole length was recorded (length categories ranged from very small through small, small-medium, medium, medium-large, large, very large and very-very-large). Corpora lutea (residual ovarian structures, by-products of egg production) were also counted. In cases where ovarian dissections were only partially complete, but the spermatheca could not be found, these other data could be used to establish whether a queen had been mated or not. Mated queens were counted as colony queens while non-mated queens were taken to be newly eclosed and thus were counted as part of the new queen fraction. This was justified because A.F.G. Bourke (unpublished data) found that of 72 queens collected from Santon Warren between April and May (1990-1991), all were mated (reported in Chan and Bourke 1994). Furthermore, A.F.G. Bourke (unpublished data) found that *L. acervorum* sexuals from Santon Warren do not mate in the natal colony (reported in Chan and Bourke 1994).

In total, from three different populations, 287 colonies were collected containing 22 322 workers and 1 064 queens, the latter of which were all dissected. These colonies produced 33 955 new brood including workers, queens and males (Appendix A).

Genetic composition of the populations

The Santon Warren population was studied genetically by Heinze *et al.* (1995b) using allozyme analysis of two polymorphic loci on samples collected in 1991 and 1992. The Roydon Wood population was studied genetically by A. Hingle (unpublished). Within-colony relatedness was examined in 20 colonies using five polymorphic allozyme loci. The results of these studies are given in Tables 3.5 and 3.6, and are discussed in the Results section §3.3.5. The genetic data suggested that inbreeding was absent from the Santon Warren population (Heinze *et al.* 1995b), and low worker egg-laying rates imply that worker male production was negligible (Bourke 1991).

L. acervorum males almost certainly mate both in large swarms and near nests (Bourke and Heinze 1994). The lack of evidence for inbreeding suggests that males disperse widely enough to render local mate competition among them unlikely. However, there is strong evidence that polygynous *L. acervorum* colonies reproduce by budding (see, for example, Stille and Stille 1993; Bourke and Heinze 1994; Chan and Bourke 1994), suggesting that local resource competition due to budding is probable (see Chan and Bourke 1994).

Estimation of mean dry weights

Samples of callow workers, alate queens and males were taken from several monogynous and polygynous colonies from Santon Warren in all three years. [Appendix B, Table B.1]. These were dried in an oven for 24 h, at 60 °C, and each ant individually weighed on an electronic microbalance. Thorax length was measured using a dissection microscope attached to a computer-based image analysis system (NIH Image). Using a basic thorax length-dry weight comparison, the dry weight for a larger sample of ants could be estimated where only the thorax was available for measurements. Samples of all three castes of ant from several monogynous and polygynous colonies in 1994 and 1995 were measured for thorax length using this technique (except in polygynous colonies in 1995 where sexuals were unavailable for measurement).

§4.3 Results

§4.3.1 Colony production schedules

Colony size

In all cases, polygynous colonies were larger than monogynous colonies in the same year and population (Figure 4.1) (sign test: $n = 6, p = 0.016$; for sample sizes of six sample groups, only a perfect score of six similar signs is significant). Polygynous colonies from Santon Warren had significantly larger worker forces than monogynous colonies but there was significant variation in colony size between years (Tables 4.1, 4.2, 4.3 and 4.7). In the Aberfoyle populations, polygynous colonies were significantly larger than monogynous colonies in both years (Tables 4.4, 4.5 and 4.7). There was no significant difference in worker number between colony classes in the Roydon Wood population (Tables 4.6 and 4.7).

New worker production

Polygynous colonies always produced a greater mass of new workers than monogynous colonies in the same year and population (sign test: $n = 6, p = 0.016$; Figure 4.2). For both Santon Warren (Tables 4.1, 4.2, 4.3 and 4.7) and Aberfoyle (Tables 4.4, 4.5 and 4.7) populations the difference was significant overall, though not always within years, while the difference was negligible in the Roydon Wood population (Tables 4.6 and 4.7).

Total sexual production

There was considerable variation in colonies' sexual productivity (Figure 4.3). Overall, monogynous colonies from Santon Warren produced a significantly greater mass of sexual brood than polygynous colonies (Table 4.7), though there was an insignificant reversal of the trend in 1994 (Table 4.2). There was also a significant effect of year on sexual productivity in both Santon Warren and Aberfoyle populations

(Table 4.7), suggesting that colonies are flexible in their production of sexuals from year to year. Overall in the Aberfoyle populations, polygynous colonies produced a greater mass of sexuals than monogynous colonies (Tables 4.4, 4.5 and 4.7). There was no significant difference in the biomass of sexuals produced by colony classes in Roydon Wood (Tables 4.6 and 4.7).

Total production

In both Santon Warren (Tables 4.1, 4.2, 4.3 and 4.7; Figure 4.4) and Roydon Wood (Tables 4.6 and 4.7; Figure 4.4) populations, there was no overall difference in the total mass of brood produced by both colony classes, although there was significant variation between years in total production of brood in Santon Warren populations (Table 4.7). Polygynous colonies from Aberfoyle produced many more brood than monogynous colonies (Tables 4.4, 4.5 and 4.7; Figure 4.4).

Percent of sexual brood

Monogynous colonies always produced a higher fraction of sexuals in their broods than polygynous colonies (sign test: $n = 6$, $p = 0.016$; Figure 4.5). Overall, this difference was significant in Santon Warren's populations (significant difference in two out of three years; Tables 4.1, 4.2, 4.3 and 4.7), and there was considerable variation between years. Neither Aberfoyle's (Tables 4.4, 4.5 and 4.7) nor Roydon Wood's (Tables 4.6 and 4.7) populations showed a significant class difference in percentage of sexuals in their broods, although there was a strong trend in Aberfoyle in 1994 (Table 4.4).

Per capita total sexual production

Per capita production refers to the mean productivity of workers in a colony, calculated as the colony's total productivity divided by the number of old workers. In five out of six sample groups monogynous colonies had higher per capita production of sexuals than polygynous colonies, while the sixth sample group had exactly equal per capita sexual production values for the two classes (Figure 4.6) (sample group refers to all colonies and individuals collected in one area in one season, while population refers to a collection region). In the Santon Warren population the overall difference was significant (Tables 4.1, 4.2, 4.3 and 4.7), but the difference was not significant in Aberfoyle (Tables 4.4, 4.5 and 4.7) or Roydon Wood populations (Tables 4.6 and 4.7).

Per capita new worker production

Per capita new worker production was greater in polygynous than monogynous colonies in five out of six sample groups, and exactly equal in the other (Figure 4.7). This difference was significant for Santon Warren sample groups (Tables 4.1, 4.2, 4.3 and 4.7), with significant variation between years (Table 4.7), although the difference was not always significant within years. The difference was also significant overall for Aberfoyle sample groups (though significant within years in one out of two cases; Tables 4.4, 4.5 and 4.7), but not for the Roydon Wood population (Tables 4.6 and 4.7).

Per capita total production

In only one sample group [Aberfoyle 1994] was there a significant class-difference in per capita total production (Table 4.7; Figure 4.8). Santon Warren sample groups had significant variation between years (Table 4.7), and monogynous colonies all had higher (but non-significant) per capita total productivity (Tables 4.1, 4.2, 4.3 and 4.7). Polygynous colonies from Aberfoyle produced more total brood per capita than monogynous colonies and the difference was significant in one out of two years (Tables 4.4, 4.5 and 4.7). Roydon Wood's monogynous and polygynous colonies produced similar total productivities per worker (Tables 4.6 and 4.7).

§4.3.2 Productivity and colony size

Regression models were carried out for total new worker and new sexual production as well as per capita production values with colony size, year and colony-class as parameters in the models. Separate regression models were carried out for each population. Population was not included in the models because there were three years data for one population, two years for another and one year for the third. Multiple regression models with population included as a factor were considered misleading as they generalised about annual trends in populations for which data were not actually collected [i.e. Aberfoyle 1993; Roydon Wood 1993, 1994].

There was an increase in total production of brood with increasing colony size in all classes of all populations (Table 4.10). Similarly, an increasing number of sexual brood were produced in increasingly larger colonies in all classes of all populations (Table 4.8). However, new worker production was found to increase with colony size in some, but not all study groups (Table 4.9). In both Aberfoyle and Santon Warren, all polygynous colonies produced more new workers as colony size increased, and there was no difference between years (Table 4.9). Monogynous colonies from Santon Warren increased production of new workers with colony size, and this was significant in two out of three years (Table 4.9). Aberfoyle's monogynous colonies decreased production of new workers with colony size, though this trend was not significant (Table 4.9). In Roydon Wood, there was no effect of colony class or colony size on new worker production (Table 4.9).

§4.3.3 Per capita productivity and colony size

Overall, there was no effect of colony size on the per capita production of new sexuals in any of the populations studied (Table 4.11). Per capita production of sexuals decreased with increasing colony size in the Roydon Wood population, but the change was insignificant (Table 4.11). In all cases, per capita new worker production fell with increasing colony size, and the difference was significant in all study groups except monogynous colonies in Santon 1993 and polygynous colonies in Aberfoyle (Table 4.13). Per capita total productivity declined with increasing colony size (Table 4.15) as a result of the corresponding decline in per capita sexual production (Table 4.13). The trend was significant in those colonies for which the decreasing per capita sexual productivity was also significant, except in monogynous colonies from Santon 1995 (regression of per capita total production on colony size: $F_{1,53} = 3.07$, $p = 0.09$).

These trends were examined further by looking at partial correlations between colony size and per capita production of new sexuals and workers controlling for total colony productivity. In 5/6 sample groups, monogynous colonies showed a negative relationship between per capita sexual production and colony size when controlling for total productivity (Table 4.12). This trend was significant for all three Santon Warren seasons. Similarly, in 6/6 sample groups, polygynous colonies showed a negative association between per capita sexual production and colony size, controlling for total productivity (Table 4.12). This was significant in two groups: Santon Warren in 1993 and Roydon Wood. The partial correlation between per capita new worker production and colony size found a negative relationship in all classes in all populations (Table 4.14). The trend was significant in 5/6 monogynous sample groups and 4/6 polygynous sample groups.

§4.3.4 Allocation to sexuals and workers with colony size

A regression of percentage of sexuals in the brood with colony size was carried out.

There was no relationship between colony size and percentage of sexuals in the brood for colonies from Roydon Wood (Table 4.16). Both monogynous and polygynous colonies also showed non-significant partial correlations between percentage of sexuals in the brood and colony size when controlling for total productivity of colonies (Table 4.17). Both colony classes from Santon Warren produced a higher fraction of sexuals in their brood at larger colonies sizes, and the change was more pronounced in monogynous colonies (Table 4.16). When controlling for the effects of total colony productivity, the partial correlation between percentage of sexuals in the brood and colony size was significant in only monogynous colonies in 1994 (Table 4.17). However, as no year effect was found in the regression model (Table 4.16), partial correlations were examined pooling for years. Across all three years, there were significant positive partial correlations between old worker number and percentage of sexuals in the brood (controlling for total productivity) for both monogynous and polygynous colonies (Table 4.17). In the Aberfoyle population, monogynous colonies significantly increased allocation to sexuals with increasing colony size (Table 4.16), and this was supported when controlling for total productivity using partial correlation pooling across years (Table 4.17). However, polygynous colonies did not increase the proportion of sexuals in their brood with colony size (Table 4.16). When controlling for the effects of total productivity, the partial correlation between colony size and percentage of sexuals in the brood was positive and nearly significant when pooling across years (Table 4.17).

§4.4 Discussion

There was considerable variation in resource allocation strategies between monogynous and polygynous colonies within years, between years and between populations. However, certain trends were common to both colony-classes in every year and population. As colony size increased, all colonies produced more sexual brood and therefore the total amount of brood produced increased with colony size. Large colonies were also found to produce more brood by Sundström (1995) and

Backus (1995). New worker production did not always increase with colony size. There was no significant change in per capita production of sexuals with increasing colony size in any class or population group. When per capita sexual production was examined controlling for total productivity, there was a negative relationship with colony size in 11 out of 12 study groups. In general, sexual production increases with colony size, but efficiency in producing the sexuals decreases in larger colonies. This reduction in efficiency of brood production also extended to new worker brood. Per capita production of new workers always fell with increasing colony size, although not always significantly. When the effects of total productivity were controlled for, there was always a negative relationship between per capita new worker production and colony size, and this was significant in 9 out of 12 cases. This showed that larger colonies produce fewer new brood per adult worker than smaller colonies when controlling for productivity effects. Sundström (1995) found a similar trend in separate populations of monogynous and polygynous ants.

The Roydon Wood population showed no differences between monogynous and polygynous colonies in any variable examined, except for colony queen number. The colony-classes were of similar sizes and produced similar mean overall numbers of new workers and new sexuals as well as per capita production values. There was also no difference in percentage of resources allocated to sexuals in the brood. Despite differences in queen number between colonies (Appendix A), polygynous colonies from this population were not found to have significantly different intra-colony relatedness values to monogynous colonies, nor was there splitting of sex ratios between the classes (Chapter 3). Sundström's (1995) study supported the prediction that lower relatedness asymmetry in polygynous colonies results in reduced queen-worker conflict over resource allocation. Furthermore, Pamilo's (1991a) model predicts that resource allocation decisions are affected by an index of colony survivorship; a function of colony size (also see Sundström 1995). As there was no significant difference in colony size or intra-colony relatedness between monogynous and polygynous colonies in Roydon Wood, the classes were not expected to differ in resource allocation strategies. Pamilo (1990; also see Crozier and Pamilo 1996) showed that when the success of dispersing queens was very low, colonies should produce only enough females to recruit back into the natal colony, and produce male-sexuals thereafter. These colonies should also concentrate on the production of new workers to maintain the colony and stock daughter colonies formed by budding. The resource allocation patterns in this population match those predicted by Pamilo when dispersion is risky. Possible reasons for this include habitat saturation and nest-site limitation (Keller 1995). Nest-site availability may have affected maximum nest-size that could be attained in this population resulting in monogynous and polygynous colonies showing no mean size difference.

The colony classes differed in their resource allocation patterns in both Santon Warren and Aberfoyle populations. In both, polygynous colonies were larger than monogynous colonies. Aberfoyle's polygynous colonies produced many more brood than monogynous colonies, both sexuals and new workers. Overall mean colony productivity did not vary between colony classes in the Santon Warren populations. Here, monogynous colonies concentrated on sexual production while polygynous colonies concentrated on new worker production. This difference is reflected in the class-mean percentage of sexuals in the brood; monogynous colonies produced a significantly more sexual biased brood than polygynous colonies from Santon Warren. A similar trend was seen in Aberfoyle populations but was not significant. Mean percentage of sexual brood was not calculated by weighting colonies by overall productivity, so fewer colonies appear to be producing a low sexual : worker brood ratio. Aberfoyle's polygynous colonies had higher per capita new worker production values than monogynous colonies. These results suggests that in both these two populations, monogynous colonies reproduce through emission of sexuals, while polygynous colonies employ both sexual emission and production of new workers for daughter buds as reproductive strategies.

Large colony size (and by extension, high colony survivorship) is a central parameter in Pamilo's (1991a) model. As colony size increased new sexual production always increased, but this was not true for new worker production. In colonies from Roydon Wood, increasing old worker number did not affect the ratio of investment in sexuals : workers. Thus, they appear to maintain a fixed allocation strategy regardless of colony size. The extreme male-bias in this population was attributed to strong local-resource competition resulting from extensive colony budding (Chapter 3). If the majority of the population's reproductive effort is through budding, the most robust strategy should be to produce enough new queens to recruit back for stocking daughter buds, with the remaining sexual effort spent on producing males. With the risk of independent nest-founding irrelevant under complete colony-budding, colonies should still invest heavily in new males in an attempt to secure some of the scarce matings with the few virgin queens available. While much of the reproductive effort in males will be wasted if females mate singly, a successful mating brings a colony relatively high fitness returns. Thus, under extensive budding all colonies should invest as much as they can afford in producing new males.

In Santon Warren sample groups and monogynous colonies from Aberfoyle, the percentage of sexuals in the brood increased with colony size. When controlling for the effects of total productivity, this trend remained, and was nearly significant for polygynous colonies from Aberfoyle as well. Pamilo's (1991a) model predicts a negative partial correlation of percentage of sexual brood and worker number (with total sexual production controlled) under worker but not queen control, when colony survival rates are low and there is high independent nest-founding success. The opposite trend was found in two out of three populations of *L. acervorum*, and no trend was found in the third. This increase in percentage of sexual brood with increasing colony size is expected to occur when there is low female nest founding success under both worker and queen control. Thus, this study does not differentiate between which party controls resource allocation. However, as no evidence was found for partial queen control of sex allocation in these populations (Chapter 3) it is unlikely that mixed-control over resource allocation to new workers and new sexuals exists either. Instead, the pattern of resource allocation in both Santon Warren and

Aberfoyle is consistent with low independent nest-founding success and some degree of colony budding and local resource competition. Sundström (1995) predicted that there should be no expected relationship between the ratio of allocation to workers and sexuals with colony size because colonies' survivorship is invariably close to 100%, and she found no such relationship in a polygynous population of *Formica truncorum*. However, this species has polygynous colonies that typically contain many thousands of workers living in large permanent structures. Polygynous colonies of *L. acervorum* are larger on the whole than monogynous colonies but rarely exceed a few-hundred workers. Moreover, colonies occupy much less permanent structures, so colony survivorship under polygyny is more likely than under monogyny, but certainly not guaranteed. Thus, it pays both colony types to invest in more workers at smaller colony sizes to ensure future survivorship, and investment in sexuals at larger colony sizes. The colony classes do show different patterns of investment overall with polygynous colonies investing greater resources into worker production. The Santon Warren population showed a more marked difference between monogynous and polygynous colonies in their division between preferring investment in sexuals and workers, respectively. This may reflect a stronger degree of colony budding in Santon Warren's polygynous colonies than in Aberfoyle's, and the fact that Santon Warren has a stronger male-biased population sex ratio is in concordance with this.

Pamilo (1991a) predicted a conflict over resource allocation to arise as a result of differences in relatedness asymmetry between queens and workers. It follows that different classes of colony should prefer different resource investment ratios (Pamilo 1991a; Sundström 1995). Santon Warren population sex ratios were split by colony class and also differed in average worker-worker relatedness. The classes also differed in resource allocation strategies. Roydon Wood's monogynous and polygynous colonies did not differ in sex ratio, mean within-colony relatedness or resource allocation strategy. This would appear to support relatedness asymmetry as a causal factor behind resource allocation decisions. However, Santon Warren's colony classes also differed in mean colony sizes while these did not vary in Roydon Wood.

Thus, mean colony size could be the main factor affecting the ratio of sexuals to workers produced. No relatedness data are available for the Aberfoyle population, but there was no splitting of sex ratios which suggests that no difference exists between classes for within-colony relatedness values. However, there was a significant difference in mean colony size between the classes. Unfortunately, the class-difference for percentage of sexuals in the brood in Aberfoyle was not significant overall, though it was in the same direction as in Santon Warren. Therefore, it becomes impossible to predict whether colony size or within-colony relatedness and queen-worker conflict was the dominant factor in resource allocation in these populations.

The pattern of resource allocation investment in this species of ant supports the predictions of Pamilo (1991a) when independent nest-founding is a risky strategy, and colony survival is relatively low. This species appears to adopt variable levels of colony budding which suggests that the strategy of dispersing single-queens may be risky. Furthermore, Heinze *et al.* (1995a) found that the queen(s) which had produced a colony's brood and workers was often absent in colonies of this species, suggesting that she had either died or departed. Colony survivorship is difficult to predict, but small colony sizes in both monogynous and polygynous colonies, along with high queen turnover suggest that levels may be low to moderate. Under these parameters, queens and workers are expected to agree over the ratio of investment to sexuals and workers. As colony size increases, workers are expected to switch over to sexual production sooner than queens, but the difference cannot be predicted, as independent nest-founding success and colony survivorship are very difficult to measure. Differences between colony classes in resource allocation strategies cannot be directly attributed to mean class-differences in colony size or differences in relatedness asymmetry. It appears that in *L. acervorum*, colony size is an important determinant of resource allocation in both classes of colony, whereas Sundström (1995) found that colony size did not affect resource allocation decisions in polygynous colonies of *Formica truncorum*. The importance of variable relatedness asymmetry and queen-worker conflict could not be established without precise colony-level relatedness estimates for these populations of *L. acervorum*. Colonies appear to respond to relatedness asymmetry values when making sex allocation decisions (Chapter 3). Environmental variables, especially independent nest-founding success almost certainly affect both allocation to male and female sexuals as well as proportional investment in new workers.

§4.5 Summary

1. In Santon Warren and Aberfoyle populations of *Leptothorax acervorum*, polygynous colonies tended to be larger than monogynous colonies. There was no difference in mean colony size between the classes in Roydon Wood population.

2. Monogynous colonies from Santon Warren and Aberfoyle concentrated on new sexual production, while polygynous colonies concentrated on new worker production. Colonies from Roydon Wood showed no preference for new sexual or new worker production according to colony class.

3. As colony size increased, total productivity always increased. Sexual productivity always increased with colony size, but new worker production did not.

4. Per capita total productivity (biomass produced per old worker) decreased with increasing colony size.

5. The proportion of investment in new sexuals increased with colony size in Santon Warren and Aberfoyle, but the trend was insignificant in polygynous colonies from Aberfoyle. There was no relationship between colony size and proportion of investment in new sexuals in colonies from Roydon Wood.

6. Evidence of worker control over resource allocation was not found, according to the predictions of a model by Pamilo (1991a). *L. acervorum* has small colony sizes and is likely to employ budding as a reproductive strategy. According to Pamilo's model, both colony survival prospects and harsh environmental conditions mean queens and workers favour increasing investment in new sexuals with increasing colony survivorship (colony size).

7. Resource allocation in this species was consistent with monogynous colonies reproducing through the emission of sexuals. Polygynous colonies appeared to reproduce partially through budding, and produced large numbers of new workers to stock daughter buds. Colonies from Roydon Wood may have reproduced almost entirely by budding, and only produced enough new queens to readopt back into colonies to head new buds.

Table 4.1. Santon Warren 1993. Mean sexual and worker production schedules for monogynous and polygynous colonies.

	OW	NW [mg]	TSP [mg]	TP [mg]	% sexuals	percapita TSPmg	percapita NWmg	percapita TPmg
Monogyne	84.10 (53.52)	25.89 (14.10)	52.40 (51.06)	78.28 (60.88)	0.52 (0.28)	0.50 (0.36)	0.37 (0.20)	0.86 (0.33)
Polygyne	114.25 (77.26)	53.33 (31.92)	20.44 (22.42)	74.40 (47.89)	0.26 (0.18)	0.17 (0.13)	0.57 (0.44)	0.74 (0.48)
d.f.	43	31	21	42	42	25	$n = 44$	42
p	0.1409	< 0.001	< 0.02	0.8145	0.0005	< 0.001	0.0761 *	0.3357

Table legend. OW = old workers (colony size),
NW[mg] = biomass of new workers [mg],
TSP[mg] = total biomass of sexuals produced [mg],
TP[mg] = total biomass produced [mg]; equals NW[mg] + TSP[mg],
% sexuals = fraction of total production dedicated to sexuals; equals TSP[mg] / TP[mg],
per capita TSPmg = average biomass of sexuals produced by each old worker; equals TSP[mg] / OW,
per capita NWmg = average biomass of new workers produced by each old worker; equals NW[mg] / OW,
per capita TPmg = average total biomass produced by each old worker; equals TP[mg] / OW,

Values given in the table are mean per colony values (± standard deviations) for monogynous and polygynous colonies within populations. Differences between means of monogynous and polygynous colonies are calculated using Student's *t*-test. Where *p*-values are marked with an asterisk, differences between means were calculated using Mann-Whitney *U*-test.

Table 4.2. Santon Warren 1994. Mean sexual and worker production schedules for monogynous and polygynous colonies.

	OW	NW [mg]	TSP [mg]	TP [mg]	% sexuals	percapita TSPmg	percapita NWmg	percapita TPmg
Monogyne	81.65 (54.69)	17.29 (11.61)	23.57 (19.63)	40.85 (25.46)	0.53 (0.23)	0.28 (0.18)	0.26 (0.19)	0.54 (0.29)
Polygyne	162.89 (87.83)	40.36 (33.65)	28.32 (26.60)	68.68 (51.38)	0.41 (0.25)	0.17 (0.13)	0.31 (0.26)	0.48 (0.31)
d.f.	38	9	38	9	38	38	38	38
p	0.0016	$0.05<p<0.1$	0.5592	> 0.05	0.1847	0.0918	0.5397	0.5660

Table legend. As in Table 4.1.

Table 4.3. Santon Warren 1995. Mean sexual and worker production schedules for monogynous and polygynous colonies.

	OW	NW [mg]	TSP [mg]	TP [mg]	% sexuals	percapita TSPmg	percapita NWmg	percapita TPmg
Monogyne	60.21	23.90	28.00	52.12	0.44	0.40	0.48	0.88
	(35.92)	(12.31)	(31.70)	(38.57)	(0.26)	(0.34)	(0.30)	(0.45)
Polygyne	65.44	44.24	7.55	51.79	0.16	0.12	0.71	0.83
	(29.80)	(30.68)	(10.02)	(33.04)	(0.16)	(0.16)	(0.49)	(0.52)
d.f.	83	19	n = 85	82	43	n = 85	21	82
p	0.5719	< 0.02	0.001	0.9735	< 0.0001	< 0.0001	0.05<p<0.1	0.6514

Table legend. As in Table 4.1.

Table 4.4. Aberfoyle 1994. Mean sexual and worker production schedules for monogynous and polygynous colonies.

	OW	NW [mg]	TSP [mg]	TP [mg]	% sexuals	percapita TSPmg	percapita NWmg	percapita TPmg
Monogyne	79.70 (41.48)	14.80 (6.92)	15.58 (14.76)	30.37 (17.58)	0.41 (0.25)	0.17 (0.14)	0.22 (0.13)	0.39 (0.17)
Polygyne	223.57 (205.31)	113.06 (133.96)	29.05 (27.11)	142.11 (155.36)	0.22 (0.15)	0.14 (0.10)	0.50 (0.15)	0.63 (0.17)
d.f.	6	6	28	6	28	28	28	28
p	> 0.05	> 0.05	0.0961	> 0.05	0.0652	0.5820	<0.0001	0.0024

Table legend. As in Table 4.1.

Table 4.5. Aberfoyle 1995. Mean sexual and worker production schedules for monogynous and polygynous colonies.

	OW	NW [mg]	TSP [mg]	TP [mg]	% sexuals	percapita TSPmg	percapita NWmg	percapita TPmg
Monogyne	75.92 (38.63)	14.80 (7.40)	17.12 (16.27)	31.92 (15.31)	0.46 (0.30)	0.19 (0.13)	0.30 (0.30)	0.49 (0.26)
Polygyne	346.00 (259.06)	77.80 (55.45)	79.75 (74.75)	157.56 (113.51)	0.39 (0.25)	0.19 (0.11)	0.31 (0.16)	0.50 (0.13)
d.f.	4	4	4	4	15	15	15	15
p	$0.05<p<0.1$	$0.05<p<0.1$	> 0.05	$0.05<p<0.1$	0.6722	0.9983	0.9394	0.9293

Table legend. As in Table 4.1.

Table 4.6. Roydon Wood 1995. Mean sexual and worker production schedules for monogynous and polygynous colonies.

	OW	NW [mg]	TSP [mg]	TP [mg]	% sexuals	percapita TSPmg	percapita NWmg	percapita TPmg
Monogyne	46.10 (22.13)	15.25 (9.59)	7.08 (10.17)	22.33 (9.33)	0.34 (0.35)	0.24 (0.30)	0.35 (0.23)	0.59 (0.32)
Polygyne	50.26 (25.07)	15.55 (8.68)	9.25 (9.44)	24.80 (14.63)	0.33 (0.22)	0.21 (0.22)	0.35 (0.21)	0.56 (0.30)
d.f.	31	31	$n = 33$	31	31	31	31	31
p	0.6537	0.9306	0.4807 *	0.6279	0.9307	0.7583	0.9806	0.7903

Table legend. As in Table 4.1.

Table 4.7. Results from two-way ANOVAs looking at the effects of colony class and different years on mean values for eight variables. Three years were examined from Santon Warren and two from Aberfoyle. Only one year's data was collected from Roydon Wood and the means for the eight variables, along with *t*-values for differences between means are given in table 6. 'OW' = old workers (colony size); NW(mg) = weight of new workers produced in milligrams; 'TSP(mg)' = total sexual production in milligrams; 'TP(mg)' = total production (new workers + total new sexuals) in milligrams; '% sexuals' = fraction of total brood devoted to new sexuals; 'per capita TSP' = mean weight of new sexual brood produced by each worker in milligrams; 'per capita NW' = mean weight of new workers produced by each worker in milligrams; 'per capita TP' = mean weight of total brood produced by each worker in milligrams. Mean values for each variable are given in Tables 4.1 through 4.6.

		population		
variable	factor	Santon Warren	Aberfoyle	Roydon Wood
OW	class	$F_{1,164} = 16.49$ $p = < 0.001$	$F_{1,43} = 27.26$ $p = < 0.001$	NS
	year	$F_{2,164} = 13.66$ $p = < 0.001$	$F_{1,43} = 2.24$ $p = 0.14$	.
	class.year	$F_{2,164} = 4.96$ $p = 0.008$	$F_{1,43} = 2.53$ $p = 0.12$	.
NW(mg)	class	$F_{1,162} = 40.80$ $p = < 0.001$	$F_{1,43} = 19.58$ $p = < 0.001$	NS
	year	$F_{2,162} = 2.53$ $p = 0.08$	$F_{1,43} = 0.94$ $p = 0.34$	.
	class.year	$F_{2,162} = 0.39$ $p = 0.68$	$F_{1,43} = 0.94$ $p = 0.34$	.
TSP(mg)	class	$F_{1,164} = 8.25$ $p = 0.005$	$F_{1,43} = 15.39$ $p = < 0.001$	NS
	year	$F_{2,164} = 4.79$ $p = 0.01$	$F_{1,43} = 7.25$ $p = 0.01$	.
	class.year	$F_{2,164} = 3.22$ $p = 0.04$	$F_{1,43} = 6.42$ $p = 0.02$	.
TP(mg)	class	$F_{1,162} = 1.10$ $p = 0.31$	$F_{1,43} = 25.08$ $p = < 0.001$	NS
	year	$F_{2,162} = 4.64$ $p = 0.01$	$F_{1,43} = 0.13$ $p = 0.72$	.
	class.year	$F_{2,162} = 1.41$ $p = 0.25$	$F_{1,43} = 0.09$ $p = 0.77$	.

Table 4.7 (continued).

variable	factor	population Santon Warren	 Aberfoyle	 Roydon Wood
% sexuals	class	$F_{1,162} = 25.35$ $p = < 0.001$	$F_{1,43} = 2.23$ $p = 0.14$	NS
	year	$F_{2,162} = 4.92$ $p = 0.008$	$F_{1,43} = 1.65$ $p = 0.21$	.
	class.year	$F_{2,162} = 1.08$ $p = 0.34$	$F_{1,43} = 0.54$ $p = 0.46$	.
per capita TSP	class	$F_{1,164} = 23.24$ $p = < 0.001$	$F_{1,43} = 0.13$ $p = 0.72$	NS
	year	$F_{2,164} = 1.60$ $p = 0.21$	$F_{1,43} = 0.74$ $p = 0.40$	.
	class.year	$F_{2,164} = 1.40$ $p = 0.25$	$F_{1,43} = 0.12$ $p = 0.73$	.
per capita NW	class	$F_{1,162} = 7.36$ $p = 0.007$	$F_{1,43} = 4.71$ $p = 0.04$	NS
	year	$F_{2,162} = 8.61$ $p = < 0.001$	$F_{1,43} = 0.77$ $p = 0.39$	.
	class.year	$F_{2,162} = 0.77$ $p = 0.46$	$F_{1,43} = 4.01$ $p = 0.05$	.
per capita TP	class	$F_{1,162} = 1.11$ $p = 0.29$	$F_{1,43} = 3.71$ $p = 0.06$	NS
	year	$F_{2,162} = 6.75$ $p = 0.002$	$F_{1,43} = 0.09$ $p = 0.77$	.
	class.year	$F_{2,162} = 0.09$ $p = 0.92$	$F_{1,43} = 3.09$ $p = 0.09$	.

Table 4.8. Results of a multiple regression model of total sexual production and colony size, with year and class as factors. As each population differed considerably in basic resource allocation schedules, separate regression models were considered for each population. In this table a single box, containing the results of a regression model, spanning both colony classes means that there was no significant effect of class on the model. If a box spans more than one year, it means that year was not a significant factor in the model. Thus, in Santon Warren (SD) there was a significant effect of class on the regression model, but year was only significant within monogynous colonies.

<table>
<tr><th>population</th><th>monogynous</th><th>polygynous</th></tr>
<tr><td>SD 1993</td><td>$F_{1,15} = 48.63, p < 0.0001$</td><td rowspan="3">$F_{1,35} = 23.22, p < 0.001$</td></tr>
<tr><td>SD 1994</td><td>$F_{1,23} = 29.37, p < 0.0001$</td></tr>
<tr><td>SD 1995</td><td>$F_{1,53} = 71.25, p < 0.0001$</td></tr>
<tr><td>AB 1994</td><td colspan="2" rowspan="2">$F_{1,32} = 70.03, p < 0.001$</td></tr>
<tr><td>AB 1995</td></tr>
<tr><td>RW 1995</td><td colspan="2">$F_{1,22} = 3.64, p = 0.07$</td></tr>
</table>

Note: The regression for the Aberfoyle population (AB) uses log (total sexual production (mg)) and log (old worker number).

Table 4.9. Results of a multiple regression model of new worker production and colony size, with year and class as factors. As each population differed considerably in basic resource allocation schedules, separate regression models were considered for each population. The layout of this table is according to rules described in the legend for Table 4.8 .

<table>
<tr><th>population</th><th>monogynous</th><th>polygynous</th></tr>
<tr><td>SD 1993</td><td>$F_{1,15} = 12.30, p = 0.003$</td><td rowspan="3">$F_{1,35} = 8.85, p = 0.005$</td></tr>
<tr><td>SD 1994</td><td>$F_{1,23} = 0.26, p = 0.62$</td></tr>
<tr><td>SD 1995</td><td>$F_{1,53} = 5.56, p = 0.02$</td></tr>
<tr><td>AB 1994</td><td rowspan="2">$F_{1,23} = 1.32, p = 0.26$</td><td rowspan="2">$F_{1,7} = 10.66, p = 0.01$</td></tr>
<tr><td>AB 1995</td></tr>
<tr><td>RW 1995</td><td colspan="2">$F_{1,22} = 2.67, p = 0.12$</td></tr>
</table>

Note: The regression for the Aberfoyle population (AB) uses log (new worker production (mg)) and log (old worker number).

Table 4.10. Results of a multiple regression model of total production and colony size, with year and class as factors. As each population differed considerably in basic resource allocation schedules, separate regression models were considered for each population. The layout of this table is according to rules described in the legend for Table 4.8 .

<table>
<tr><th>population</th><th>monogynous</th><th>polygynous</th></tr>
<tr><td>SD 1993</td><td>$F_{1,15} = 62.24, p < 0.0001$</td><td rowspan="3">$F_{1,35} = 23.84, p < 0.001$</td></tr>
<tr><td>SD 1994</td><td>$F_{1,23} = 19.41, p = 0.0002$</td></tr>
<tr><td>SD 1995</td><td>$F_{1,53} = 61.58, p < 0.0001$</td></tr>
<tr><td>AB 1994</td><td rowspan="2">$F_{1,23} = 7.22, p = 0.01$</td><td rowspan="2">$F_{1,7} = 57.44, p < 0.001$</td></tr>
<tr><td>AB 1995</td></tr>
<tr><td>RW 1995</td><td colspan="2">$F_{1,22} = 7.30, p = 0.01$</td></tr>
</table>

Note: The regression for the Aberfoyle population (AB) uses log (total production (mg)) and log (old worker number).

Table 4.11. Results of a multiple regression model of per capita total sexual production and colony size, with year and class as factors. As each population differed considerably in basic resource allocation schedules, separate regression models were considered for each population. The layout of this table is according to rules described in the legend for Table 4.8.

<table>
<tr><th>population</th><th>monogynous</th><th>polygynous</th></tr>
<tr><td>SD 1993</td><td>$F_{1,15} = 2.87, p = 0.11$</td><td rowspan="3">$F_{1,35} = 0.19, p = 0.67$</td></tr>
<tr><td>SD 1994</td><td>$F_{1,23} = 0.68, p = 0.42$</td></tr>
<tr><td>SD 1995</td><td>$F_{1,53} = 2.14, p = 0.15$</td></tr>
<tr><td>AB 1994</td><td colspan="2" rowspan="2">$F_{1,32} = 0.05, p = 0.82$</td></tr>
<tr><td>AB 1995</td></tr>
<tr><td>RW 1995</td><td colspan="2">$F_{1,22} = 2.98, p = 0.10$</td></tr>
</table>

Note: The regression for the Aberfoyle population (AB) uses log (old worker number).

Table 4.12. Partial correlation coefficients between colony size (number of old workers) and per capita production of all sexuals, controlling for total colony productivity.

population	monogynous	polygynous
SD 1993	$r = -0.72$, d.f. = 14, $p < 0.002$	$r = -0.63$, d.f. = 15, $p < 0.01$
SD 1994	$r = -0.61$, d.f. = 22, $p < 0.002$	$r = -0.32$, d.f. = 5, $p > 0.05$
SD 1995	$r = -0.55$, d.f. = 52, $p < 0.001$	$r = -0.03$, d.f. = 8, $p > 0.05$
AB 1994	$r = -0.48$, d.f. = 13, $0.05 < p < 0.10$	$r = -0.49$, d.f. = 2, $p > 0.05$
AB 1995	$r = 0.17$, d.f. = 6, $p > 0.05$	$r = -0.45$, d.f. = 1, $p > 0.05$
RW 1995	$r = -0.50$, d.f. = 3, $p > 0.05$	$r = -0.54$, d.f. = 15, $p < 0.05$

Table 4.13. Results of a multiple regression model of per capita new worker production and colony size, with year and class as factors. As each population differed considerably in basic resource allocation schedules, separate regression models were considered for each population. The layout of this table is according to rules described in the legend for Table 4.8.

population	monogynous	polygynous
SD 1993	$F_{1,15} = 0.87$, $p = 0.37$	$F_{1,35} = 14.29$, $p < 0.001$
SD 1994	$F_{1,23} = 8.55$, $p = 0.008$	
SD 1995	$F_{1,53} = 23.94$, $p < 0.0001$	
AB 1994	$F_{1,23} = 31.28$, $p < 0.001$	$F_{1,7} = 2.46$, $p = 0.16$
AB 1995		
RW 1995	$F_{1,22} = 9.58$, $p = 0.005$	

Note: The regression for the Aberfoyle population (AB) uses log (old worker number).

Table 4.14. Partial correlation coefficients between colony size (number of old workers) and per capita production of new workers, controlling for total colony productivity.

population	monogynous	polygynous
SD 1993	$r = -0.41$, d.f. = 14, $p > 0.05$	$r = -0.74$, d.f. = 15, $p < 0.001$
SD 1994	$r = -0.71$, d.f. = 22, $p < 0.001$	$r = -0.88$, d.f. = 5, $p < 0.01$
SD 1995	$r = -0.68$, d.f. = 52, $p < 0.001$	$r = -0.70$, d.f. = 8, $p < 0.05$
AB 1994	$r = -0.78$, d.f. = 13, $p < 0.001$	$r = -0.89$, d.f. = 2, $p > 0.05$
AB 1995	$r = -0.91$, d.f. = 6, $p < 0.002$	$r = -0.46$, d.f. = 1, $p > 0.05$
RW 1995	$r = -0.64$, d.f. = 3, $p < 0.05$	$r = -0.66$, d.f. = 15, $p < 0.005$

Table 4.15. Results of a multiple regression model of per capita total production and colony size, with year and class as factors. As each population differed considerably in basic resource allocation schedules, separate regression models were considered for each population. The layout of this table is according to rules described in the legend for Table 4.8.

<table>
<tr><th>population</th><th>monogynous</th><th>polygynous</th></tr>
<tr><td>SD 1993</td><td>$F_{1,15} = 1.25, p = 0.28$</td><td rowspan="3">$F_{1,35} = 10.90, p = 0.002$</td></tr>
<tr><td>SD 1994</td><td>$F_{1,23} = 6.05, p = 0.02$</td></tr>
<tr><td>SD 1995</td><td>$F_{1,53} = 3.07, p = 0.09$</td></tr>
<tr><td>AB 1994</td><td rowspan="2">$F_{1,23} = 10.98, p = 0.003$</td><td rowspan="2">$F_{1,7} = 2.76, p = 0.14$</td></tr>
<tr><td>AB 1995</td></tr>
<tr><td>RW 1995</td><td colspan="2">$F_{1,22} = 19.02, p < 0.001$</td></tr>
</table>

Note: The regression for the Aberfoyle population (AB) uses log (old worker number).

Table 4.16. Regression of % sexuals in brood with colony size (number of old workers).

population	monogynous	polygynous
SD 1993	$F_{1,95} = 43.63$, $p < 0.001$	$F_{1,35} = 5.71$, $p = 0.02$
SD 1994		
SD 1995		
AB 1994	$F_{1,23} = 21.70$, $p < 0.001$	$F_{1,7} = 1.19$, $p = 0.31$
AB 1995		
RW 1995	$F_{1,21} = 0.23$, $p = 0.64$	

Note: The regression for the Aberfoyle population (AB) uses log (old worker number).

Table 4.17. Partial correlation coefficients between colony size (number of old workers) and percentage of sexuals in the brood, controlling for total colony productivity. As the regression model of percentage of sexuals in the brood with colony size (Table 4.16) found no year effect for both Santon Warren and Aberfoyle populations, the partial correlations were examined pooled for year within colony classes for these two populations.

population	monogynous	polygynous
SD 1993	$r = -0.20$, d.f. = 14, $p > 0.05$	$r = 0.003$, d.f. = 15, $p > 0.05$
SD 1994	$r = 0.41$, d.f. = 22, $p < 0.05$	$r = 0.59$, d.f. = 5, $p > 0.05$
SD 1995	$r = 0.21$, d.f. = 52, $p > 0.05$	$r = -0.03$, d.f. = 8, $p > 0.05$
AB 1994	$r = 0.46$, d.f. = 13, $0.05 < p < 0.10$	$r = 0.18$, d.f. = 2, $p > 0.05$
AB 1995	$r = 0.82$, d.f. = 6, $p < 0.02$	$r = -0.08$, d.f. = 1, $p > 0.05$
RW 1995	$r = -0.16$, d.f. = 3, $p > 0.05$	$r = -0.09$, d.f. = 15, $p > 0.05$
SD pooled	$r = 0.30$, d.f. = 94, $p < 0.005$	$r = 0.39$, d.f. = 34, $p < 0.02$
AB pooled	$r = 0.60$, d.f. = 22, $p < 0.002$	$r = 0.47$, d.f. = 6, $p > 0.05$

Note: partial correlations for Aberfoyle populations were calculated using log (old worker number) and log (total production).

Figure 4.1. Mean colony size (with standard error bars) for all years in all populations sampled. Population abbreviations are 'SD' = Santon Warren; 'AB' = Aberfoyle; 'RW' = Roydon Wood. White bars represent monogynous colonies, coloured bars represent polygynous colonies.

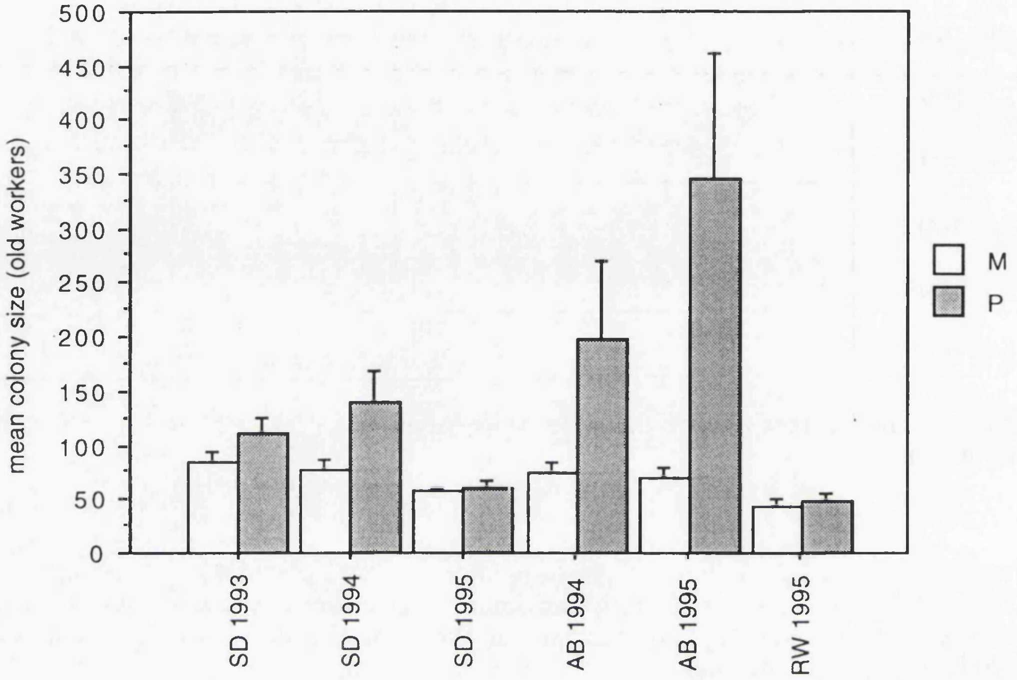


Figure 4.2. Mean dry weight of new workers produced by colonies (with standard error bars) for all years in all populations sampled. Figure layout as in Figure 4.1.

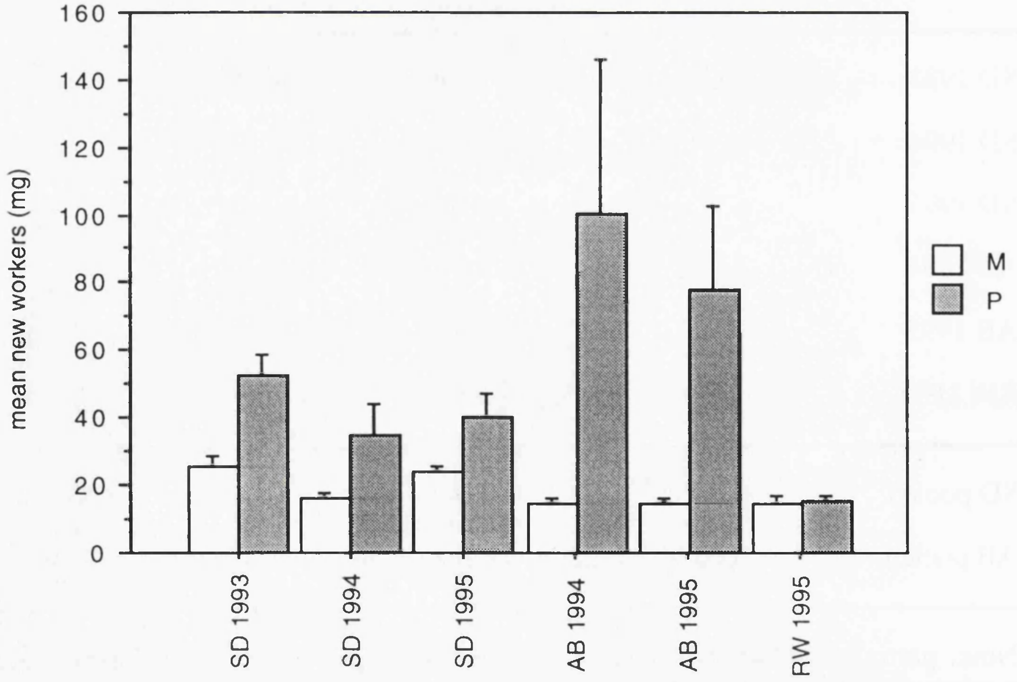


Figure 4.3. Mean dry weight of all new sexuals produced by colonies (with standard error bars) for all years in all populations sampled. Figure layout as in Figure 4.1.

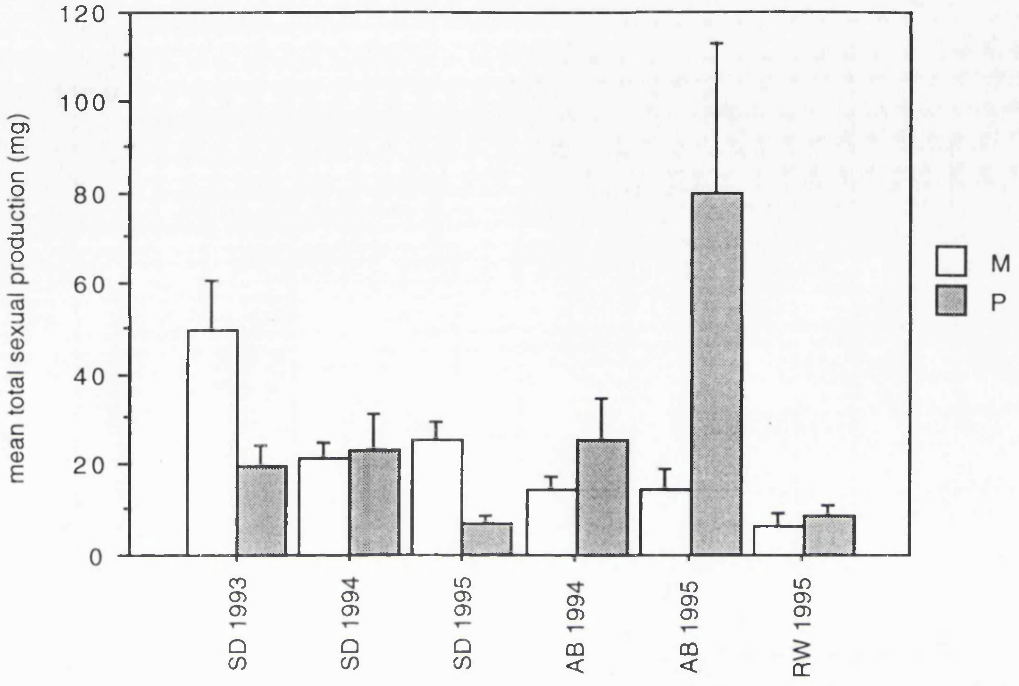


Figure 4.4. Mean dry weight of all new brood produced by colonies (with standard error bars) for all years in all populations sampled. Figure layout as in Figure 4.1.

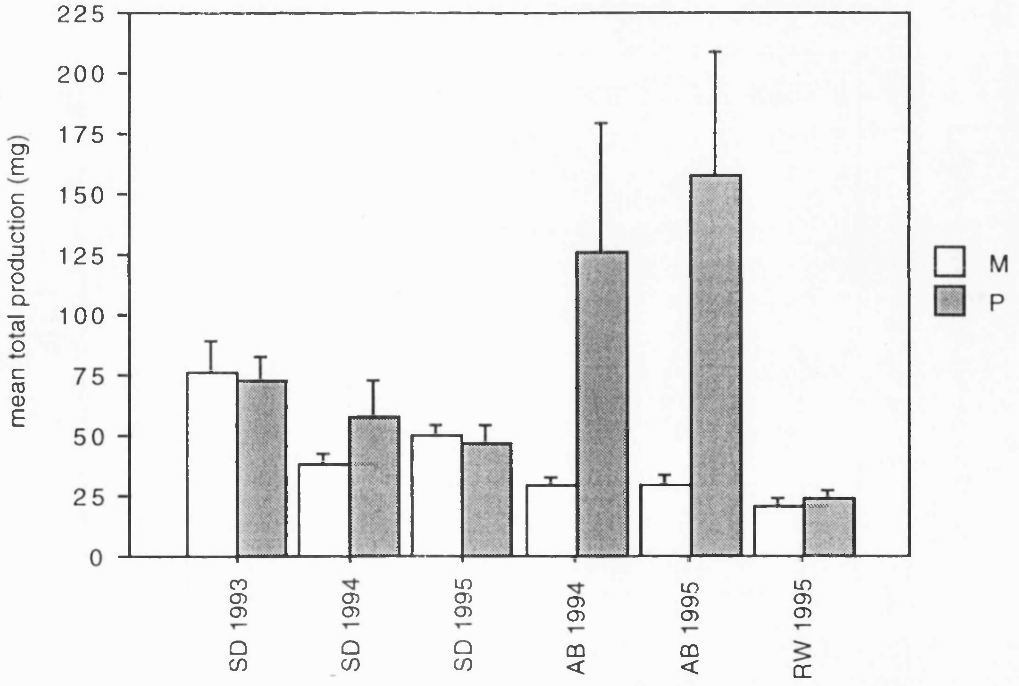


Figure 4.5. Mean fraction of total brood dry weight allocated to sexuals by colonies (with standard error bars) for all years in all populations sampled. Figure layout as in Figure 4.1.

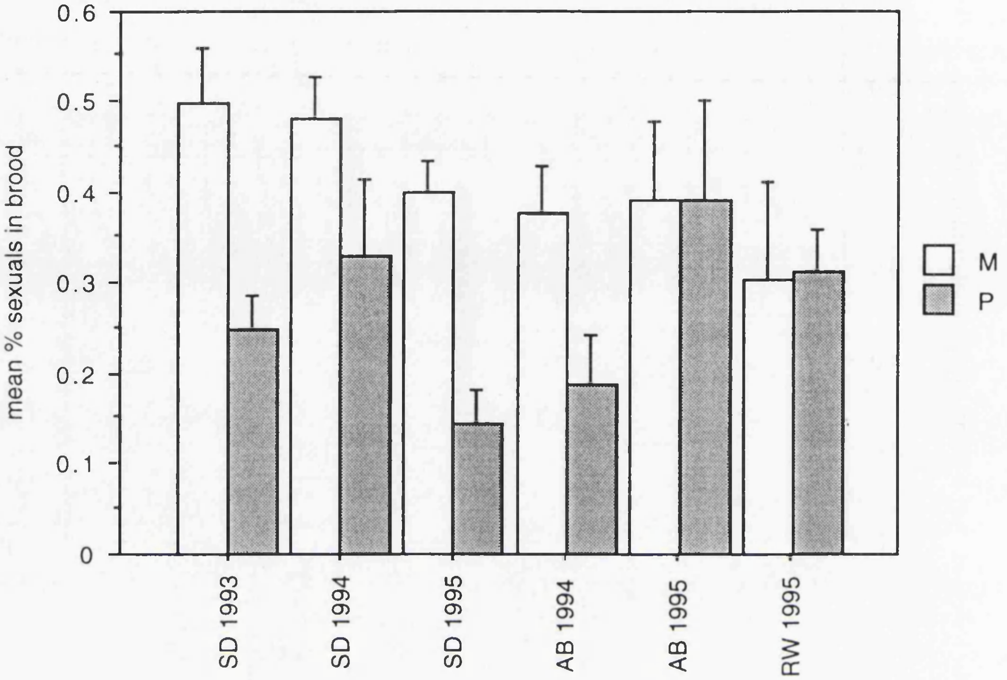


Figure 4.6. Mean dry weight of new workers produced by each old worker (with standard error bars) for all years in all populations sampled. Figure layout as in Figure 4.1.

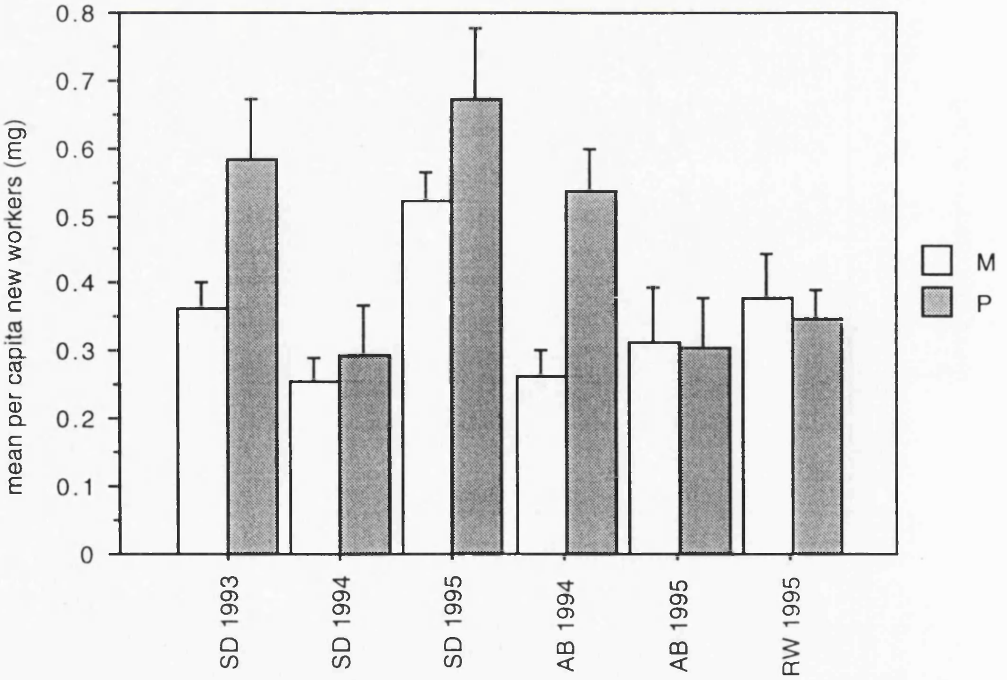


Figure 4.7. Mean dry weight of all new sexuals produced by each old worker (with standard error bars) for all years in all populations sampled. Figure layout as in Figure 4.1.

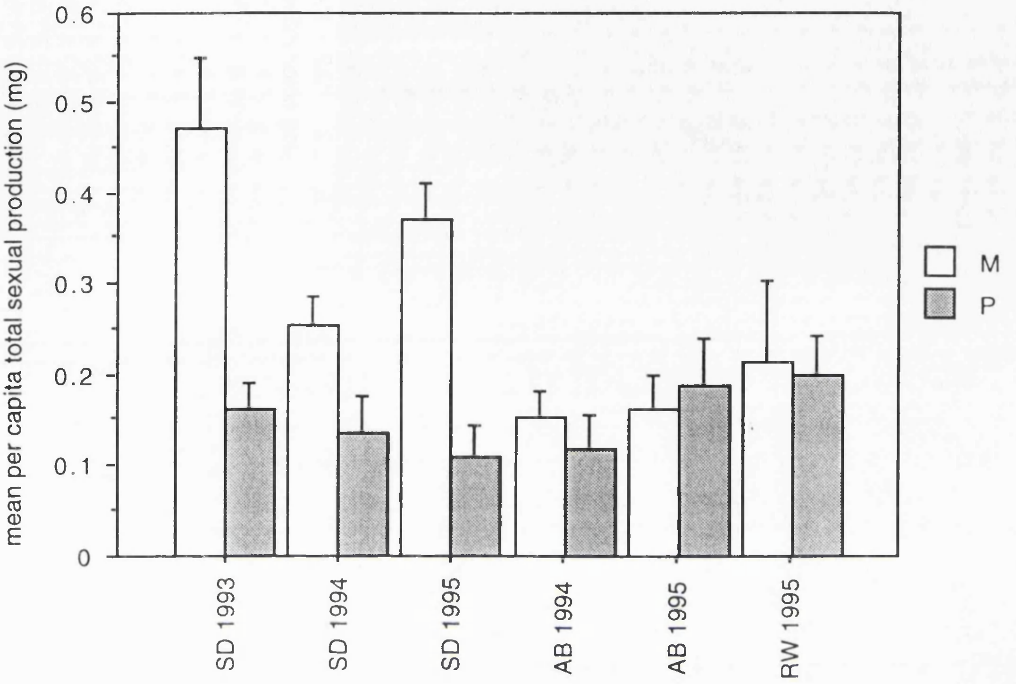


Figure 4.8. Mean dry weight of all new brood produced by each old worker (with standard error bars) for all years in all populations sampled. Figure layout as in Figure 4.1.

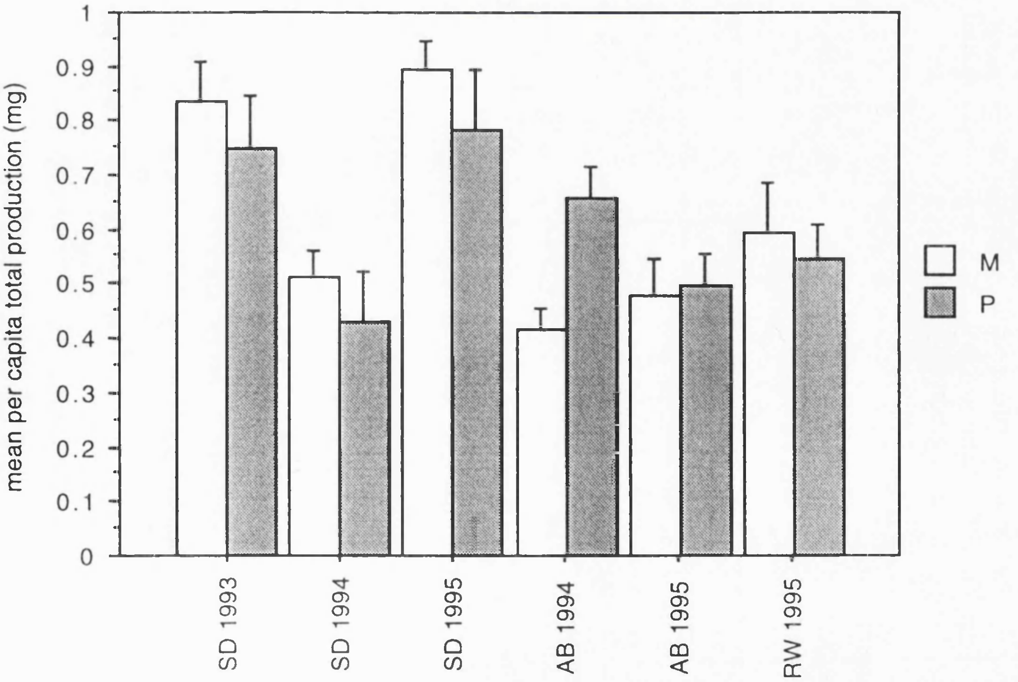
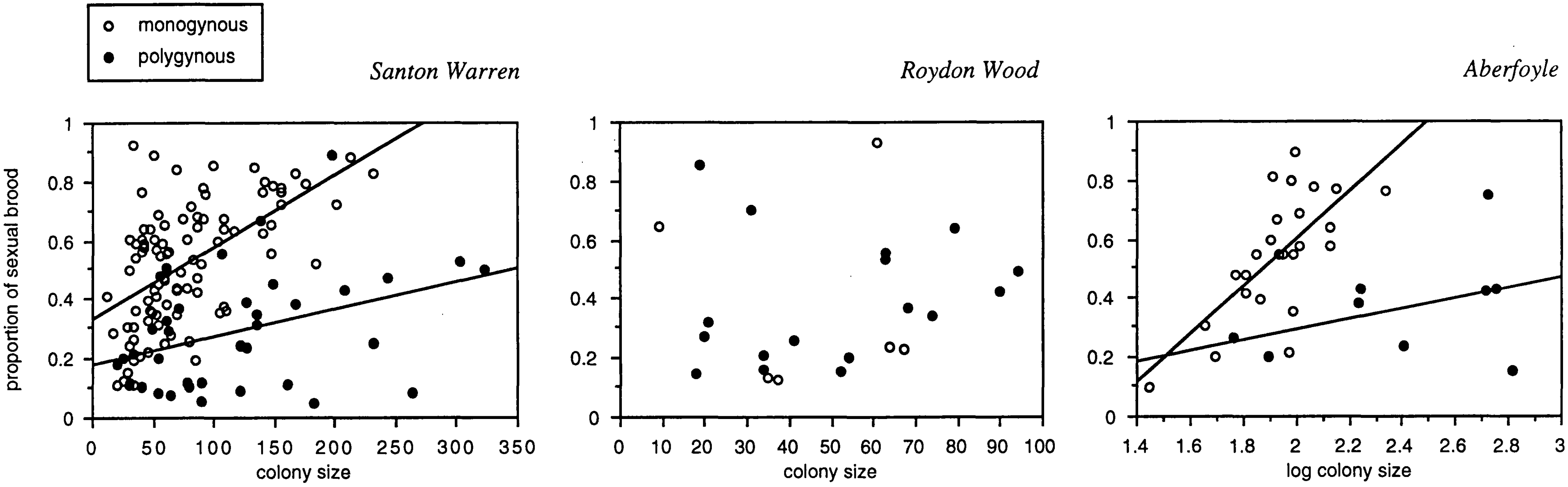


Figure 4.9. Plot of the proportion of sexual brood against colony size (number of old workers) for all three populations. Proportion of sexual brood is calculated as the dry weight of sexual brood over the entire dry weight of brood produced by colonies. Multiple regression models are given in Table 4.16.



regression equations are given for significant results:
Santon Warren, monogynous: $y = 0.002x + 0.335$; $r^2 = 0.32$, polygynous: $y = 0.001x + 0.181$; $r^2 = 0.14$.
Royden Wood: both classes pooled: non-significant (see Table 4.16); $r^2 = 0.01$.
Aberfoyle, monogynous: $y = 0.806x - 1.008$; $r^2 = 0.49$, polygynous: non-significant; $r^2 = 0.15$.

Chapter 5

CONCLUSION

§5.1 Colony-level sex allocation

Split sex ratio theory (Boomsma and Grafen 1990, 1991) predicts that the party controlling sex allocation should bias its colony sex ratio according to its relative relatedness asymmetry. The observed sex allocation pattern in the two populations for which relatedness data were available supports worker controlled sex allocation in response to relatedness asymmetry (assuming that within-colony relatedness is a reliable estimator of a colony's relatedness asymmetry). Heinze *et al.* (1995b) found monogynous colonies from Santon Warren (sampled in 1991 and 1992) to have higher average worker-worker relatedness than in polygynous colonies, and accordingly, monogynous colonies produced stronger female-biased broods than polygynous colonies. Roydon Wood's colonies did not differ in mean worker-worker relatedness (A. Hingle, unpublished), or sex investment ratio according to colony gyny class. This result adds further credence to Boomsma and Grafen's theory of sex ratios split by relatedness asymmetry, already supported by a study of the ant species *Formica truncorum* with multiply-mated queens (Sundström 1994a) and the multiply-queened ant *Myrmica tahoensis* (Evans 1995).

In two out of three study populations, local resource competition appeared to affect sex allocation at the colony level, and resource abundance appeared to affect the third. Local resource competition is expected to result in diminishing returns on investment in new queens at high levels of total sexual productivity, and this was seen in polygynous colonies from Santon Warren and all Roydon Wood colonies. Thus, productive polygynous colonies from Santon Warren produced a strong male bias compared with less productive polygynous colonies. In Roydon Wood, the degree of local resource competition may have been so severe that colonies tended to produce only a small number of new queens for readoption, and males thereafter, as predicted by Frank (1987). In the Aberfoyle population, colonies that had a high relative total sexual productivity produced a strong female bias. Nonacs (1986b) suggested that resource abundance in these colonies allowed them to raise more costly sexual brood than poorly resourced colonies. Thus, factors aside from relatedness asymmetry are influencing sex allocation in different ways in different populations. Rising male bias with increasing total sexual productivity has previously been demonstrated in ants by Sundström (1993, 1995) in *F. truncorum* and by Chan and Bourke (1994) in *L. acervorum*. Resource abundance was shown to affect a range of species by Nonacs (1986b).

Sex ratio compensation (Taylor 1981) describes an adjustment in the sex ratio of a class of colony in response to the sex allocation pattern of another class of colony in the same population. Thus, if one class of colony is specialising in the production of a particular sex in a population, the other sex will have a high relative mating success and should be favoured by what Boomsma and Grafen (1991) termed, 'the balancing class'. When two classes of colony in a population systematically contribute different sex ratios to the same generation, the population sex ratio will be split. Polygynous colonies from Santon Warren appeared to produce male-biased broods as a result of workers' response to relative relatedness asymmetry as well as local resource competition. Monogynous colonies may have produced female bias in response to their own high relative relatedness asymmetry, or in response to polygynous colonies producing male bias, or a combination of these effects. It is difficult to know how sex ratio compensation operates in real populations, especially when brood development times are long, as in *L. acervorum*, and the relative frequency of gyny classes changes from year to year. Demonstrating that sex ratio compensation exists requires manipulating the cues that workers use to base sex allocation decisions on, and current understanding of these cues is still speculative.

§5.2 Population-level sex allocation

The population sex investment ratio is the sum of the sex investment ratios of its constituent colonies. Each colony must be sensitive to the sex allocation pattern for the population overall because this affects the relative mating success of the sexes. Facultatively polygynous ant populations vary in the frequency of polygynous colonies, and this is predicted to affect the extent of male bias encountered. This trend was encountered in the study species, *L. acervorum*. A study by Elmes (1987a, b) on the polygynous ant *Myrmica sulcinodis* also found a link between degree of polygyny and male bias, but most previous associations have been cross-species studies. Polygyny is frequently associated with colony budding, which leads to neighbouring daughter buds encountering local resource competition. Evidence of this in two populations was seen as diminishing returns on new queen production at higher levels of total sexual productivity. In the Aberfoyle population, an apparent absence of local resource competition and low levels of polygyny resulted in a strongly female-biased population sex investment ratio as predicted under worker control by Trivers and Hare (1976).

That factors excluding relatedness asymmetry were important in determining population sex investment ratios was clear. Santon Warren and Roydon Wood population did not differ in overall worker-worker relatedness, but produced very different sex investment ratios. Quantifying the level of environmental effects such as habitat patchiness and cost of dispersal may not be feasible (Keller and Vargo 1993). It may be that competition for limited nest sites and high mortality of solitary nest founding queens leads to increased levels of polygyny. In turn, budding may increase, with a corresponding rise in population male bias due to strong local resource competition. Again, the degree of budding in a population may be difficult to measure. Budding was shown to influence the degree of male bias in the ant genus *Rhitidoponera*, in a study by Ward (1983). The effects of resource abundance on population sex allocation may be easier to quantify than local resource competition and colony budding. For example, populations that experience resource abundance may have lower numbers of potential forage, such as invertebrate biomass (Elmes 1987a; Herbers 1990), than populations that do not experience resource abundance effects.

Both extreme male bias as well as female bias in population sex investment ratios of this species were encountered. This is indicative of the plasticity of social Hymenopteran sex ratios in response to a number of variables. With so many factors potentially influencing the fitness returns from sex allocation, the ability to adjust sex ratios is expected to be naturally selected. Diploid species may be strongly constrained in sex allocation by sex determination mechanisms. Haplodiploid Hymenoptera are clearly capable of interpreting information about the potential influences on sex allocation, and responding to it at the colony and population levels.

§5.3 Resource allocation

Both colony size and relatedness asymmetry are expected to affect the allocation of resources between sexual and new worker fractions. Three study populations varied in their patterns of resource allocation, but this variation was less extreme than the sex allocation patterns they adopted. Colony size was shown to play a significant role in how colonies allocated resources in Santon Warren and Aberfoyle, with all colonies allocating more resources to new sexuals at larger colony sizes. Colony class also affected how resources were allocated in these two populations, with monogynous colonies concentrating on sexual production, and polygynous colonies concentrating on new worker production, although this pattern proved non-significant in the Aberfoyle population. Roydon Wood was exceptional in that neither colony size, nor colony gyny class were found to affect resource allocation decisions.

In the two populations in which colony size affected resource allocation, both colony classes tended to increase investment in new sexuals with increasing colony size. A model by Pamilo (1991a) does not differentiate between queen and worker control when the proportion of sexual brood increases with colony survivorship. Sundström (1995) tested Pamilo's model and found the proportion of sexual brood to decrease in monogynous, but not polygynous colonies of the ant *Formica truncorum*, in support of worker control over resource allocation. Workers' relatedness asymmetry means they are more likely to favour investment in new sexuals than queens do, and the degree of this conflict is expected to fall with worker relatedness asymmetry in polygynous colonies. In agreement with this, polygynous colonies concentrated on the production of new workers and monogynous colonies produced more new sexuals. However, both workers and queens in monogynous colonies may have preferred less new worker production because reproduction by colony budding, which requires large numbers of new workers, is not an option for them.

In Roydon Wood, all colonies were very small, with none containing more than one-hundred workers. In comparison, colony size in the other two populations frequently exceeded one-hundred workers. Thus, nest site limitation may have constrained colony growth, and the high frequency of polygynous colonies in this population also suggested harsh ecological conditions. The fact that proportional investment in new sexuals was not affected by colony size may indicate that there was no significant change in the survival prospects of colonies over the small size range in this population. Thus, colony growth may not be a means of improving colony survival prospects in this population.

Available relatedness data for two study populations found colony classes to differ in average worker-worker relatedness in Santon Warren (Heinze *et al.* 1995b) but not Roydon Wood (A. Hingle, unpublished). Assuming that within-colony relatedness is a reliable indicator of relatedness asymmetry, this is in agreement with colony classes basing resource allocation decisions at least partially upon relatedness asymmetry (Pamilo 1991a). Is it possible to determine whether colony size or relatedness asymmetry was more important in these populations' resource allocation decision-making processes? Recall that sex allocation patterns were also consistent with within-colony relatedness in these two populations. In Aberfoyle, the two colony classes did not differ in sex investment ratios, suggesting that they may not have differed in relatedness asymmetry, but they did differ in mean colony size. The percentage of sexual brood produced by the two colony classes in Aberfoyle was highly variable with no overall difference between them. This would implicate relatedness asymmetry as a more important factor in resource allocation than colony size. Unfortunately, without data on within-colony relatedness for this population, the various effects of relatedness asymmetry and colony size on resource allocation could not be confirmed.

§5.4 *Who controls allocation?*

Kin selection theory allows the prediction of preferred sex and resource allocation schedules under both queen and worker control of allocation. However, many factors are predicted to affect Hymenopteran sex ratio patterns. Furthermore, several factors causing both male and / or female bias (reviewed in Bourke and Franks 1995) may be operating on sex allocation patterns at the same time. Any number of these factors could combine to produce a wide spectrum of population and colony level sex allocation patterns. The three study populations produced very different sex allocation patterns. One population sex investment ratio was very male-biased, another was very female biased, with the third being not significantly different from 1 : 1 with sex ratios split according to colony class. Thus, is it still possible to determine which party (or parties) controlled allocation in this species?

A model by Pamilo (1991a) could not differentiate between queen and worker control over resource allocation. In fact, when the probability of independent nest-founding is low, and colony survival prospects are low, queen and worker resource allocation interests are predicted to converge. Polygynous colonies concentrating on new worker production may have resulted from a reduced relatedness asymmetry, and thus reduced desire for diploid brood to develop into sexuals. A more likely explanation is that both queens and workers in polygynous colonies favoured raising new workers to stock daughter buds.

Split sex ratios in the direction predicted by Boomsma and Grafen's (1990, 1991) theory supported worker control of sex allocation in Santon Warren. However, polygynous colonies also appeared to respond to local resource competition in producing male-biased broods. This response is predicted to be favoured by queens as well as workers. Furthermore, monogynous colonies production of female-biased broods may have resulted from workers response to relative relatedness asymmetry, but also may have stemmed from sex ratio compensation for the male bias in polygynous colonies. When one class of colony responds to sex ratio specialisation by another class of colony, the interests of queens and workers converge.

Two pieces of evidence support worker control of allocation in this species. The first is a lack of influence of queen number over sex investment ratios in polygynous colonies from all three populations. There was no sign that queens gained partial control of sex allocation (Herbers 1984, 1990) when the relative number of colony workers, or the number of sexual brood in colonies was taken into account. Under facultative polygyny, Pamilo (1990) predicted that preferred sex ratios had to take into account the parameter '*q*', which varies with the extent of non-dispersing queens. Under even low levels of queen recruitment, the queen's preferred sex investment ratio is never predicted to rise above 0.50, whereas that of workers may still vary between strong male and female bias. Because the parameter *q* could not be measured (no measure of *q* has been made for any species), the adjusted queen's preferred population sex investment ratio could not be calculated. The population sex investment ratio from Santon Warren in 1995 was 0.68, which did not differ significantly from 0.5, but may have differed from the adjusted queen's preferred population sex investment ratio, and Aberfoyle's population sex investment ratio in 1995 was even more female-biased than this.

Worker control of sex and resource allocation in this species was supported in general, and no evidence of queen control was found. In many cases, however, queens' and workers' interests may have converged. These results add to the almost unequivocal support for worker control of sex and resource allocation in social Hymenoptera (see Bourke and Franks 1995; Crozier and Pamilo 1996, for reviews; also see Alexander and Sherman 1977; Herbers 1984, 1990; Sherman and Shellman-Reeve 1994; Banschbach and Herbers 1996a, b).

§5.5 *Future work*

The precise prediction of sex investment ratios is the holy grail of Hymenopteran sex allocation studies. Ideally, relatedness data are required for whole populations, as well as intra- and inter-colony relatedness data to demonstrate that workers actually respond to within colony relatedness asymmetry when making sex allocation decisions. Recent techniques in the field of genetics such as the analysis of microsatellite DNA variation are allowing more detailed understanding of colony and population genetic structures. But the technique is still relatively slow, laborious and costly. The structure of study populations need to be elaborated in terms of frequencies of occurrence of different colony types, and in terms of effective population size, along with more information on relative costs of producing different castes (Boomsma *et al.* 1995; Crozier and Pamilo 1996). An understanding of the effects and strengths of environmental influences on sex allocation needs to be obtained specifically for each population. As Herbers (1990) noted, significant variation between years supports the need for long term studies. Finally, these field-based studies need to be supported by experimental evidence.

As the theory surrounding this field expands, more factors affecting sex allocation come into focus. The field of resource allocation is still in its infancy in terms of theoretical predictions and attempts to test what theory is available. For an all encompassing study like the one suggested above to attempt to fully understand sex and resource allocation in a population of Hymenoptera is not yet feasible, if it ever will be. Crozier and Pamilo (1996) suggested a series of approaches to uncovering the factors affecting allocation in social insects. They proceeded from comparisons between species down to colony-by-colony genetic portraits of populations. Cross-species comparisons may still be the most concrete evidence of worker-controlled sex allocation in social Hymenoptera. However, detailed, single-species studies allow a better understanding of the processes directly affecting sex allocation in Hymenoptera, and how these vary over time and between populations.

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Appendix A

Composition and annual production of **Leptothorax acervorum** *colonies.*

Table A.1	Santon Warren 1993
Table A.2	Santon Warren 1994
Table A.3	Santon Warren 1995
Table A.4	Aberfoyle 1994
Table A.5	Aberfoyle 1995
Table A.6	Roydon Wood 1995

DQ = number of dealate, colony queens,

OW = number of original, old workers,

NW = number of new workers produced,

F = number of new queens produced,

M = number of males produced.

* = data not available. In several colonies, queens were not successfully dissected. Therefore, these individuals could not be correctly designated either dealate queens or new queens.

Table A.1: Santon Warren 1993

colony number	DQ	OW	NW	F	M
SD 93 17	8	29	87	5	0
SD 93 18	1	41	30	11	16
SD 93 19	11	71	100	9	36
SD 93 20	5	127	117	11	47
SD 93 21	1	43	33	4	27
SD 93 22	1	93	58	91	34
SD 93 23	11	136	226	32	58
SD 93 24	1	18	52	1	0
SD 93 25	1	54	25	8	0
SD 93 26	1	59	90	15	39
SD 93 27	1	31	18	19	0
SD 93 28	4	90	141	5	9
SD 93 29	3	56	*	12	0
SD 93 30	1	140	111	185	68
SD 93 31	1	147	152	131	69
SD 93 32	43	263	294	4	16
SD 93 33	1	142	96	119	151
SD 93 34	1	87	96	81	41
SD 93 35	1	176	108	170	122
SD 93 36	1	202	115	79	128
SD 93 37	5	141	161	1	2
SD 93 38	3	28	79	0	0
SD 93 39	8	183	234	8	1
SD 93 40	8	20	60	3	7
SD 93 41	25	168	309	10	141
SD 93 42	39	31	2	2	0
SD 93 43	7	136	258	9	82
SD 93 44	1	86	111	116	49
SD 93 45	3	107	54	10	43
SD 93 46	9	61	176	5	61
SD 93 47	2	71	29	0	1
SD 93 48	1	134	54	143	63
SD 93 49	1	36	42	1	1
SD 93 50	0	21	33	1	1
SD 93 51	5	148	146	67	18
SD 93 52	5	161	143	4	10
SD 93 53	1	40	39	77	11
SD 93 54	1	89	67	51	0
SD 93 55	19	208	191	35	76
SD 93 56	4	80	265	11	13
SD 93 57	1	80	139	31	3
SD 93 58	6	323	207	45	114
SD 93 59	3	54	44	6	2
SD 93 60	1	47	66	2	1
SD 93 61	1	88	72	0	0
SD 93 62	4	29	158	3	2
SD 93 63	5	49	42	1	13

Table A.2: Santon Warren 1994

colony number	DQ	OW	NW	F	M
SD 94 11	0	53	90	31	2
SD 94 12	1	86	94	35	13
SD 94 13	1	39	31	3	0
SD 94 14	0	185	116	87	0
SD 94 15	1	11	2	0	0
SD 94 16	1	31	16	8	3
SD 94 17	11	126	273	15	51
SD 94 18	1	19	12	0	0
SD 94 19	1	45	95	21	11
SD 94 20	1	104	99	22	16
SD 94 21	1	109	40	36	14
SD 94 22	1	61	49	5	16
SD 94 23	1	232	39	93	38
SD 94 24	2	198	8	24	24
SD 94 25	0	108	38	38	16
SD 94 26	0	213	18	61	33
SD 94 27	1	110	81	20	12
SD 94 28	1	156	74	121	48
SD 94 29	5	121	7	1	1
SD 94 30	10	244	80	8	48
SD 94 31	0	99	22	56	33
SD 94 32	1	49	9	5	0
SD 94 33	1	33	36	4	2
SD 94 34	0	141	56	43	21
SD 94 35	12	26	43	4	4
SD 94 36	1	83	59	33	14
SD 94 37	1	69	1	0	33
SD 94 38	1	75	45	56	8
SD 94 39	*	37	16	*	0
SD 94 40	0	64	43	4	0
SD 94 41	1	58	65	49	17
SD 94 42	0	22	7	0	0
SD 94 43	0	50	11	14	47
SD 94 44	1	29	19	0	0
SD 94 45	1	59	58	14	22
SD 94 46	4	38	36	0	0
SD 94 47	0	28	3	2	1
SD 94 48	1	10	17	1	0
SD 94 49	1	36	41	20	14
SD 94 50	2	44	9	0	0
SD 94 51	1	41	55	16	43
SD 94 52	2	139	85	13	122
SD 94 53	4	78	117	11	0
SD 94 54	*	162	81	*	18
SD 94 55	1	69	109	43	14
SD 94 56	4	231	211	49	1
SD 94 57	10	303	205	54	119
SD 94 58	*	39	49	*	0
SD 94 59	1	35	8	6	2

Table A.3: Santon Warren 1995

colony number	DQ	OW	NW	F	M
SD 95 01	1	23	17	2	0
SD 95 02	1	25	60	3	3
SD 95 03	1	109	79	11	22
SD 95 04	1	45	66	5	8
SD 95 05	2	121	134	7	26
SD 95 06	1	65	45	11	1
SD 95 07	1	168	96	197	123
SD 95 08	3	62	7	2	2
SD 95 09	0	61	41	19	9
SD 95 10	1	59	66	83	4
SD 95 11	0	35	55	2	0
SD 95 12	1	50	103	4	0
SD 95 13	1	148	109	214	65
SD 95 14	1	92	45	108	2
SD 95 15	1	21	0	0	3
SD 95 16	4	54	83	1	5
SD 95 17	5	51	38	1	0
SD 95 18	1	91	95	19	119
SD 95 19	6	89	248	9	1
SD 95 20	1	87	53	29	4
SD 95 21	3	51	73	0	2
SD 95 22	1	23	45	1	0
SD 95 23	1	49	103	23	16
SD 95 24	1	20	81	5	2
SD 95 25	10	125	264	1	2
SD 95 26	1	156	95	108	66
SD 95 27	2	10	12	0	0
SD 95 28	1	37	142	0	0
SD 95 29	4	21	19	0	0
SD 95 30	5	122	305	18	4
SD 95 31	1	43	80	4	0
SD 95 32	*	24	14	*	0
SD 95 33	1	117	118	125	18
SD 95 34	1	26	29	2	0
SD 95 35	1	29	55	10	7
SD 95 36	3	71	118	0	4
SD 95 37	1	17	39	0	0
SD 95 38	2	65	128	4	4
SD 95 39	4	40	93	2	6
SD 95 40	1	3	5	0	0
SD 95 41	1	42	114	68	45
SD 95 42	1	47	96	108	10
SD 95 43	1	54	98	55	1
SD 95 44	1	33	131	6	5
SD 95 45	1	46	*	20	6
SD 95 46	1	147	74	46	18
SD 95 47	*	24	136	*	0
SD 95 48	1	30	40	9	0
SD 95 49	1	55	70	55	4
SD 95 50	0	6	35	0	0
SD 95 51	*	11	24	*	5

Table A.3 continued: Santon Warren 1995

colony number	DQ	OW	NW	F	M
SD 95 52	1	50	93	47	2
SD 95 53	1	11	16	0	0
SD 95 54	*	*	*	*	*
SD 95 55	0	4	0	0	0
SD 95 56	3	56	36	14	10
SD 95 57	4	34	51	2	9
SD 95 58	1	17	40	8	3
SD 95 59	1	45	122	0	0
SD 95 60	1	33	24	1	5
SD 95 61	1	30	64	2	4
SD 95 62	1	72	85	58	0
SD 95 63	1	35	72	1	4
SD 95 64	1	69	137	49	26
SD 95 65	1	29	71	9	0
SD 95 66	0	54	69	100	7
SD 95 67	0	11	27	10	3
SD 95 68	*	*	*	*	*
SD 95 69	3	45	51	0	4
SD 95 70	1	46	49	9	13
SD 95 71	1	81	55	69	26
SD 95 72	1	103	136	112	29
SD 95 73	1	34	45	1	3
SD 95 74	1	84	42	7	0
SD 95 75	0	45	14	1	0
SD 95 76	10	61	110	6	82
SD 95 77	1	70	25	82	8
SD 95 78	1	12	8	0	0
SD 95 79	1	51	49	28	24
SD 95 80	1	62	52	28	19
SD 95 81	1	77	63	36	30
SD 95 82	1	75	0	9	51
SD 95 83	0	33	11	86	10
SD 95 84	0	61	55	36	12
SD 95 85	0	155	58	60	80
SD 95 86	0	36	25	10	0
SD 95 87	13	38	242	3	0
SD 95 88	5	31	112	10	0
SD 95 89	1	5	0	0	0
SD 95 90	*	*	*	*	*
SD 95 91	1	43	76	55	19
SD 95 92	1	48	70	28	0
SD 95 93	1	34	75	22	1
SD 95 94	3	62	163	6	47
SD 95 95	1	52	130	44	19
SD 95 96	1	39	43	5	3
SD 95 97	1	69	93	9	25
SD 95 98	1	42	13	8	8
SD 95 99	1	52	91	52	31
SD 95 100	1	109	93	101	35
SD 95 101	1	78	136	42	31
SD 95 102	1	59	128	30	0

Table A.4: Aberfoyle 1994

colony number	DQ	OW	NW	F	M
AB 94 01	0	65	27	6	11
AB 94 02	1	102	39	41	19
AB 94 03	1	94	26	0	1
AB 94 04	1	29	36	0	1
AB 94 05	1	99	16	79	17
AB 94 06	1	215	37	77	7
AB 94 07	0	64	88	43	1
AB 94 08	0	31	20	3	0
AB 94 09	10	172	244	69	41
AB 94 10	1	49	45	8	0
AB 94 11	1	30	28	0	3
AB 94 12	8	254	232	46	4
AB 94 13	2	23	39	0	3
AB 94 14	1	85	22	30	1
AB 94 15	125	654	1160	101	51
AB 94 16	1	28	8	3	0
AB 94 17	1	86	67	20	36
AB 94 18	5	15	35	0	0
AB 94 19	10	174	134	56	16
AB 94 20	1	94	42	8	0
AB 94 21	1	89	47	31	9
AB 94 22	0	80	59	15	46
AB 94 23	1	20	39	0	0
AB 94 24	1	134	58	26	46
AB 94 25	1	40	4	0	0
AB 94 26	0	41	48	0	1
AB 94 27	1	97	73	25	3
AB 94 28	1	98	49	37	4
AB 94 29	1	102	23	8	14
AB 94 30	6	78	146	0	29
AB 94 31	1	73	49	1	21
AB 94 32	20	210	287	0	4
AB 94 33	1	14	30	0	0
AB 94 34	1	48	57	0	4

Table A.5: Aberfoyle 1995

colony number	DQ	OW	NW	F	M
AB 95 01	102	525	174	262	111
AB 95 02	1	34	51	1	3
AB 95 03	1	70	35	24	6
AB 95 04	1	39	17	0	0
AB 95 05	0	30	59	0	0
AB 95 06	1	68	20	0	3
AB 95 07	1	141	42	60	40
AB 95 08	1	39	78	2	0
AB 95 09	1	135	48	36	9
AB 95 10	1	115	19	38	8
AB 95 11	25	514	429	185	42
AB 95 12	18	565	338	164	19
AB 95 13	1	95	30	46	37
AB 95 14	1	59	52	24	9
AB 95 15	2	68	75	4	1
AB 95 16	5	58	86	9	14
AB 95 17	1	28	78	0	6
AB 95 18	1	45	36	3	8
AB 95 19	1	82	14	42	0

Table A.6: Roydon Wood 1995

colony number	DQ	OW	NW	F	M
RW 95 01	6	63	54	5	48
RW 95 02	6	63	73	7	59
RW 95 03	3	68	51	3	20
RW 95 04	2	52	52	2	5
RW 95 05	1	9	12	0	15
RW 95 06	1	35	57	1	5
RW 95 07	10	94	88	7	60
RW 95 08	2	13	9	1	3
RW 95 09	1	61	7	0	69
RW 95 10	1	64	65	1	13
RW 95 11	2	79	24	1	33
RW 95 12	10	74	39	1	15
RW 95 13	0	12	2	0	12
RW 95 14	9	31	28	0	53
RW 95 15	10	20	44	0	13
RW 95 16	7	68	27	0	2
RW 95 17	5	54	84	1	16
RW 95 18	1	8	14	0	0
RW 95 19	1	37	81	5	3
RW 95 20	3	25	26	0	2
RW 95 21	1	67	39	7	1
RW 95 22	3	18	43	0	6
RW 95 23	4	90	50	11	17
RW 95 24	2	67	93	2	2
RW 95 25	3	34	71	4	6
RW 95 26	1	63	54	3	0
RW 95 27	2	34	45	3	6
RW 95 28	10	21	19	1	6
RW 95 29	2	19	8	0	37
RW 95 30	1	66	54	1	0
RW 95 31	3	41	25	0	7
RW 95 32	2	5	1	0	0
RW 95 33	3	48	17	0	3
RW 95 34	1	47	61	1	2
RW 95 35	2	31	26	0	0
RW 95 36	9	80	43	3	1

Appendix B

Calculation of mean dry weight values for all three castes.

Callow workers

A total of 63 callow workers was sampled in 1993 and 1994 and measured for dry weight and thorax length (Table B.1). There was no significant difference in mean thorax length between monogynous and polygynous colony classes, or between years (ANOVA, year: $F_{1,60} = 1.03, p > 0.05$; class: $F_{1,60} = 1.07, p > 0.05$; year*class: $F_{1,60} = 2.51, p > 0.05$). However, there was a strong effect of year on log dry weight of callow worker (ANOVA, year: $F_{1,60} = 93.45, p < 0.0001$; class: $F_{1,60} = 1.33, p > 0.05$; year*class: $F_{1,60} = 5.43, p < 0.05$). This was likely to have stemmed from the fact that workers from 1993 were sampled immediately after eclosion into adults, while the 1994 samples were taken from adults that were no longer callow (and therefore had already gained post-eclosion weight). It was decided to use weight data from the 1993 samples of callow workers only as these represented a more accurate estimate of investment in dry weight of callow workers than the 1994 samples did. There was a significant regression of log dry weight on log thorax length in the 1993 samples ($F_{1,24} = 13.89, p < 0.005$). Thorax-only measurements were made for 261 workers from three consecutive years (Table B.1), and there was no significant difference in mean thorax length between monogynous and polygynous colony classes, or between years (ANOVA, year: $F_{2,256} = 1.36, p > 0.05$; class: $F_{1,256} = 0.96, p > 0.05$; year*class: $F_{2,256} = 2.28, p > 0.05$). The overall mean log thorax length for callow workers from all samples was 0.389. Using the regression equation for log dry weight versus log thorax length for callow workers from 1993:

$$\text{log dry weight} = -1.383 + 2.392 * \text{log thorax length},$$

gives an overall mean log dry weight of -0.453, which transforms to a mean dry weight for callow workers of 0.353 mg.

Alate queens

A total of 60 alate queens was taken from colonies in 1993 and 1994 (Table B.1) and measured for dry weight and thorax length. There was no difference between years or classes for either mean log dry weight (ANOVA, year: $F_{1,57} = 3.23$, $p > 0.05$; class: $F_{1,57} = 0.79$, $p > 0.05$; year*class: $F_{1,57} = 2.11$, $p > 0.05$) or mean log thorax length (ANOVA, year: $F_{1,57} = 0.09$, $p > 0.05$; class: $F_{1,57} = 0.33$, $p > 0.05$; year*class: $F_{1,57} = 1.17$, $p > 0.05$). After pooling data for year and class, there was a significant regression of log dry weight on log thorax length ($F_{1,59} = 27.54$, $p < 0.0001$). The overall mean log thorax length taken from measurements of 196 alate queens (Table B.1) was 0.452. Using the equation from the regression of log dry weight versus log thorax length for alate queens:

$$\text{log dry weight} = -1.701 + 3.109 * \text{log thorax length},$$

gives an overall mean log dry weight of -0.296, which transforms to a mean dry weight for alate queens of 0.506 mg.

Males

A total of 70 males was taken from colonies in 1993 and 1994 (Table B.1) and measured for dry weight and thorax length. There was a significant effect of colony class, but not year on both mean log dry weight (ANOVA, year: $F_{1,67} = 2.85$, $p > 0.05$; class: $F_{1,67} = 7.99$, $p < 0.01$; year*class: $F_{1,67} = 6.09$, $p < 0.05$) and mean log thorax length (ANOVA, year: $F_{1,67} = 1.30$, $p > 0.05$; class: $F_{1,67} = 7.13$, $p < 0.01$; year*class: $F_{1,67} = 9.82$, $p < 0.005$). Mean dry weight values for males were calculated for monogynous and polygynous colonies separately.

monogynous colonies

The regression of log dry weight on log thorax length was significant ($F_{1,34} = 17.31$, $p < 0.0005$). The overall mean log thorax length taken from measurements of 131 alate queens over three years (Table B.1) was 0.549, and there was no effect of year (ANOVA: $F_{2,129} = 1.66$, $p > 0.05$). Using the regression equation for log dry weight versus log thorax length for monogynous males:

$$\text{log dry weight} = -1.182 + 1.620 * \text{log thorax length},$$

gives an overall mean log dry weight of -0.293, which transforms to a mean dry weight for males in monogynous colonies of 0.510 mg.

polygynous colonies

The regression of log dry weight on log thorax length was significant ($F_{1,34} = 77.87$, $p < 0.0001$). The overall mean log thorax length of 75 males in polygynous colonies taken from the two available years (Table B.1) was 0.531, and there was no difference between years (*t*-test: $t = 1.54$, d.f. = 73, $p > 0.05$). Using the regression equation for log dry weight versus log thorax length for polygynous males:

$$\text{log dry weight} = -1.797 + 2.720 * \text{log thorax length},$$

gives an overall mean log dry weight of -0.353, which transforms to a mean dry weight for males in polygynous colonies of 0.444 mg.

Table B.1. Numbers of ants of three different castes sampled for weight and thorax measurements for calculating dry weights. Samples were taken from both colony classes in all three years, although weight data were not available for all ants measured. Calculation of mean dry weights is given in Appendix B.

colony	class	workers	queens	males	length	weight
SD 93 21	M	-	-	5	✓	✓
SD 93 44	M	5	5	5	✓	✓
SD 93 46	P	5	-	5	✓	✓
SD 93 48	M	-	5	5	✓	✓
SD 93 51	P	5	5	5	✓	✓
SD 93 53	M	-	5	-	✓	✓
SD 93 54	M	5	5	-	✓	✓
SD 93 55	P	5	5	5	✓	✓
SD 94 12	M	10	10	10	✓	x
SD 94 17	P	9	-	10	✓	✓
SD 94 19	M	10	10	8	✓	x
SD 94 21	M	10	10	10	✓	x
SD 94 24	P	10	10	10	✓	x
SD 94 26	M	10	10	10	✓	✓
SD 94 30	P	10	8	10	✓	x
SD 94 31	M	10	10	10	✓	✓
SD 94 34	M	9	10	10	✓	x
SD 94 52	P	10	10	10	✓	✓
SD 94 56	P	10	10	10	✓	x
SD 94 57	P	10	9	10	✓	x
SD 95 03	M	9	9	9	✓	x
SD 95 07	M	10	10	10	✓	x
SD 95 08	P	10	-	-	✓	x
SD 95 13	M	10	10	10	✓	x
SD 95 16	P	10	-	-	✓	x
SD 95 17	P	10	-	-	✓	x
SD 95 18	M	10	10	10	✓	x
SD 95 19	P	10	-	-	✓	x
SD 95 23	M	10	10	10	✓	x
SD 95 25	P	10	-	-	✓	x
SD 95 26	M	9	10	9	✓	x
SD 95 30	P	10	-	-	✓	x

Appendix C

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Split sex ratios in a multiple-queen ant population

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SUMMARY

Split sex ratio theory is an important extension of sex allocation theory. It suggests that colony sex ratios in social insects vary because workers control sex allocation and respond to variations in their comparative relatedness with females and males (relatedness asymmetry). In a population of the ant *Leptothorax acervorum*, 21 monogynous (single-queen) colonies produced a female-biased sex ratio (62% females), and 24 polygynous (multiple-queen) colonies produced a male-biased sex ratio (28% females). Within the polygynous colonies, queen number did not affect sex ratio (with colony productivity statistically controlled). As colony productivity rose, the sex ratio either did not change (monogynous colonies) or became more male-biased (polygynous colonies). The fraction invested in sexuals rose with increasing colony size and productivity in monogynous but not in polygynous colonies, which invested less in sexuals. These findings suggest that split sex ratios in *L. acervorum* stem from two processes. The first is workers' response to the variation in relatedness asymmetry caused by variable queen numbers. The second is sex-ratio compensation by monogynous colonies for male-biased production in polygynous colonies. This arises because polygynous colonies reproduce, partly, by colony budding and so have daughter colonies subject to local resource competition.

1. INTRODUCTION

Trivers & Hare (1976) combined Fisher's (1930) sex ratio theory with Hamilton's (1964) inclusive fitness theory to explain population sex investment ratios in the social Hymenoptera (ants, bees and wasps). The stable population sex ratio for the party controlling sex allocation is predicted to equal its relatedness asymmetry (life-for-life relatedness with females: life-for-life relatedness with males) (Boomsma & Grafen 1990, 1991). For example, if workers control sex allocation, the stable population sex ratio in the simplest case (one singly-mated queen per colony, sterile workers, and random mating) should be female-biased (3:1 females:males), since workers' relatedness with females equals 0.75 and with males equals 0.25.

Population sex ratios of ant species with different social structures broadly conform to Trivers and Hare's (1976) predictions, assuming worker control (Trivers & Hare 1976; Nonacs 1986*a*; Boomsma 1989; Pamilo 1990). However, the idea of worker control remains controversial (e.g. Alexander & Sherman 1977; Sherman & Shellman-Reeve 1994). In addition, the colony sex ratios of ant populations vary more than would be expected by chance alone. Specifically, colonies tend to produce single-sex broods (Nonacs 1986*a*).

Boomsma & Grafen (1990, 1991) explained these split sex ratios (Grafen 1986) as a result of between-colony variation in the workers' relatedness asymmetry. This variation may occur through variable levels of queen mating frequency or queen number within populations. Boomsma & Grafen (1990, 1991) showed that workers should bias the sex ratio of the brood towards that sex to which they are comparatively more closely related than the average for the population. For example, if queen number varies, workers should favour raising females in monogynous (single-queen) colonies and males in polygynous (multiple-queen) colonies (Boomsma & Grafen 1990, 1991; Boomsma 1993).

Evidence for split sex ratio theory has been found in social bees and wasps (Boomsma 1991; Mueller 1991; Queller *et al.* 1993; Packer & Owen 1994). In an ant species with partial multiple mating, split sex ratios were recently demonstrated by Sundström (1994*a*). As expected, colonies headed by a singly mated queen produced female-biased broods, whereas those headed by a multiply mated queen had male-biased broods (see also Bourke & Chan 1994; Sherman & Shellman-Reeve 1994; Sundström 1994*b*). In polygynous ants, split sex ratio theory is supported by the observation that colony queen number and male bias in the brood sex ratio tend to rise together (Boomsma 1993). Examples occur in studies by Ward (1983), Elmes (1987), and, on *Leptothorax* species, by Herbers (1984, 1990).

However, sex ratios could be split in polygynous ants for reasons other than variation in workers' relatedness asymmetry. First, male bias could rise as queen control increases in the more polygynous colonies, since queens always prefer less female-biased broods than workers (Herbers 1984, 1990). Secondly, resource abundance may lead productive colonies to favour female production (Nonacs 1986*a*, *b*). Thirdly, productive colonies could favour greater female bias because their males experience stronger local mate competition (Frank 1987). Fourth, polygynous colonies often

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reproduce by budding, in which groups of queens and workers found new colonies near the parent colony. Budding leads to local resource competition between the daughter colonies and hence to male-biased sex allocation (Frank 1987; Pamilo 1991). Therefore the existence of a fraction of colonies that bud within a population could lead to split sex ratios (Boomsma 1993; Nonacs 1993).

This paper describes split sex ratios in a polygynous ant, *Leptothorax acervorum*. We provide evidence that they do not result from either partial queen control, resource abundance effects, or local mate competition. Instead, they appear to be due to a combination of variation in workers' relatedness asymmetry and local resource competition due to colony budding.

2. MATERIALS AND METHODS

Leptothorax acervorum was studied in a population from Santon Warren, Norfolk, U.K. (Bourke 1991). This population is facultatively, secondarily polygynous (new queens are acquired by adoption). Colonies occupy dead twigs and so can be collected whole (except for foraging workers) by fragmenting the twig and aspirating the ants and brood (Bourke 1991). Single colonies occupy single twigs (monodomy), allowing colony-level sex allocation to be examined with relative ease.

The population has been studied genetically by Heinze *et al.* 1994) using allozyme analysis of two polymorphic loci on samples collected in 1991 and 1992. As in other polygynous *L. acervorum* populations (Stille *et al.* 1991; Heinze *et al.* 1994), queens within colonies were related on average (queen–queen relatedness was 0.26), indicating that many queens are readopted by their natal colony. Worker–worker relatedness (±s.e.) was significantly higher in monogynous colonies (0.50±0.06; $n = 34$ colonies) than in polygynous ones (0.28±0.09; $n = 19$ colonies) (Heinze *et al.* 1994). We therefore infer that workers' relatedness asymmetry was truly lower in polygynous colonies than in monogynous ones. Worker–worker relatedness presumably fell below 0.75 in monogynous colonies despite the likelihood that queens mate singly (Stille *et al.* 1991; Heinze *et al.* 1994) because some had previously been polygynous. The genetic data also suggested that inbreeding was absent from the Santon Warren population (Heinze *et al.* 1994). Finally, low worker egg-laying rates imply that worker male production was negligible (Bourke 1991).

L. acervorum males almost certainly mate both in large swarms and near nests (Bourke & Heinze 1994). The lack of evidence for inbreeding suggests that males disperse widely enough to render local mate competition among them unlikely. However, there is strong evidence that polygynous *L. acervorum* colonies reproduce by budding (see, for example, Stille & Stille 1993; Bourke & Heinze 1994), suggesting that local resource competition due to budding is probable.

Forty-seven colonies of *L. acervorum* were collected on a single day, 17 June 1993. Since all colonies found were collected, the sample was considered a random population sample. Twigs containing colonies were returned to the laboratory in plastic bags. All ants and brood were then aspirated, transferred to standard nests, and kept at room temperature under a standard feeding and watering regime (Bourke 1991).

Between 24 June and 7 July, before the maturation of any worker pupae (except in a few colonies), the first of two censuses was carried out. All adults and pupae were counted. To ensure accuracy, counts in both censuses were performed by transferring individual adults or brood items with fine forceps. In the first census, all pupae and sexuals (young winged queens and males) were removed from the colonies. The colonies were then kept at room temperature for 4 months, after which a second census was performed to measure the number and caste of any larvae maturing into adulthood since the first census (almost all these became workers). Comparison of the two censuses gave each colony's original composition and its total annual production of new workers, new queens and males.

During the second census, all dealate queens were removed from the colonies. Dealate queens have shed their wings and are potential colony queens. Their reproductive status was established by ovarian dissection (Bourke 1991). All queens with sperm in the sperm receptacle were counted as colony queens. By contrast, any virgin queens among the dissected dealates were classified as new queen production, and were assumed to derive from young queens that had shed their wings in the nest following collection. This was justified because all dealate queens from colonies collected in April and May in previous years (1990–1991) proved to be mated on dissection ($n = 72$ queens; A. F. G. Bourke, unpublished data). For the same reason, any dealate queens present on collection that died before dissection were classified as mated queens. The mated queens were unlikely to have included any newly produced queens that had mated in the nest after collection, because laboratory experiments suggest that mating in the nest does not occur in *L. acervorum* from the study population (A. F. G. Bourke, unpublished data).

To measure dry mass, 25 callow (newly emerged) workers, 30 alate (young, winged) queens and 30 males were removed from arbitrarily chosen colonies. After drying in an oven at 60 °C for 24 h, each ant was weighed on an electronic microbalance.

3. RESULTS

Of 47 colonies, one was queenless, 21 (45%) were monogynous and 25 (53%) were polygynous (see table 1). For further analysis, the queenless colony was classified as monogynous. In the polygynous colonies, the harmonic mean of queen number was 5.3 (see table 1).

The average dry masses of callow workers, alate queens and males were, respectively, 0.37 mg, 0.51 mg and 0.51 mg. Although significant between-colony variation in these dry masses was detected, this information could not be used because only six colonies were sampled for dry mass. However, the variation was apparently due to rare colonies producing exceptionally small individuals. Since females and males had identical dry mass at the population level, numerical sex ratios were taken to equal investment sex ratios in this population (Boomsma 1989).

Two of the 47 colonies (one monogynous and one polygynous) produced no sexuals (see table 1). The population sex ratio (95% confidence limits) was 0.51 (0.43–0.58) ($n = 45$ colonies). (We express all sex ratios as the proportion of females.) These values were calculated by weighting each colony by its total sexual productivity divided by the average sexual productivity of all colonies. The weights were then treated as frequencies in frequency-structured data. This method (J. J. Boomsma, personal communication) reflects the greater contribution of the more productive colonies to the population sex ratio.

Table 1. *Composition and annual production of 47* Leptothorax acervorum *colonies*

(DQ, number of dealate, colony queens; OW, number of original, old workers; NW, number of new workers produced; F, number of new queens produced; M, number of males produced.)

colony number	DQ	OW	NW	F	M
monogynous and queenless colonies ($n = 22$)					
93.50	0	21	33	1	1
93.18	1	41	30	11	16
93.21	1	43	33	4	27
93.22	1	93	58	91	34
93.24	1	18	52	1	0
93.25	1	54	25	8	0
93.26	1	59	90	15	39
93.27	1	31	18	19	0
93.30	1	140	111	185	68
93.31	1	147	152	131	69
93.33	1	142	96	119	151
93.34	1	87	96	81	41
93.35	1	176	108	170	122
93.36	1	202	115	79	128
93.44	1	86	111	116	49
93.48	1	134	54	143	63
93.49	1	36	42	1	1
93.53	1	40	39	77	11
93.54	1	89	67	51	0
93.57	1	80	139	31	3
93.60	1	47	66	2	1
93.61	1	88	72	0	0
subtotal	21	1854	1607	1336	824
polygynous colonies ($n = 25$)					
93.47	2	71	29	0	1
93.29	3	56	—	12	0
93.38	3	28	79	0	0
93.45	3	107	54	10	43
93.59	3	54	44	6	2
93.28	4	90	141	5	9
93.56	4	80	265	11	13
93.62	4	29	158	3	2
93.20	5	127	117	11	47
93.37	5	141	161	1	2
93.51	5	148	146	67	18
93.52	5	161	143	4	10
93.63	5	49	42	1	13
93.58	6	323	207	45	114
93.43	7	136	258	9	82
93.17	8	29	87	5	0
93.39	8	183	234	8	1
93.40	8	20	60	3	7
93.46	9	61	176	5	61
93.19	11	71	100	9	36
93.23	11	136	226	32	58
93.55	19	208	191	35	76
93.41	25	168	309	10	141
93.42	39	31	2	2	0
93.32	43	263	294	4	16
subtotal	245	2770	3523	298	752
grand total	266	4624	5130	1634	1576

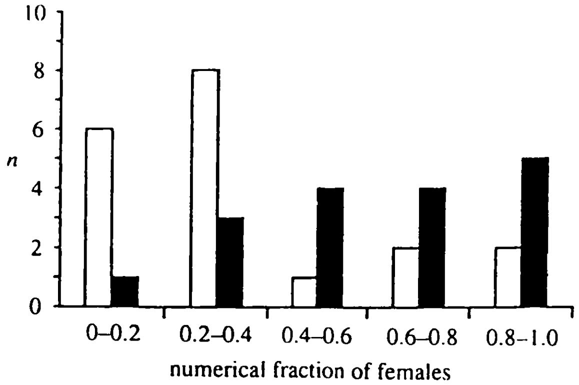

Figure 1. Frequency distribution of sex ratio (numerical fraction of females) in 17 monogynous colonies (black bars) and 19 polygynous colonies (white bars); n, number of colonies. Colonies producing five or fewer sexuals are omitted.

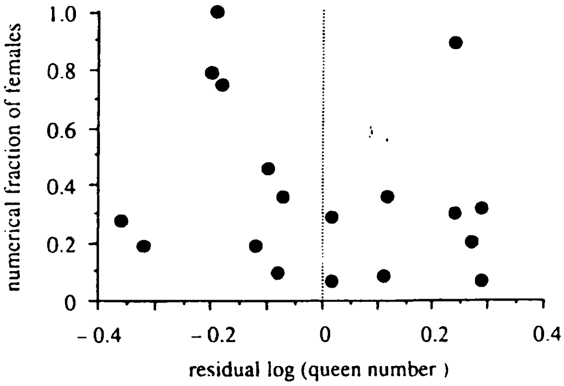

Figure 2. Plot of sex ratio versus residual log queen number in 18 polygynous colonies. There was no significant regression of sex ratio on residual log queen number ($F_{1,16} = 0.83$, $p > 0.3$). The residuals were calculated by regressing log queen number on sexual productivity ($F_{1,16} = 8.70$, $p < 0.01$). There was no significant regression of log queen number on old worker number ($F_{1,16} = 1.87$, $p > 0.1$).

Monogynous colonies tended to produce female-biased broods and polygynous ones to produce male-biased broods (see figure 1). The mean sex ratio (95 % confidence limits) of the monogynous colonies was 0.62 (0.54–0.70) ($n = 21$ colonies). By contrast, the mean sex ratio (95 % confidence limits) of the polygynous colonies was 0.28 (0.19–0.38) ($n = 24$ colonies). (These values were calculated by the same method as used for the population sex ratio.) These means were significantly different (t-test: $t = 2.23$, d.f. $= 32$, $p < 0.05$). Therefore, in this population, sex ratio was split by queen number.

We investigated the effects of queen number on sex allocation within the polygynous colony class. There was no significant regression of sex ratio on queen number ($F_{1,16} = 3.13$, $p > 0.05$). (This and all following analyses of sex allocation omitted six polygynous and five monogynous colonies producing five or fewer sexuals each, and one outlying colony with a queen number that was exceptionally high for those polygynous colonies producing six or more sexuals (colony 93.32 in table 1). Queen number was transformed by taking logarithms.) There was also no significant regression of sex ratio on residual queen number ($F_{1,16} = 0.83$, $p > 0.3$) calculated from the regression of log queen number on total sexual productivity (figure 2). A similar analysis was carried out non-parametrically by calculating Kendall rank-order partial correlation coefficients (Siegel & Castellan 1988). Again, sex ratio was not associated with queen number, controlling for the number of old workers and total sexual production respectively ($T = -0.28$ and -0.12, respectively, both $n = 18$, both $p > 0.05$). If the sex ratio had become more male-biased as queen number increased, this could either have stemmed from greater queen control, or from workers responding to the higher queen numbers by raising more males. However, queen number evidently did not affect sex allocation within the polygynous class. This suggests that the sex ratio difference between the classes did not result from greater queen control under polygyny.

In the monogynous colony class, the partial correlation coefficient of sex ratio with colony size (number of old workers), with total sexual production held constant, was not significant ($r = -0.01$, d.f. $= 14$, $p > 0.05$). Thus falling worker numbers (controlling for productivity) did not result in lower female bias. This further suggested that queens do not gain increasing control of sex allocation at high queen-to-worker ratios (cf. Nonacs 1986*b*). In short, queen-controlled allocation seems unlikely in the study population.

We also examined sex allocation as a function of colony productivity. In monogynous colonies, there was no significant partial correlation of sex ratio with total sexual production, with the number of old workers held constant ($r = -0.09$, d.f. $= 14$, $p > 0.05$). However, in the polygynous colonies, the corresponding partial correlation was significantly negative ($r = -0.51$, d.f. $= 15$, $p < 0.05$). Thus, in monogynous colonies sex ratio did not change as sexual productivity increased, whereas in polygynous colonies it grew more male-biased. In keeping with these findings, within the monogynous colonies biomass invested in females and biomass invested in males both rose with total sexual productivity ($b = 0.57$ and 0.43, respectively, $F_{1,15} =$ 94.7 and 55.5, respectively, both $p < 0.001$), but the gradients of these two regressions were not significantly different ($F_{1,30} = 2.57$, $p > 0.05$). However, in the polygynous colonies, biomass invested in females and biomass invested in males both rose significantly with increasing total sexual productivity ($b = 0.21$ and 0.79, respectively, $F_{1,16} = 8.42$ and 119.3, respectively, both $p < 0.01$), and the gradient of the regression for females was significantly smaller than the corresponding gradient for males ($F_{1,32} = 32.1$, $p < 0.001$).

These findings indicate that, in both colony classes, sex ratio was unaffected by resource abundance; if it were, female bias should always have increased with productivity (Nonacs 1986*b*). They also rule out an interaction of colony productivity and local mate competition, which predicts a flat relation between male production and productivity (Frank 1987). On the other hand, if there is local resource competition due to budding in polygynous colonies, one would expect diminishing returns on female production with increasing productivity (Frank 1987; Pamilo 1991). The observed pattern of sex allocation was consistent with this (cf. Sundström 1993).

Finally, dry mass productivity per worker did not differ significantly in monogynous and polygynous colonies (productivity per worker per colony (s.d.) equals 0.85 (0.35) mg and 0.79 (0.49) mg, respectively; $t = 0.51$, d.f. $= 41$, $p > 0.6$). However, monogynous colonies invested significantly more of their overall production in sexuals than did polygynous colonies (fraction of investment in sexuals (s.d.) equals 0.49 (0.29) and 0.26 (0.19), respectively; $t = 3.25$, d.f. $=$ 35, $p < 0.01$). The two colony types also differed in their productivity schedules. The fraction of investment in sexuals rose with increasing colony size (number of old workers) and total dry mass productivity in monogynous colonies (multiple regression: $r^2 =$ 49.4%, $F_{2,19} = 11.2$, $p < 0.01$), but not in polygynous colonies ($r^2 = 0\%$, $F_{2,21} = 0.9$, $p > 0.4$). These findings are as expected if monogynous colonies concentrate on reproduction by the emission of sexuals, producing workers only for colony growth, whereas polygynous colonies also reproduce by budding and so invest in worker production for both colony growth and for stocking daughter buds.

4. DISCUSSION

These results show that *L. acervorum* colonies within a single population facultatively pursue different sex ratio and life history strategies. Although colonies may change class over time, monogynous colonies concentrate on female production and probably reproduce mainly by emission of sexuals, whereas polygynous colonies concentrate on male production and probably also reproduce by colony budding. There was no evidence that sex ratio splitting stemmed either from partial queen control of sex allocation (Herbers 1984, 1990), or from resource abundance effects (Nonacs 1986*a*, *b*), or from local mate competition (Frank 1987). Instead, sex ratio splitting was consistent with worker control of sex allocation and the workers' response to a variation in relatedness asymmetry caused by variable queen numbers (Boomsma & Grafen 1990, 1991; Boomsma 1993). If this occurs, then the lack of an influence of queen number on sex ratio within the polygynous class suggests that workers can assess the class of colony they are in (monogynous or polygynous) without being able to assess the precise number of queens (cf. Ratnieks 1991). However, the observed split in sex allocation was also consistent with polygynous, budding colonies concentrating on male production because of local resource competition between their daughter colonies (Frank 1987; Pamilo 1991), and monogynous colonies responding to this excess of males by producing mainly females (sex ratio compensation). Split sex ratios due to this process could occur under either queen or worker control, but the absence of other effects of queen number on sex allocation suggest this process was worker controlled.

Can one separate the effects of workers' relatedness asymmetry and colony budding on the sex ratio? This is difficult because both factors may influence sex allocation simultaneously. Consider the class-specific and population sex ratios that would be expected if only one factor affected allocation. If there was no budding, and queens controlled sex allocation completely, no sex ratio splitting should occur because the queens' relatedness asymmetry does not vary with queen number. Given no budding and complete worker control of allocation, sex ratios should be split and the population sex ratio should either equal, or lie between, the relatedness asymmetries of the two classes (Boomsma & Grafen 1990, 1991). However, in the study population, the predicted class-specific sex ratios cannot be calculated (and hence neither can the expected population sex ratio) because the precise relatedness asymmetries were unknown. Another complication is that, when polygyny arises through the adoption of related queens, the expected sex ratio among the dispersing sexuals must be devalued by a parameter (q) that equals the proportion of fitness derived from females that is attributable to dispersing queens (Pamilo 1990). The value of this parameter was also unknown. Furthermore, it might differ systematically between monogynous and polygynous colonies. Under queen control the expected population sex ratio is simply $q/(q+1)$ (Pamilo 1990); since q is by definition less than one, this must always be below 0.5 (male biased).

If polygynous colonies reproduced solely by budding, one would expect their sex investment ratio to be very heavily male biased, but more so under queen than under worker control. This is the case even if the (unknown) fraction of workers that make up a bud are counted as female investment (Pamilo 1991). Under queen control, the monogynous colonies should then overproduce females to restore the population sex ratio to the queens' stable value, whereas under worker control it should be restored to the workers' stable value.

It is more likely that polygynous colonies reproduce both by sexual emission and by budding. In this case queens favour a class-specific sex ratio between a very male-biased value and $q/(q+1)$, whereas workers favour one between a male-biased value and that dictated by their relatedness asymmetry, devalued by q. Since the value of q was unknown, an observed population sex ratio of approximately 1 : 1 cannot be used to distinguish between queen and worker control. However, if queen control was present, one might have expected an effect of queen number on sex allocation within the polygynous colonies, which did not occur. Therefore, the likeliest conclusion is that split sex ratios in *L. acervorum* stem from a combination of the effects of variation in the workers' relatedness asymmetry and of colony budding.

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Appendix D

Bourke, A. F. G., and G. L. Chan. 1994. Split sex ratios in ants with multiple mating. *Trends in Ecology and Evolution* **9**: 120-122.

Split sex ratios in ants with multiple mating

In a famous paper, Trivers and Hare[1] extended Fisher's sex-ratio theory[2] to cover the social Hymenoptera (ants, bees and wasps). Trivers and Hare added two new elements to Fisher's theory. First, following Hamilton's kin selection theory[3], they explicitly included genetic relatedness in the expression for the fitness payoff gained from raising progeny. Secondly and more controversially[4], they proposed that individuals other than parents – namely workers – might control sex allocation. Recently, Boomsma and Grafen's theory of split sex ratios[5–10] has allowed a powerful test of the idea of worker control. It has also offered an explanation for the puzzlingly large amount of between-colony variation in Hymenopteran sex ratios. In a new paper[11], Liselotte Sundström (Dept of Zoology, University of Helsinki) describes work with ants that provides impressive support for split sex-ratio theory, worker control and sex-ratio theory in general.

Split sex-ratio theory

Fisher's theory, as modified by Trivers and Hare, predicts that the stable population sex-investment ratio occurs when the per capita fitness payoffs to the party controlling allocation (per unit cost) are equal for investment in both sexes[12]. If this condition holds, there is no selection for the overproduction of either sex, so the sex ratio remains the same. The fitness payoffs may be measured as the products of 'life-for-life' relatedness (i.e relatedness incorporating sex-specific reproductive value) and mating success (per capita number of mates)[7]. If mating is random, mating success is inversely proportional to abundance, meaning that the male:female ratio of mating successes equals the female:male sex ratio. It follows that, at equilibrium, the female:male population sex ratio equals the relatedness asymmetry (RA), where this is defined as life-for-life relatedness with females divided by that with males[5,6]. This yields the familiar result that in ants with one singly mated queen per colony, the workers' stable sex ratio equals 3:1 [i.e. relatedness with diploid sisters (0.75) divided by relatedness with haploid brothers (0.25)][1].

Significantly, this argument predicts only the population sex ratio. Since mating success is set by relative abundance in a population-wide swarm, individual sex ratios may take any value at equilibrium, provided they collectively yield the stable population level[6]. Therefore, in ants, colony sex ratios might be expected to have a random, unimodal distribution about a population average. But, instead, they are often bimodally distributed, with colonies producing mainly females or mainly males[13]. In addition, the many factors that potentially affect population sex ratios[13,14] pose problems for testing worker control conclusively just by comparing populations[15,16].

Split sex-ratio theory both explains bimodal colony sex ratios, and tests for worker control at the within-population level[5–9]. Sex ratios are split if discrete classes in a population contribute systematically different sex ratios to the same generation of progeny[7]. In the present context, these classes consist of sets of colonies with differing RA. Within ant populations, worker RA varies with queen number, queen mating frequency and the level of worker male-production. Split sex-ratio theory predicts that workers should raise mainly or solely females in colonies whose RA is high relative to the female:male population sex ratio, and mainly or solely males if RA is relatively low[5,6]. So, if worker RA varies and sex allocation is worker-controlled, colony sex ratios should be bimodally distributed and dependent on relative RA. This assumes that workers can assess RA. If they cannot, the population sex ratio should equal the mean per colony RA[6], and any bimodality in colony sex ratios[17] should be uncorrelated with worker RA. Queen RA is unaffected by queen number and mating frequency, since queens are equally related with sons and daughters. So, if queens control allocation, colony sex ratios should be unimodally distributed about the queens' equilibrial level.

Why should workers be responsive to the relative RA? Recall that RA equals the stable population sex ratio because then the per capita fitness payoffs from both sexes are equal. Therefore, if RA exceeds the population sex ratio, females have a relatively high mating success and so yield a greater fitness payoff than males. So, workers favour female production. Similarly, if RA falls below the population sex ratio, males yield more fitness per capita and so are favoured[6]. As RA classes respond to the population sex ratio, their responses alter this ratio itself. The population sex ratio stabilizes when it equals the RA value of one of the classes, since that class then has no incentive to respond further (males and females yield equal fitness), and the other class, as its response, can do no better than produce single-sex broods. Alternatively, when neither class is sufficiently abundant to bias the population sex ratio to its own RA value, the population ratio equilibrates between the RA values of each class. This situation can be stable because both classes then produce single-sex broods whose collective outcome equals the very population ratio to which each class is responding[5,6].

Previous evidence for split sex ratios has come from bees and wasps in which, for example, RA varies according to whether workers raise sibs or their sisters' offspring[8,18,19]. Additional support has come from ants with variable queen numbers[9]. The relative fractions of single-sex broods in species with different social structures are also consistent with the theory[5]. However, because it involves fewer confounding factors, one of the best systems with which to test the theory would be a population of singly queened colonies in which queen mating frequency varies. Sundström's new work[11] is the first to examine just such a system for split sex ratios.

Split sex ratios in wood ants

Sundström studied a population of the wood ant *Formica truncorum* on islands off S.W. Finland. In an unusually thorough and long-term investigation, the sex ratios and genetic composition of individual colonies were tracked over four consecutive years by brood sampling and allozyme analysis, respectively. The genetic work confirmed that colonies were singly queened, mating was random and worker male-production was absent. So, the only source of RA variation, also established with allozyme analysis, was double and triple mating by queens in some colonies. Consistent with split sex-ratio theory, individual colonies produced similar sex ratios in successive years, and those with a singly mated queen concentrated on female production, while those with a multiply mated queen produced mainly males (Fig. 1). The population sex-investment ratio was 0.67 (proportionate investment in females). The female bias of this value, and the fact that colony sex ratios were split in a manner dependent on workers' RA, excludes queen-controlled sex allocation in this population. The dependence of colony sex ratio on RA also rules out worker-controlled allocation with no worker assessment of RA.

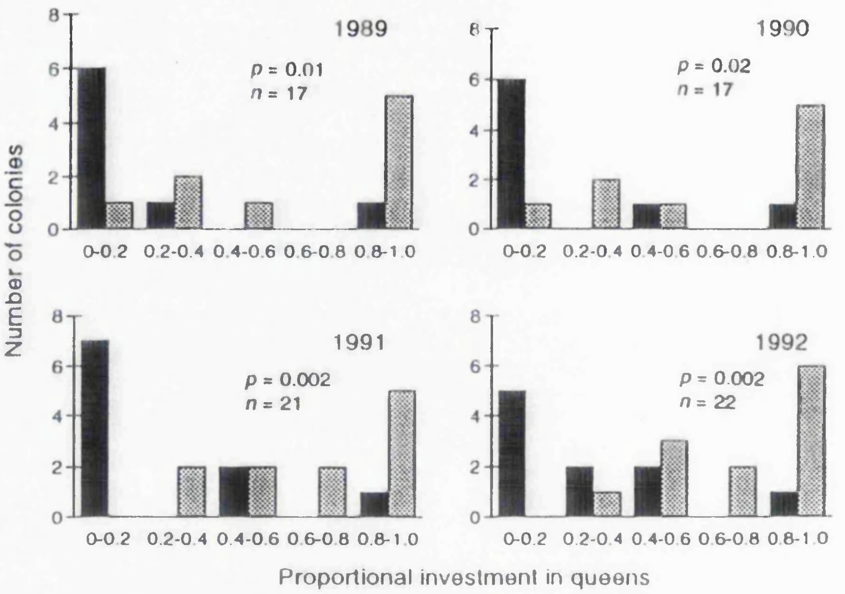

Fig. 1. Frequency distribution of colony sex ratios in a population of *Formica truncorum* over four years. Colonies were headed by either a singly mated queen (grey bars) or a multiply mated queen (black bars). Colonies in these two classes had significantly different sex ratios in each year, with relative investment in females falling with rising mating frequency [Mann–Whitney rank sum test, *p* values and sample size (*n* colonies) as shown on each graph]. *Reprinted, with permission, from Ref. 11.*

In more detail, 13 (57%) of 23 colonies sampled were judged to have a singly mated queen (allozyme analysis showed that worker–female relatedness equalled 0.72). The other 10 colonies (43%) had a doubly or triply mated queen (worker–female relatedness was 0.43). It is a fair assumption that workers can detect multiple mating in their queen but not assess her exact mating frequency. This allows the colonies with double and triple mating to be treated as one class[11]. The RA of the singly mating class was therefore $0.72/0.25 = 2.9$ (worker–male relatedness equals 0.25 independently of mating frequency). Similarly, the RA of the multiply-mating class was $0.43/0.25 = 1.7$. The average per colony sexual productivity of the two classes was equal. Consequently, following a method of Boomsma and Grafen[6], the expected sex ratio (as the proportion of investment in females) of (1) the singly mating class is 1.0 (all females), (2) the multiply mating class is 0.14 (male-biased), and (3) the population is 0.63 (the RA of the multiply mated class expressed as a fraction). These predictions follow directly from the theory outlined above. Specifically, the multiply mating class is relatively large enough to 'balance' the all-female production of the singly mating one, producing a male-biased class-specific sex ratio that decreases the population sex ratio until equilibrium is reached at a level equalling the RA of the multiply mating class[6].

The observed population ratio (0.67) and the predicted level (0.63) were satisfyingly close. Also, as already stated, sex ratios were split in the directions predicted. However, the singly mating class clearly did not produce females exclusively (Fig. 1). There are several possible explanations for this, one being that workers may make occasional mistakes in judging colony RA and so rear an inappropriate sex ratio[10]. The mating frequency in a few colonies may also have been misclassified by the observer, since some queens probably mated with two males bearing the same marker allele[11].

Conclusion

Sundström's results provide the best evidence yet of split sex ratios arising from workers' responses to their variable RA. Moreover, they also support worker-controlled sex allocation[1], the idea that within-population sex ratio variation may often stem from variable RA[5,6] and Fisherian theory in general[1,2,12]. It would now be interesting to check similar systems, in other species, for split sex ratios. In addition, testing for worker assessment of RA using methods which are independent of the examination of sex allocation is also desirable. Split sex-ratio theory has clearly given new impetus to the study of sex ratios and social evolution in insects.

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