University of Bath

PHD

The social biology of the slave-making ant Harpagoxenus sublaevis

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Award date:
1987

Awarding institution:
University of Bath

Link to publication

The Social Biology of the Slave-making Ant
Harpagoxenus sublaevis

submitted by Andrew Bourke
for the degree of Ph.D.
of the University of Bath
1987

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To my family

Summary

This thesis deals with the social biology of the slave-making ant Harpagoxenus sublaevis. H.sublaevis workers can increase their inclusive fitness by procuring Leptothorax slaves to rear their kin, or by producing male offspring parthenogenetically. I describe work exploring the consequences of worker reproduction for colony social structure, temporal division of labour, and productivity in H.sublaevis. I also use data from a field H.sublaevis population to test the genetic relatedness hypothesis of sex ratio determination, taking into account potential confounding factors such as intra-colony genetic relatedness and population mating structure. In addition, I review the occurrence and significance of worker reproduction throughout the advanced social Hymenoptera.

I also deal with issues concerning social parasitism in ants, reviewing the origin of slave-making (I conclude it arose via the temporary parasitism route) and describing an investigation of the host-parasite relations between H.sublaevis and Leptothorax (H.sublaevis appears to be the permanent winner in an asymmetric interspecific "arms race" with its slave species).

My principal findings and conlusions are as follows. First, reproductive H.sublaevis workers, despite being full sisters (intra-colony relatedness is maximal) form competitive dominance orders in which rank correlates with ovarian development. Queens inhibit worker egg-laying and dominance activity, and most worker-derived males are produced by orphaned (queenless) workers. The social structure of H.sublaevis colonies therefore represents the current state of a kin-selected queen-worker conflict over male parentage. Second, the level of relative sex investment in the H.sublaevis study

population confirms the genetic relatedness hypothesis of sex ratio. Third, throughout the advanced social Hymenoptera, as in *H.sublaevis*, worker reproduction and concomitant queen-worker conflict over male parentage have been and remain potent influences on colony organization and function. Hence this thesis highlights the importance of intra-group reproductive conflict in social evolution.

Acknowledgements

I am extremely grateful to Nigel Franks who, as supervisor of this thesis, contributed to it in innumerable, valuable ways. I also thank Stuart Reynolds for his interest and encouragement, and similarly my fellow research students in the Animal Physiology and Ecology Group at Bath - Nick Platt, Frances Judson, Ian Gilham, Margaret Jones, David Hobdey, and Andrew Timmins.

For extremes of kindness and hospitality during field trips to Sweden, I thank Per and Steffi Douwes. Per Douwes also selflessly introduced Nigel Franks and myself to *Harpagoxenus sublaevis* collecting sites in Sweden (in particular to the Bröms-Kristianopel pinewoods), and shared with us his wide knowledge of leptothoracine and other ants. In addition, I thank Arne Lundberg for his generous hospitality on our visit to the Uppsala area, and Jan Tengö, Folke Larsson, and all people working at the Ecological Station of Uppsala University, Öland (Director: Lennart Ågren), for making our stays there so enjoyable.

Tom van der Have and Koos Boomsma gave me a kind welcome to the Department of Population and Evolutionary Biology, University of Utrecht, and made many valuable suggestions for this work. As stated in chapter 5, the electrophoretic analysis described in this thesis was carried out in collaboration with Tom van der Have, without whom this part of the project would not have been possible.

I am also greatly indebted to Alfred Buschinger, for his published work on *H.sublaevis* and its relatives, for generously making available his leptothoracine culture techniques, and for help over points of information in discussion and correspondence.

I acknowledge the Ecological Station of Uppsala University, Öland, and the Department of Population and Evolutionary Biology, University of Utrecht, for use of laboratory facilities.

The work described in this thesis was carried out while I held a University of Bath Research Studentship. Additional funding for overseas collecting and research visits came from the Balfour-Browne Fund of the University of Cambridge, The British Council, and the Ecological Station of Uppsala University, Öland, as well as from grants from the Royal Society, the Nuffield Foundation, and the British Broadcasting Corporation, awarded to Nigel Franks.

Chapter 1

Introduction

The theory of kin selection (Hamilton 1964,1972) is central to modern studies of sociality and altruism in animals. The simplest kind of society is a family of parents and offspring, characterized by parental self-sacrifice (altruism) on behalf of the young. Hamilton's insight was to realise that if there could be selection for parental care, there must also exist selection for the care of relatives other than offspring. Therefore arguments about kin selection concern not whether the concept is true or false, but whether in practice kin selection is important in explaining altruism towards relatives other than offspring (Dawkins 1979). Nearly all researchers agree kin selection plays a major - but not omnipotent - part in the maintenance of societies throughout the animal kingdom, and perhaps especially among the social insects, whose hallmark is the care of kin (Wilson 1975a). Hence the permanent value of Hamilton's contribution.

But the revolution in evolutionary thought inspired by Hamilton goes deeper than this. Its fundamental tenet is gene selectionism (Williams 1966, Dawkins 1976). Individual selection for parental care implies the existence of kin selection because parental care and altruism towards kin other than offspring are both aspects of the same, gene-selected phenomenon. Both arise because a gene for altruism will be favoured by selection if its bearers recognize and care for individuals with a high probability of containing the same gene. Close relatives, including offspring, have a high probability of sharing an individual's genes. Relatedness is also the most plausible basis for recognition of genetic co-bearers, simply because relatives tend to inhabit the same nest. Thus kin selection promotes altruism towards offspring or other close relatives because the gene for altruism is caring for copies of itself. In gene selectionism, all genes for adaptive traits promote their own survival through the agency of organisms.

Recently, biologists have invoked the idea of a hierarchy of levels at which natural selection may operate, with individuals constituting the principal level. However, gene and individual selection do not both lie on such a single dimensional hierarchy of levels of selection. Rather, all adaptive characteristics of organisms which in everyday language are attributed to selection at the individual level, strictly speaking result from gene selection. This is because natural selection concerns the differential survival of replicating entities: genes are replicators, but individuals are not (Dawkins 1982). We therefore speak of individual-level selection for linguistic convenience. But this is not to say the hierarchical viewpoint is wrong, or that all evolutionary phenomena can be reduced to processes at the gene level. Other kinds of selection may indeed occur at different grades of biological organization. Thus the present mix of species on earth may partly result from a process whereby former species prone to splitting have left more species descendants than other, slower speciators. There may have been "species selection" (Stanley 1979). However, though this process may account for the composition of faunas, and for macro-evolutionary trends, it cannot account for complex adaptations in individuals (Dawkins 1986), which are the subject matter of the animal sociologist.

In social insects, biologists have frequently also suggested the existence of selection at the colony level. But the validity of this concept appears more problematic. Frequently the individual social insect's genetic interest and the (hypothetical) colony good coincide, so it is not always obvious that features of individuals are gene-selected adaptations rather than adaptations for the good of the colony. This could explain why colony-level selection is so often invoked. But when the individual and the colony good do not concur, such as when dominance activity in laying ant workers disrupts brood care (Cole 1986), the prevalence of individual interests is obvious. Hence, writing about the social insect which to many epitomizes the sacrifice of the individual to the collective, one authority states: "In summary, I know of no observation on honey bee biology which unequivocally demonstrates the action of colony-level selection at the expense of individual interests" (Seeley 1985:7). Therefore colony-level selection, even if it occurs, seems unimportant compared with individual (gene) selection.

The consequence of gene selectionist thinking is that where biologists previously saw co-operation and harmony, such as in the relations between a mated pair, or between parent and offspring, or among members of an insect society, they now see competition and discord (Trivers 1972,1974, Trivers and Hare 1976). Therefore, what makes social insects especially fascinating to the evolutionist is not that they exhibit the tension between different levels of selection, individual versus colony. As already explained, individual- is shorthand for gene selection, and colony-level selection is a concept of doubtful utility: in social insects probably all the characteristic phenomena, particularly worker sterility, are explicable from the gene selectionist viewpoint. Instead, social insects, in addition to their historical importance in the development of kin selection theory, continue to provoke interest because they display in heightened form the subtle conflicts of reproductive (gene-propagating) strategy that arise between and within co-existing individuals. For example, the reproductive strategies favoured by Hymenopteran queens and workers often differ over the sex investment ratio each entails (Trivers and Hare 1976). This is between-individual conflict. But conflicts within a single individual can also arise. Workers in many advanced social Hymenoptera, though incapable of mating, possess ovaries and so can produce male offspring parthenogenetically. Each worker is therefore subject to a conflict between selection to rear kin, and selection to bear young. The resolution of this conflict profoundly affects the worker's behaviour and the society to which it belongs.

The slave-making ant Harpagoxenus sublaevis forms societies pervaded by such conflicts. Their effects on worker behaviour, sex investment ratio, colony productivity, and life history strategy, constitute the principal themes of this thesis. As already implied, the importance of within-colony reproductive differences in social Hymenoptera has been appreciated for some years (Trivers and Hare 1976). But the widespread use of electrophoretic techniques to measure genetic relatedness in nature, and thereby test precisely hypotheses regarding expected levels of conflict, has occurred only relatively recently. An outstanding example of this approach is the work of Ward (1983a,b), who found sex investment ratios matched those expected on the basis of genetic relatedness and worker control of investment in colonies of Rhytidoponera ants. Furthermore, few studies have combined analysis of genetic colony structure, sex ratio, and production schedules, with a parallel investigation of the behaviour of individual workers with different reproductive strategies, as I attempt here. Hence a principal~~e~~ aim of this thesis is to understand the behaviour of H.sublaevis individuals in terms of their social and genetic environment. In this thesis I also examine the resolution of one kind of within-colony conflict - the queen-worker conflict over male parentage - by comparative method, in a review of worker reproduction throughout the advanced social Hymenoptera.

General principles in evolution perhaps become better understood when their more unusual manifestations are examined. Hence other themes in the thesis will involve the attempt to explain features of the biology of H.sublaevis and allied species in terms of widely applicable evolutionary processes. These themes include the evolution of intra- and inter-specific social parasitism in ants (especially the evolution of slavery), the possibility of species radiation by host race formation in slave-makers, and the application of the "arms race" concept (Dawkins and Krebs 1979) to the slave-maker/slave relation.

The arrangement of the thesis is as follows. In the next two chapters I introduce first the slave-making ants, then H.sublaevis itself, to provide the background for the rest of the study. Chapter 2 is also where I discuss how slavery in ants evolved. Chapters 4 and 5 describe my work on worker reproduction, queen-worker conflict, genetic colony structure, sex ratio, and productivity in H.sublaevis. In chapters 6 and 7, I return to issues concerning social parasitism: in chapter 6, I discuss the relationship between H.sublaevis and its hosts, including sections on host race formation and arms races in slave-makers; chapter 7 concentrates on one remarkable adaptation for slave-making in H.sublaevis. A review of worker reproduction in the advanced social Hymenoptera appears in chapter 8. Finally, I conclude with a summary and a discussion of an outline life history model for H.sublaevis in chapter 9.

Chapter 2

The Slave-making Ants

Harpagoxenus sublaevis is one of about thirty-five known species of slave-making ant. This chapter first describes ant slavery in general, then considers how this and other forms of inter-specific exploitation in ants evolved. This is both interesting in terms of issues it raises, and necessary because it could help explain particular social features of *H. sublaevis* described in later chapters.

Slavery in ants is a form of social or brood parasitism. Slave-makers exploit the labour of other ant species for rearing their young by raiding neighbouring host (slave) species colonies and stealing their workers. These slaves are captured as brood, usually pupae. Adults cannot be integrated into slave-maker colonies because of colony specific odour differences in adult ants, which provoke mutual hostility. But neither colony- nor (at least between closely related taxa) species-specific odour differences apparently exist in brood. The absence of brood discriminators evidently makes slavery possible (Hölldobler and Michener 1980), since slavery involves rearing conspecific brood from other colonies as well as slave-maker brood. Similarly, since the slaves which eclose from captured brood perform work for their captors, slavery almost certainly also involves an imprinting - like phenomenon in young ants (Le Moli 1980, Jaisson 1985) (see Ch.6).

Table 2.1 lists the known inter-specific slave-making ants. Slave-making is clearly a polyphyletic trait. Ant phylogeny is uncertain, but the number of times slave-making has independently evolved is probably not less than seven (five times in the Leptothoracini

[Buschinger 1986], once respectively in the Tetramoriini and Formicinae) (Table 2.1). Therefore the selective forces responsible for the evolution of slavery must have been widespread.

Recently several authors have reported cases of facultative intra-specific slave raiding. Ants have been observed raiding conspecific colonies for brood, some of which has survived in the captors' nests. Examples include Myrmecocystus mimicus (Hölldobler 1976, 1979), Leptothorax curvispinosus (Wilson 1975b), L.ambiguus, and L.longispinosus (Alloway 1980). Alloway (1980) also observed similar interactions between colonies of non-conspecific pairs of the three Leptothorax species. But the frequency of Leptothorax colonies in the field containing individuals of one of the other species is very low (less than 1.0%: Alloway 1980), so facultative inter-specific slavery appears unimportant in these ants. The frequency of intra-specific slavery is obviously harder to measure owing to the difficulty of determining the origin of conspecific ants in any one colony. At the end of this chapter I discuss whether these cases of intra-specific slavery bear directly on the evolution of inter-specific slavery as has been claimed (Alloway 1980).

The inter-specific slave-makers all conform to "Emery's rule". This states that the closest phylogenetic relatives of Hymenopteran social parasites are frequently their hosts, which usually number one or a few species (Emery, quoted by Wilson 1971: 360). As I will describe later, this important generalization is in many cases best explained by supposing that a remarkable mode of speciation has occurred in the ancestors of social parasites.

Slave-making species share another characteristic. As far as is known, all slave-maker colonies are founded by colony usurpation by single slave-maker queens (Buschinger 1970, Buschinger, Ehrhardt and Winter 1980). Typically a newly-mated slave-maker queen enters a host species colony and kills or expels all its adult members, or at least the resident queen(s). The brood thus captured subsequently provides the slave-maker colony's first slaves. By contrast, queens of non-parasitic ant species generally found colonies by what is known as the claustral method, in which solitary queens rear their first workers with energy derived from wing muscle histolysis (Wilson 1971). Hence any theory to explain the evolution of slavery must account for the constant association, in several unrelated lineages, between slave raiding and parasitic or non-independent colony foundation.

Other forms of Hymenopteran social parasitism

As previously implied, slave-making is not the only kind of social parasitism in ants. It is now necessary to describe the others, namely temporary social parasitism and workerless inquilinism. This is because the evolution of any one of these cannot be considered independently of the others. Temporary social parasitism occurs when a queen founds a colony by usurpation like a slave-maker, but then produces workers which instead of slave raiding gradually take over colony tasks as the initial stock of host workers diminishes through natural causes. Workerless inquilinism takes two forms. In the first, parasite queens again found colonies by usurpation and killing host queens, but then produce sexuals exclusively. The colony life span of these parasites is consequently limited by the longevity of the host workers. In the second, commoner kind of workerless inquilinism, parasite queens infiltrate rather than usurp host colonies. They do not kill host queens, but produce sexuals alongside them. The parasitized colony in this case persists as long as host queens, or longer if requeening occurs. Examples of all these kinds of ant social parasite are given by Wilson (1971) and Dumpert (1981).

Slave-making does not occur outside ants, but temporary social parasitism and workerless inquilinism are widespread in social wasps and bees (Wilson 1971). All these parasitic life-styles have their parallels in nest usurpation among non-social insects (e.g. Eickwort 1975), and also in brood parasitism among fish (e.g. Sato 1986) and birds. Clearly, the exploitation of other species' labour for rearing young is a relatively common mode of life in animal groups with parental or familial care.

Evolution of workerless inquilinism

Before discussing how slavery evolved, I will first consider how workerless inquilinism (without host queen elimination) might have arisen in two groups of ants. I discuss this topic both because it raises novel biological points concerning speciation, and also because it introduces some of the principles arising in the discussion of the evolution of slavery.

The first group I consider is the genus *Leptothorax*. *Leptothorax kutteri* is a workerless inquiline which coexists with the queens in the polygynous (multi-queened) colonies of its host *L.acervorum*. It conforms strictly to Emery's rule, being morphologically extremely like *L.acervorum* apart from its smaller size. In *Leptothorax* species, polygyny commonly results from the adoption of additional queens into the colony (Buschinger 1968c, Alloway et al. 1982). Buschinger (1965) suggested that *L.kutteri* evolved when in the common ancestor of *L.kutteri* and *L.acervorum* queens arose which, being genetically incapable of worker production, survived by infiltrating conspecific colonies and parasitically producing sexuals. They were preadapted for colony infiltration by the habit of queen adoption. Reproductive isolation of such forms (the details of which are unclear - but see below) resulted in the new workerless inquiline species L.kutteri.

This hypothetical evolutionary pathway is remarkable in suggesting the evolution of a social parasite by sympatric speciation from its host stock. Biologists generally believe that sympatric speciation occurs far less frequently than allopatric speciation, if ever, because they doubt reproductive isolation can readily arise without prior geographic separation (White 1978). However, in workerless inquiline ants and other socially parasitic Hymenoptera, it is hard to explain the almost universal close relationship of parasite and host (Emery's rule - see above) without invoking sympatric speciation. Certainly, in some instances, Emery's rule may simply reflect the fact that social parasites need a similar biology to their hosts. But this interpretation predicts only a relatively close relationship of parasite and host, not the precise one-to-one phylogenetic correspondence that frequently occurs (see the Myrmica example below). It seems unparsimonious and unrealistic to invoke allopatric speciation followed by secondary sympatry in all these cases. Therefore, a sympatric route for the evolution of L.kutteri seems the most plausible.

Emery's rule for Hymenopteran social parasites may in fact be only an especially clear-cut instance of a more general phenomenon. West-Eberhard (1986) has presented evidence that in numerous types of organism, phyletic divergence (speciation) has followed from the fixation of alternative adaptive phenotypes within a lineage, without major genetic change. By alternative adaptive phenotypes are meant any of the many kinds of behavioural or morphological polymorphisms occurring within species and believed to have arisen through intraspecific competition. West-Eberhard believes that fixation (i.e. exclusive expression) of such phenotypes facilitates speciation by reinforcing the differences between those individuals exhibiting the alternative adaptation and the parent population. Therefore, the inclusion in Buschinger's scheme for the evolution of L.kutteri of an initial genetic loss of the ability to produce workers (compelling mutant queens to become parasites) may be unnecessary. Instead, as the result of selection pressures for alternative modes of colony foundation to be described later, parasitism may have originated in the ancestors of L.kutteri as an alternative adaptive phenotype, to use West-Eberhard's terminology. Pursuing West-Eberhard's scheme, such incipient parasitism and the subsequent speciation of L.kutteri may have been intimately connected. In other words, speciation occurred partly because of the behavioural shift towards parasitism.

However, there is another solution to the problem of how L.kutteri split from L.acervorum sympatrically. In L.acervorum the haploid chromosome number is 13, but in L.kutteri and two further, related workerless inquiline parasites of L.acervorum - L.goesswaldi and Doronomyrmex pacis - this number ranges from 23 to 28 (Buschinger 1981, Douwes and Buschinger 1983). This raises the possibility that the ancestor of the three parasitic species arose from the L.acervorum host stock by doubling of the chromosome set. Speciation by polyploidy has been suggested in a few other Hymenoptera (Crozier 1977, White 1978), although it has been doubted in ants (Crozier 1975). If L.kutteri arose by such a process, its initial sympatric reproductive isolation would be simply explained. Further, West-

Eberhard's hypothesis would not apply in this case: the parasitic habit would not have been a motivating factor in speciation, but would have come after that event. However, since a polyploid derivative of *L.acervorum* could presumably have adopted a free-living habit, it still remains necessary, even assuming polyploidy occurred, to invoke selection pressures for parasitism to explain the course *L.kutteri* took.

The possibility of a polyploid origin of the *L.kutteri* complex deserves further, cytogenetic investigation. But it may be concluded that the chromosome numbers of the host and parasitic lineages diverged by some other chromosomal mechanism, after speciation. I now discuss another group of ant hosts and parasites which again supports the idea of a sympatric origin of inquiline species.

The group in question is the genus *Myrmica*. In several polygynous members of this genus are found large and small queens (macro- and microgynes) with different reproductive biologies (Brian and Brian 1955, Elmes 1973,1976). In two cases where microgynes occur in the same colonies as macrogynes, microgynes have recently been shown (*M.sabuleti*, Elmes 1978) or are almost certain (*M.rubra*, Pearson and Child 1980) to be separate workerless inquiline species. By contrast, microgynes in *M.ruginodis* apparently lack species status (Pearson 1981). In *M.ruginodis* the two queen forms occur in different types of colony: macrogynes head monogynous (singly-queened) colonies, but microgynes occur together in polygynous ones. Both *M.ruginodis* colony types can occur alongside each other, but they tend to have different microhabitat preferences (Brian and Brian 1955). To account for these facts, Pearson (1981) suggested the socially parasitic microgynes in *M.sabuleti* and *M.rubra* arose through a process beginning with a M.ruginodis - like situation. First, ecological factors (e.g. habitat differences) led to the co-existence of monogynous and polygynous forms. This was followed by miniaturization of queens in the polygynous colonies. Finally, the microgynes became social parasites of the macrogynes. Also, at some point, speciation occurred. Pearson implies speciation would have been allopatric, but even if his pathway is correct in the other details, for the reasons already given it seems more plausible that speciation was sympatric. This is in fact the view of Elmes (1978), who also differs from Pearson in his proposed evolutionary route to microgyne parasitism.

Elmes (1978) suggested the microgyne parasite of M.sabuleti arose by the same route as L.kutteri evolved according to Buschinger (see above), i.e. directly from the host stock, without initial ecological separation of host and incipient parasite. Therefore, the existence of two colony types in M.ruginodis may be an evolutionary phenomenon not necessarily connected to the evolution of social parasitism: conspecific monogynous and polygynous forms occur elsewhere in ants, without queen dimorphy (e.g. Solenopsis invicta, Ross and Fletcher 1985). Elmes further suggested that each member of the exclusively inquiline genus Sifolinia, all of which also parasitize Myrmica, arose via microgyne ancestors direct from its respective host Myrmica species (implying Sifolinia is a polyphyletic genus). However, as in L.kutteri, it is not possible to know whether speciation in these cases occurred through some genetic process, or according to West-Eberhard's "alternative adaptation" hypothesis.

To summarize this somewhat involved discussion of the evolution of workerless inquilinism. The simplest way to account for Emery's rule in workerless inquilines is to suppose the parasites have arisen sympatrically from their host stock. But this raises questions concerning the interdependence and relative timing of speciation and the evolution of parasitic behaviour. Speciation could have occurred prior to the evolution of (inter-specific) parasitism, e.g. by polyploidy (possible in L.kutteri), or during ecological separation (Pearson's hypothesis) (both still sympatric modes). Alternatively, it could have occurred after the evolution of (intra-specific) parasitism. In this case speciation could have resulted either by some unknown means following a genetic loss mutation in ancestral parasitic queens (Buschinger's hypothesis), or through fixation of a facultative parasitic phenotype (West-Eberhard's "alternative adaptation" hypothesis). At present, it does not seem possible to discriminate between these various hypotheses. Different ones may apply in different cases. However, the idea inter-specific arose from intra-specific parasitism (Buschinger or West-Eberhard routes) suggests a search for intra-specific inquilinism in ants. In all these pathways, we still need to explain why selection should have favoured parasitism (intra- or inter-specific) at all.

Evolution of slave-making

To return now to the slave-making ants. How slave-making evolved is a classic, if rarefied, problem in ant biology. Buschinger (1970) gives the early references on the subject. Both this and a more recent paper, Buschinger (1986), review the origin and evolution of all forms of social parasitism in ants. Below I outline the four main theories for slave-making, then describe how they can be synthesized.

1. Brood predation hypothesis: Darwin (1859) proposed that predatory ants could have evolved into slave-makers after accidentally

acquiring extra labour when uneaten ant brood prey items eclosed in their nests. But this idea does not explain non-independent colony foundation (see above) by slave-makers (undescribed in Darwin's time) and is therefore incomplete.

2. *Territoriality hypothesis*: Wilson (1975b,c) and Alloway (1980) suggested that slave raids began not as predatory forays but as territorial interactions, which occur in many ants and may involve brood capture. Supporting evidence includes close similarities in the organization of raiding behaviour in the slave-maker *Harpagoxenus canadensis* and of territorial battles in its related *Leptothorax* hosts (Stuart and Alloway 1982,1983). To account for non-independent colony foundation, Alloway (1980) hypothesized that the ancestors of slave-makers (at least in leptothoracine ants) were polygynous as well as territorial. Polygyny and concomitant queen adoption could have been preadaptations for colony foundation by usurpation (in the same way these conceivably preadapted *L.kutteri* for colony infiltration). However, this refinement of the territoriality theory is retrospective, and does not explain why slave-raiding is obligatorily associated with non-independent colony foundation.

3. *Temporary parasitism hypothesis*: Wheeler (1905) suggested that slave-makers evolved from temporary parasites whose workers acquired the habit of slave-raiding by the brood predation route. Since temporary parasite queens found colonies by usurpation (see above), this idea does explain the ubiquity of non-independent colony foundation in slave-makers. Alloway (1980) criticized this hypothesis on the grounds that some taxa have temporary parasitic representatives but no slave-making ones, and vice versa. On the other hand, the genus *Formica* contains both kinds of parasite, so Alloway's reasoning is

inconclusive.

4. Polydomy-polygyny hypothesis: Buschinger (1970) first emphasized that non-independent colony foundation is the common factor in all the social parasitic life histories, and therefore the key to explaining their evolution. I have already discussed how he explained inquilinism in species like L.kutteri as the result of a genetic loss of the ability to bear worker offspring in queens preadapted for non-independent colony foundation by polygyny (Buschinger 1965). Similarly, to account for slave-making, Buschinger (1970) suggested that queens arose genetically incapable of producing adequate numbers of workers in species which were both polygynous and polydomous. Polygyny would have preadapted these forms for non-independent colony foundation as in inquilines, and polydomy (the occupation of multiple nests by single colonies) for slave-making. This is because in species with multiple nests, brood transport between nests is frequent. Hence nests with queens only able to produce low worker numbers could have begun selfishly importing brood, a habit which could in turn have resulted in slave raiding. Therefore, unlike the other hypotheses except perhaps Alloway's refinement of the territoriality theory, Buschinger's hypothesis again suggests sympatric speciation of the parasite from the host stock. Evidence in its favour includes the fact most slave-makers have polygynous hosts (Buschinger 1986).

In my view all the above hypotheses contain an element of truth, and can be reconciled by considering the selective forces acting upon ant foundress queens. Preadaptations, whether involving behavioural traits or genetic loss mutations, cannot explain the evolution of particular traits without the involvement of selective forces. Further, invoking preadaptations carries the risk of teleological reasoning.

In ants, competition among queens to found colonies successfully undoubtedly resulted in the present diversity of colony foundation methods (Hölldobler and Wilson 1977). One such method involves young queens returning to their maternal nests (conspecific queen adoption, leading to polygyny as previously discussed). There is now empirical evidence that such behaviour results from competition for nest-sites (Herbers 1986). Adoptees escape the vulnerable solitary phase of claustral foundresses. They can also achieve an earlier age of reproduction by parasitizing the existing workforce and producing sexuals exclusively. Hence selection to be an adoptee could be strong enough to induce young queens to attempt to enter foreign conspecific nests, instead of returning to their own. Such queens would have to acquire a means of surmounting the foreign colony's nestmate recognition barrier to be adopted (see Ch.7). They could presumably perfect such a method under selection for successful infiltration: hence this hypothetical pathway does not require the simultaneous acquisition of multiple adaptations in incipient parasite queens, as is sometimes maintained. Reproductive isolation of such forms from the ancestral species would result in a host-parasite pair like L.acervorum and L.kutteri, as previously discussed. Here then is an evolutionary rationale for inquilinism in terms of selective forces on foundress queens.

Inside a host colony an incipiently parasitic queen benefits from worker production by the other queens, but loses from their sexual production. Unless she can preferentially suppress sexual production, the parasite must decide (in evolutionary terms) whether to treat the other queens as hosts or competitors. Inquilines which co-exist with host queens have evidently been selected to follow the first option. But, equally clearly, some parasitic queens have followed the second, and kill the queens whose colonies they enter, even when there are several host queens per colony (e.g. _Epimyrma stumperi_, Kutter cited by Dumpert 1981:172). The absence of host queens dictates the kind of life history the parasites then pursue. This is also true of parasitic queens which fight their way into host colonies, since they kill or eject all adult occupants. These parasites gain the benefit of early reproduction (sexual production) as do infiltrators, but avoid the necessity of subtly overcoming nestmate recognition systems. However, like infiltration, colony foundation by fighting is perfectible.

If they kill host queens and therefore terminate the host worker supply, parasite queens have at least three available reproductive options. First, they could produce sexuals in a "big bang" until all host workers die out. This is the strategy of workerless inquilines which kill host queens. Second, for example if long-lived colonies are ecologically advantageous, they could produce their own workers and hence be temporary parasites. Third, also to prolong the colony's life, they could replenish the colony's labour force by producing workers which capture additional host workers, i.e. slave-raiders.

I therefore suggest that slave-making ants evolved from ancestors which, under selection for early reproduction etc., developed parasitic, non-independent colony foundation involving elimination of host queens; then, under selection to extend colony longevity, evolved a worker habit of procuring extra host workers by raiding. This suggestion is closest to the temporary parasitism hypothesis of those outlined above. It also resembles Buschinger's hypothesis in deriving non-independent colony foundation from the habit of queen adoption associated with polygyny. Hence it further resembles Buschinger's route by suggesting slave-makers evolved sympatrically from the ancestors of their hosts. However, I do not consider these ancestors were necessarily polydomous. Instead, I agree with the other authors in assigning a role to brood predation and territoriality in the evolution of slave-making. It is highly likely the elements of worker raiding behaviour derive from these phenomena. But which was involved in particular cases probably differs in different lineages. This would explain the resemblance of Harpagoxenus canadensis raids to Leptothorax territorial interactions (see above), the corresponding rarity of brood predation in leptothoracines (Alloway 1980), and conversely the frequency of brood predation in formicine slave-makers (Topoff, LaMon, Goodloe and Goldstein 1984).

In addition, the above scheme agrees with Buschinger (1970) in viewing slave-making as one of a set of divergent life histories commencing with non-independent colony foundation. The evolution of non-independent colony foundation in queens must have preceded the evolution of slave-raiding in workers, for only in this way can we explain the complete slave-making syndrome, i.e. the repeated independent evolution of queen colony foundation by usurpation obligatorily combined with worker slave-raiding. However, the route I propose differs from all the others in establishing selective forces rather than preadaptations as the evolutionary impetus to queen usurpation behaviour and slavery. Its feasibility is evidenced by the common occurrence of non-independent colony foundation without slavery in other parasitic ants. But if the route is correct, we must accept that some intermediate steps to slave-making are no longer represented. Otherwise, the absence of temporary parasites in leptothoracines must be taken as counter-evidence.

To conclude this chapter, I return to the question of intra-specific slavery mentioned near the start. As already described, the above route to inter-specific slave-making suggests, as in the inquilines, a sympatric derivation of slave-makers from their host stock. However, slavery could conceivably have evolved by an allopatric, temporary parasitic route, and the sympatric element is also not essential to the main points about the precedence of non-independent colony foundation and the importance of selective forces. Nevertheless, the possibility of sympatric speciation giving rise to slave-makers raises the following question about the significance of the intra-specific slave raiders. Do these species represent the early stages of inter-specific slave-making? If intra-specific slave raiding is accompanied by intra-specific usurpation by colony founding queens (this is at present unknown), it is clear the answer to the question is positive, and the intra-specific slave raiders could provide valuable insights into the evolution of inter-specific slave-making. If not, then intra-specific slavery as so far described is an interesting but unrelated issue.

Table 2.1 Inter-specific Slave-making Ants

Group	Genus	Species	Additional references
Leptothoracini (Myrmicinae)	*Leptothorax*	*L.duloticus*	
	Harpagoxenus	*H.americanus*	Not a true congener: See Ch.3.
		H.canadensis	Stuart and Alloway 1982, 1983, Stuart 1984
		H.sublaevis	
		H.zaisanicus	
	Epimyrma	Several spp., e.g. *E.ravouxi*	Buschinger and Winter 1983
	Myrmoxenus	*M.gordiagini*	Buschinger,Winter and Faber 1983;probably an *Epimyrma*:Jessen 1986
	Chalepoxenus	Several spp., e.g. *C.muellerianus*	
Tetramoriini (Myrmicinae)	*Strongylognathus*	Several spp., e.g. *S.alpinus*	
Formicinae	*Formica* subgenus *Raptiformica*	Several spp., e.g. *F.sanguinea*	Some are facultative slave-makers.
	Polyergus	*P.rufescens*	
		P.lucidus	
		P.breviceps	Topoff,LaMon,Goodloe and Goldstein 1984
		P.samurai	
	Rossomyrmex	*R.proformicarum*	
Dolichoderinae	*Conomyrma*	*C.bicolor*	Bernstein 1978: doubtful cases because whether captured workers genuinely behave like slaves (i.e. rear mixed brood) is apparently unknown.
		C.insana	

Sources: Buschinger 1970, 1981, Buschinger, Ehrhardt and Winter 1980, Wilson 1971, and contained references.

Chapter 3

The Biology of Harpagoxenus sublaevis

This chapter focuses on the general biology of Harpagoxenus sublaevis, and so provides the necessary background for the rest of the thesis. I first consider the morphology, systematics and distribution of H.sublaevis. I next concentrate on the life cycle of its colonies, then end with discussions of the genetics of queen dimorphy and caste, and the adaptive significance of queen winglessness. Throughout, I draw extensively on the findings of Alfred Buschinger and his associates (especially Ursula Winter) in West Germany, who from 1966 onwards have been largely responsible for building up our knowledge of the biology of H.sublaevis. References to the 19th and early 20th century authors who worked with H.sublaevis, notably Adlerz and Viehmeyer, are given by Buschinger (1966a).

H.sublaevis workers (length 3.5-5.5mm.) are morphologically extremely well adapted for their parasitic habits (see Collingwood 1979 for complete descriptions of H.sublaevis and its Leptothorax hosts and relatives). The relevant adaptations include (1) the greater body size of H.sublaevis compared to its hosts, (2) the disproportionately large head, housing the musculature for (3) the broad, toothless and scissor-like mandibles, ideal for severing the appendages of hostile host species ants. They also include (4) two lateral grooves (scrobes) on the head in which the antennae may be protectively folded during fights, and (5) a spine which helps strengthen the vulnerable postpetiolar segment joining the gaster (terminal part of the abdomen) with the rest of the body.

Queen H.sublaevis, unusually for ants, are with rare exceptions wingless and very similar to workers in size and external morphology. Queens therefore share with workers the adaptations for fighting just described, which they require for colony foundation. The significance of queen winglessness will be discussed at the end of the chapter. The definitive difference between H.sublaevis queens and workers is in their reproductive anatomy. Both castes possess ovaries, but queens have a spermatheca (sperm storage receptacle) which workers lack. Since arrhenotoky (parthenogenetic origin of males) is the rule in Hymenoptera, H.sublaevis workers can produce offspring, but they are always male.

Male H.sublaevis, as in most ants, are unspecialized in form. They are always winged and resemble the general type for Leptothorax subgenus Mychothorax, to which H.sublaevis is affiliated and its hosts belong (see below). A convenient identification feature of H.sublaevis males is the black cell on the frontal margin of their forewings, which is absent in the otherwise very similar males of their Leptothorax hosts.

H.sublaevis is one of four members of its genus. In full, the genus consists of H.americanus (Emery) and H.canadensis M.R.Smith from North America, H.sublaevis (Nylander) from Europe, and H.zaisanicus Pisarski from Mongolia. All four species live with Leptothorax ants, and in each the females share the distinctive external morphology already described in H.sublaevis. H.zaisanicus is known only from four type workers, and its slave-making habits are entirely inferred (Pisarski 1963). The three other species are, by contrast, well known obligate slave-makers. However, although H.americanus resembles H.sublaevis and H.canadensis in the ways already mentioned, this species also differs from the other two in several important features which they, on the other hand, share (see Table 3.1).

The conclusions which follow from the pattern of resemblances and differences between Harpagoxenus species depicted in Table 3.1 are extremely interesting. First, H.americanus is sufficiently unlike the other Harpagoxenus to justify its imminent removal from the genus (Buschinger 1981, pers. comm.). Second, in taxonomically important features, H.americanus resembles its Myrafant hosts, and H.sublaevis / H.canadensis their Mychothorax hosts, more closely than H.americanus resembles H.sublaevis / H.canadensis. These slave-makers therefore provide a striking instance of "Emery's rule" that the closest relatives of Hymenopteran social parasites are their hosts (see Ch.2). Third, it follows that the shared slave-making habits and other strong (adaptive) similarities between H.americanus and H.sublaevis / H.canadensis are the result of convergence, following independent evolution from two sets of free-living ancestors. This remarkable conclusion will again be discussed in later chapters.

The distribution of H.sublaevis embraces central and northern Europe, excluding the British Isles (see list of localities in Buschinger 1966a). The species is particularly associated with mountain and forest habitats. Its nests necessarily occur in the same sites as those of its hosts, namely fallen dead twigs, under the bark of tree stumps, under stones, or in rock fissures. Similarly, since the slave-makers obtain their food exclusively in regurgitated form from their slaves (see below), their food must ultimately be the same as Leptothorax ants collect in a free state, which is small insect prey (Dobrzański 1966, Collingwood 1979:72).

Life cycle of H.sublaevis colonies

1. Colony foundation and early colony growth

The life cycle of H.sublaevis colonies begins with non-independent colony foundation by the queens (Buschinger 1968a,1974b; Ch.2). In summer, young newly-mated queens (singly, and on foot) seek and enter Leptothorax nests. They dismember the adult Leptothorax with their mandibles, and also attack them with a chemical weapon of glandular origin described in chapter 7. The smaller and less robust Leptothorax ants, equipped only with serrated mandibles unspecialized for cutting, and with insufficiently powerful stings, are often evidently no match for the slave-maker queens. However, many queens undoubtedly perish attempting colony foundation. Successful ones kill or expel all the adult Leptothorax single-handed, and so become sole possessors of the Leptothorax brood.

At this stage slave-maker queens apparently face competition from other colony-founding slave-maker queens for the captured brood. Buschinger (1974b) inferred this from the discovery of a field slave-maker colony headed by a queen with partially severed limbs (evidence of slave-maker attack). Such competition is perhaps expected, since a queen can possibly overpower a solitary slave-maker more easily than a nestful of slaves. The possible influence of competition between foundress queens on the spatial distribution of H.sublaevis colonies is discussed in chapter 6.

Following successful colony foundation, H.sublaevis queens begin to lay eggs. These are raised by the Leptothorax workers which shortly eclose from the captured brood. The first H.sublaevis workers appear in the following summer, and the colony becomes a mixed society of adult slave-makers and slaves.

As far as is known, the slaves carry out all the work of food gathering and brood care. When Stuart and Alloway (1985) presented food-deprived _H.sublaevis_ colonies with a food source, the slave-maker workers performed only 0.7% of food gathering trips. They never responded to recruitment to the food source by the slaves. Therefore trophallaxis (liquid food transfer) with slaves and larvae, commonly observed in slave-maker nests (see Ch.4), is almost certainly the sole means by which slave-maker queens and workers obtain nourishment. The lack of brood care by slave-maker workers is suggested both by direct observation and a finding of Adlerz. When he removed all the slaves from a colony of _H.sublaevis_, the larvae eventually shrivelled and died (quoted in Wheeler 1910:493).

2. _Slave raids_

H.sublaevis workers conduct slave raids to build up and maintain their colony's labour force (Ch.2). A description of these remarkable events now follows. In summer when pupae are present in both _H.sublaevis_ and _Leptothorax_ brood, single slave-maker workers search the vicinity of their nests for nests of _Leptothorax_ (scouting). They can apparently detect the proximity of _Leptothorax_ nests from chemical cues on the substrate (Buschinger, Ehrhardt and Winter 1980:249). A successful scout returns to the slave-maker nest and performs an excitation display which induces the other slave-maker workers to gather at the entrance (Buschinger and Winter 1977). Next the scout stands at the entrance in a "tandem calling" posture (with gaster raised and sting extruded), inviting a nestmate to touch the caller's gaster with its antennae, whereupon the pair sets off towards the _Leptothorax_ nest in a "tandem run" (Buschinger and Winter 1977).

Tandem running is a primitive form of recruitment in ants in which one ant leads a nestmate to a target location by proceeding there with the recruit behind in more or less constant antennal-gastral contact (occurrence reviewed by Wilson 1971:248, Dumpert 1981:76). In the slave-making leptothoracines tandem running occurs not only in H.sublaevis, but also in H.canadensis and Chalepoxenus muellerianus. The hosts of these three species also employ tandem running, in recruitment to food sources and new nest-sites (Dobrzański 1966, Möglich, Maschwitz and Hölldobler 1974, Möglich 1978,1979, Buschinger, Ehrhardt and Winter 1980). In both L.acervorum and H.sublaevis recruits for tandem runs are attracted by "tandem callers" releasing poison gland secretion from their extruded stings. In addition, in both species the contact between the follower's antennae and the leader's gaster is a necessary tactile signal for the initiation and maintenance of the tandem run (Möglich, Maschwitz and Hölldobler 1974, Buschinger and Winter 1977). Both kinds of signal are effective interspecifically, since sometimes in slave-maker colonies mixed tandems, with either the slave-maker leading the slave or vice versa, are observed (Buschinger and Winter 1977). Therefore tandem recruitment and the associated communication systems were not developed by H.sublaevis for slave raiding, but were primitively present in its non-parasitic ancestors.

By means of tandem recruitment, scouts and their recruits collect a slave-maker force at the Leptothorax nest entrance. The slave-makers then fight their way into the nest, dismembering the Leptothorax ants and attacking them chemically as do the queens during colony foundation (see Ch.7). Yet despite their fighting prowess, slave-maker workers may be killed on slave-raids (see Ch.4). When the slave-makers have killed or ejected all adult occupants of the target nest, brood transport begins (Buschinger 1968a, Buschinger, Ehrhardt and Winter 1980, Winter 1979).

Brood transport is performed by single slave-makers running repeatedly from the Leptothorax to the slave-maker nest bearing individual brood items. Although slave-makers should arguably only collect worker pupae, which require no feeding and quickly metamorphose into useful slaves, they in fact take worker, queen and male pupae, as well as medium and large larvae (Buschinger, Ehrhardt and Winter 1980). During brood transport, two remarkable kinds of behaviour reportedly occur in the defeated Leptothorax nest. First, the slave-makers appear to smear some of the captured brood with Dufour's gland secretion, probably to make the brood repellent to would-be Leptothorax rescuers (Buschinger, Ehrhardt and Winter 1980; Ch.7). Second, one slave-maker discards the Leptothorax eggs and small larvae at the nest entrance, thereby preventing these inferior brood items from being carried to the slave-makers' nest (Buschinger, Ehrhardt and Winter 1980). The duration of slave-raids, from the first tandem recruitment to the last brood transport, is very variable, and can be several hours (Winter 1979; Ch.4).

Slave-maker colonies raise all captured brood to adulthood. As far as is known, none is eaten under natural conditions (Buschinger 1984). On eclosion the Leptothorax workers perform colony tasks as do the first slaves captured by slave-maker queens. Interestingly, any Leptothorax queens that eclose from the captured brood, after having their wings removed by the slave-makers, remain in the nest apparently as slaves (Buschinger, Ehrhardt and Winter 1980). Certainly they are never reproductive in slave-maker colonies (see Ch.6). Captured Leptothorax males, in contrast to queens, are frequently only tolerated until they eclose, when they are killed (suggesting that species-characteristic odours are only expressed by adults in these ants) (see Ch.6).

3. Sexual production and mating behaviour

H.sublaevis colonies are perennial and very long-lived, possibly lasting 12 or 13 years (see below). Each winter the adults hibernate in a cluster around the entirely larval brood. In spring and summer the queens lay eggs (Buschinger 1966b). The resulting larvae develop remarkably slowly. In general, queens take two years (two hibernations) to grow from egg to adult, while males take one year (Buschinger 1973b, pers. comm., Winter and Buschinger 1986). Pupation and eclosion also occur in the summer (Buschinger 1966b). Although new queens may eclose as early as the second year of brood production (Buschinger 1974b, Winter and Buschinger 1986), young colonies concentrate on producing worker slave-makers, to increase the slave force correspondingly (Buschinger 1978b; Ch.5). Following the growth phase, as far as is known colonies produce queens, males and workers each summer until the queen dies and all her brood is reared, and exclusively males (from reproductive workers) thereafter (see below). How investment is allocated between these categories, and the proportion of worker- to queen-produced males, are two of the major topics of this thesis (see chapters 4,5 and 9).

The young H.sublaevis queens find a mate as follows (from Buschinger 1968a,b,1971b,1972,1973a,1982,1983, Buschinger and Alloway 1979). Leaving the maternal nest one evening shortly after eclosion, young H.sublaevis queens climb onto promontories such as twigs, adopt a posture with gaster raised and sting extruded identical to that of workers in tandem calling, and emit a male attractant pheromone from the poison gland. H.sublaevis males fly in search of the "calling" queens (which they detect from 3-4m. away) and briefly copulate with them upon contact. The males then presumably search for additional females, since in the laboratory they mate with as many as ten. Queens by contrast mate only once. The evidence for this is both behavioural - after their first mating queens rarely call again - and electrophoretic (see Ch.5). Queens which fail to attract a mate call again on subsequent evenings. All queens from one year's production in nature leave the home nest within the same year, since in dissections of members of hibernating colonies from the field Buschinger and Winter (1978) found virgin queens almost totally absent.

"Female calling syndrome", as the mating behaviour of *H.sublaevis* queens is termed, contrasts strongly with the massive, synchronous nuptial flights of males and queens of most ant species (reviewed by Wilson 1971, Hölldobler and Bartz 1985). But outside ants female calling is a well-known phenomenon, notably in termites and moths (Jacobson 1965, Thornhill and Alcock 1983, Lewis 1984). In ants female calling is found in the primitive ponerines (Hölldobler and Haskins 1977, Haskins 1978, Hölldobler and Bartz 1985) and, among leptothoracines, in the other social parasitic species of (or close to) *Leptothorax* subgenus *Mychothorax* as well as *H.sublaevis*. It also occurs in the hosts of *H.sublaevis*, *L.muscorum* and *L.gredleri*, but not (at least in some populations) in *L.acervorum* (Buschinger 1971a,b, 1974a,1975a, 1982, Buschinger and Alloway 1979, P.Douwes pers. comm.).

Female calling in *H.sublaevis* did not evolve because of wing loss, since all the calling relatives of *H.sublaevis* just mentioned have winged queens. Also, the rare winged form of *H.sublaevis* queen exhibits female calling. Wing loss in *H.sublaevis* queens therefore evolved after female calling behaviour (see final section of chapter). The occurrence of female calling in the free-living L.muscorum and L.gredleri suggests that this is the primitive mating behaviour of Leptothorax subgenus Mychothorax. In species like these with small and diffuse colonies, female calling is likely to have been advantageous, since nuptial flights would have been difficult to co-ordinate. Consequently female calling is not a specifically social parasitic adaptation in H.sublaevis but rather, as in the case of tandem recruitment, pre-dates the species' parasitic habits.

Since nearly all H.sublaevis queens walk to their mating site, they possibly risk mating with a male from the same colony. The question of whether the queens preferentially outbreed (by dispersal, or by rejecting related males) is important in determining whether local mate competition influences sex investment ratios in H.sublaevis (see Ch.5). Adlerz (in Wheeler 1910:493) apparently noted a reluctance of H.sublaevis queens to mate with males from the same colony. On the other hand, from my observations mating between nestmates does occur, although I saw this in captive colonies with no other available option. The behavioural evidence for inbreeding avoidance is therefore inconclusive. Fortunately the electrophoretic analysis described in chapter 5 provides a firmer conclusion: there is no genetic evidence for inbreeding in H.sublaevis.

The mating period in H.sublaevis is relatively short, since adult males live no more than 14 days (Winter and Buschinger 1986). The mated queens go in search of Leptothorax nests in which to found new colonies and restart the colony cycle. But before the cycle ends for the mature colony, the colony reaches a final and possibly extremely important stage, the period of orphanage.

4. Colony orphanage

H.sublaevis queens, in sharp contrast to males, are very long-lived. In captivity adult queens live for up to 7 years (Buschinger pers. comm.). Since in this time they may undergo 14 artificially compressed breeding cycles, they probably live even longer than 7 years in nature. In fact after finding roughly 10% newly-founded (i.e. first year) colonies in the field, Buschinger (1974b), assuming that mortality is concentrated in old queens, estimated the average longevity of queens to be 10 years. H.sublaevis workers on the other hand live only 2 or 3 years (Buschinger pers. comm.). Consequently a colony of H.sublaevis theoretically lives for 10 years in a queenright condition (i.e. with the maternal queen present) and for a further 2 or 3 years as an orphaned society following the queen's death. The bulk of worker male production almost certainly occurs in the period of orphanage (Ch.5). In chapters 4 and 8 I will advocate the importance of colony orphanage for worker reproduction in H.sublaevis.

Orphaned colonies are also capable of mounting slave raids (Buschinger pers. comm.). Therefore in nature their productivity may not be limited by the number of Leptothorax slaves remaining after the queen's death. But since they produce no new slave-maker workers, the inevitable fate of orphaned colonies is eventually to dwindle and die.

Genetic influence on queen dimorphism and caste

I now consider an extremely intriguing aspect of the biology of H.sublaevis, the genetic influence on queen dimorphism and caste (see Buschinger 1966b,1975b,1978a,b, Buschinger and Winter 1975, Winter and Buschinger 1986). As already mentioned, H.sublaevis queens occur in two forms, winged (gynomorphic) and unwinged (worker-like or ergatoid). Ergatoids by far outnumber gynomorphs. For example, in a population of H.sublaevis near Nuremburg only 6 out of 600 colonies were headed by a gynomorph (Buschinger 1978b). Breeding experiments established that the H.sublaevis queen dimorphism is partially genetically controlled. The results were consistent with the existence of a dominant allele (E) which prevents female larvae becoming gynomorphs. Only ee individuals can be gynomorphs. But larvae of all genotypes can become workers or ergatoid queens, as summarized below:

Genotype	Phenotype
EE	Worker, ergatoid queen
Ee	Worker, ergatoid queen
ee	Worker, ergatoid queen, gynomorphic queen

Winter and Buschinger (1986) found that as well as influencing queen dimorphism, the E/e system also affects queen/worker caste determination. The allele E, by increasing their developmental time, predisposes female larvae to become workers rather than (ergatoid) queens. Conversely, e biases individuals towards becoming queens, not workers. H.sublaevis is therefore the first known ant species with genetically mediated caste determination (Winter and Buschinger 1986).

This finding is particularly significant because in social Hymenoptera caste determination is most frequently considered environmental (especially nutritional). The other well-attested case of genetic control of caste is in the stingless bee genus Melipona, in which queens only develop from female larvae heterozygous at two caste-determining loci (work of W.E.Kerr, reviewed by Wilson 1971, Crozier 1977, Oster and Wilson 1978). The *Harpagoxenus* system clearly differs in that female larvae of all genotypes can become queens. The E/e alleles bias larvae to one caste or another in proportions which cannot be exactly predicted. Nevertheless, the discovery of a genetic basis for caste determination in a second eusocial Hymenopteran group is especially timely because one recent hypothesis to account for widespread multiple mating by social Hymenopteran queens is that multiple mating serves to increase genetic variation in the workforce with respect to caste (Crozier and Page 1985).

Adaptive significance of queen winglessness

Despite, from Adlerz onwards, nearly a century of collecting of *H.sublaevis* in Sweden (where all *H.sublaevis* I studied originated), gynomorphic (ee) queens have never been found there. Therefore the allele e is presumably absent in Swedish populations, and all females are EE (see also chapters 5 and 6). This absence suggests a general, adaptive advantage of queen winglessness in *H.sublaevis*, notwithstanding the more complex situation in non-Swedish populations. I wish to close this chapter by speculating what this advantage could be.

To begin with, in a species with non-independent colony foundation, wing musculature for claustral rearing of the first brood is clearly unnecessary (Wilson 1971:138). Other ants without claustral colony foundation, such as army ants (whose colonies reproduce by fission), also have wingless queens (Wilson 1971). Furthermore, queens exhibiting female calling syndrome obviously do not need wings for nuptial flight. Ponerine ant queens which engage in sexual calling lack wings (Hölldobler and Haskins 1977). Since wing loss would therefore not have affected either *H.sublaevis* queens' mode of colony foundation or their mating behaviour, the wings *H.sublaevis* queens once all possessed were in a sense already superfluous. They may even have been an encumbrance. Darwin (1859:176) recorded that oceanic islands were typically inhabited by beetles with vestigial wings, and suggested the beetles had evolved this way to avoid being blown out to sea. In fact oceanic islands are also characterized by free-living ant species with ergatoid queens (Wilson 1971:138). By analogy, I suggest *H.sublaevis* queens lost their wings to avoid being blown from the ecological island of their host population, and consequently to increase their chances of encountering host colonies during dispersal.

Supporting evidence for this suggestion comes from other socially parasitic ants. *H.canadensis* and *H.americanus* queens admittedly have wings, but queens of the formicine slave-makers *Polyergus rufescens* (Collingwood 1979:155) and *P.breviceps* (Wheeler 1916) are occasionally ergatoid. Queens of the leptothoracine inquiline *Epimyrma kraussei* have superfluous wings, since they shed them unused after sib-mating in the nest (Winter and Buschinger 1983). Males of the inquiline *Anergates atratulus* are totally wingless and also mate in the nest (Collingwood 1979:80). More generally, winglessness (often accompanied by inbreeding at the site of emergence) is found in many insects living in restricted or transient habitats (Hamilton 1979). But in *H.sublaevis* the trend has clearly not reached the extreme state of the cases just mentioned, since queens leave the colony to mate. To conclude, queen winglessness in *H.sublaevis*, like many (though not all) of its features, is evidently a trait largely due to the species' parasitic habits.

Table 3.1 Morphological and behavioural characteristics in *Harpagoxenus* (from Buschinger 1981, Buschinger and Alloway 1979)

	H.americanus	*H.canadensis* and *H.sublaevis*
Similarities:	Obligate slave-maker of *Leptothorax* ants	Obligate slave-makers of *Leptothorax* ants
	Female external morphology	Female external morphology
Differences:	Hosts = *Leptothorax* subgenus *Myrafant* Smith, namely *L.ambiguus*, *L.longispinosus*, *L.curvispinosus*	Hosts = *Leptothorax* subgenus *Mychothorax* Ruzsky: *L.acervorum*, *L.muscorum*, *L.gredleri* (*H.sublaevis*); *L.muscorum*-like spp. (*H.canadensis*).
	Group recruitment on slave raids	Tandem recruitment on slave raids
	No female sexual calling behaviour	Female sexual calling behaviour
	H.americanus males not attracted to female sexual pheromone of *H.canadensis* or *H.sublaevis*	*H.canadensis* males attracted to female sexual pheromone of *H.sublaevis*, and vice versa.
	Dissimilar karyotype: 11 pairs of chromosomes	Similar karyotypes: 18 pairs of chromosomes in *H.canadensis*, 20 in *H.sublaevis*
	Wing venation of alate female resembles that in *Myrafant*	Wing venation of alate female unlike that in *Myrafant*
	Males have short antennae as in *Myrafant*	Males have long antennae as in *Mychothorax*

Chapter 4

Dominance Orders, Worker Reproduction, and Queen-Worker Conflict in Harpagoxenus sublaevis

Introduction

Hamilton (1964) proposed that asymmetries in relatedness between close kin caused by haplodiploid sex determination could account for Hymenopteran worker sterility, a phenomenon that has disquieted evolutionists from Darwin (1859) onwards. In haplodiploid systems males arise from unfertilized eggs and are haploid, whereas females arise from fertilized eggs and are diploid. The important consequences for relatedness (r) in social Hymenoptera are that full sisters are more closely related to each other (r=0.75) than to daughters (r=0.5) and less closely related to brothers (r=0.25) than to sons (r=0.5). Kinship theory therefore argues that workers under a single, once-mated queen should either (1) reject personal reproduction and rear a female-biased brood of the queen's reproductive daughters and sons, or (2) retain a reproductive capability and raise a more evenly-balanced brood of the queen's daughters and their (workers') sons. Queens oppose both options, in (1) because queens prefer equal investment in their sexual offspring, in (2) because queens favour their own over the workers' sons (Hamilton 1964,1972, Trivers and Hare 1976, Oster and Wilson 1978) (see also Ch.8).

Much subsequent work on kinship theory concentrated on the non-reproductive worker option (1), because this part of kinship theory was such a strikingly original solution to the evolutionary puzzle of worker sterility. However, several recent developments suggest the reproductive worker option (2) deserves renewed attention. First, a number of authors have concluded from various models that eusociality (co-operative brood care, overlapping female generations, reproductive division of labour) would have evolved more easily if early workers produced males (Aoki and Moody 1981, Iwasa 1981, Bartz 1982, Pamilo 1984). Second, empirical studies reveal a growing number of cases of worker reproduction among social Hymenoptera (see Ch.8). Third, evidence is accumulating that queen-worker conflict over worker male production is a major feature of Hymenopteran societies (West-Eberhard 1981). For all these reasons (see also Ch.8), the question of how far the organization of Hymenopteran societies is shaped by selection for worker reproduction is assuming importance in social insect biology.

One way to examine this question is to investigate behaviour (e.g. Cole 1986). If workers take the reproductive option, queens (because their interests conflict with the workers') should act to inhibit them (queen control). The expected behaviour of reproductive workers will also differ greatly (beyond obvious differences) compared to the behaviour of sterile workers. Sterile workers, because helping costs them nothing in lost offspring, predictably exhibit extremes of co-operative and altruistic (self-sacrificial) behaviour in rearing kin (Oster and Wilson 1978). By contrast, the behaviour of fertile workers will be partially directed towards competition and self-preservation. One effect of this will be to constrain the temporal division of labour - i.e. the pattern of task allocation over time - in the worker caste, since fertile workers should be unwilling to perform tasks involving personal risk (Wilson 1985).

This chapter describes my work with Harpagoxenus sublaevis concerning the influence of worker reproduction on worker behaviour, queen control, and division of labour. The next chapter concentrates on associated genetic and demographic issues, namely the level of worker male production, intra-colony relatedness, colony productivity, and the sex investment ratio. As stated in chapter 1, my aim is therefore to understand the reproductive behaviour of *H.sublaevis* workers and queens in terms of their social and genetic environment. *H.sublaevis* is ideal for this work because it combines a simple colony structure (single, once-mated queen: see Ch.5) with workers capable of male production (Buschinger 1978b, Buschinger and Winter 1978; Ch.3). Also, because slave-maker workers help rear kin by the indirect method of raiding for the colony's slave labour force (which performs all brood care: see Ch.3), the reproductive choices of slave-maker workers are conveniently reflected in their behaviour during slave raids: sterile workers should raid as their only means of helping rear kin and thereby increasing their inclusive fitness, but fertile workers should refrain from raiding because of the risk it involves to their personal fitness, since raiding can be fatal (see Ch.3 and below).

I carried out observations and experiments on six *H.sublaevis* colonies collected from the field. In colony 1 (queenright, i.e. containing the maternal queen) I discovered that potentially fertile *H.sublaevis* workers form competitive, linear dominance orders as previously found in only two other ant species (*Leptothorax allardycei*, Cole 1981,1986; *Harpagoxenus americanus*, Franks and Scovell 1983). In colonies 2 and 3 (both queenless) I found that orphaned workers also exhibit dominance orders, and further investigated reproductive competition among workers with removal experiments. In colony 4 (queenright) I tested the hypothesis that queens oppose worker reproduction, by removing then replacing the queen. Franks and Scovell (1983) found high-ranking *H.americanus* workers never scouted in search of slave colonies to raid. In colonies 5 and 6 (both queenright) I tested the hypothesis that potentially fertile *H.sublaevis* workers participate reluctantly in both scouting and slave-raiding.

Methods

Field collections Colonies 1-2 and 4-6 came from coastal pinewoods between Bröms and Kristianopel, Blekinge, S.E. Sweden. Colony 3 came from an inland site at Önnarp, near Roke, Skåne, S.Sweden. In both localities colonies of *H.sublaevis* and its *Leptothorax* slave species occur plentifully in dead twigs on the ground. Single *H.sublaevis* colonies occupy single twigs (monodomy). Whole colonies were therefore collected in June or July (1983-1985) by fragmenting twigs and aspirating the ants and brood. At the time of study (in the first or second artificial summer after collection: see below) the adult composition of the six colonies was as shown in Table 4.1.

Culture methods The colonies were housed in nests made of two 5 x 7.5cm. plain glass slides separated by a cardboard wall (internal nest dimensions 2.5 x 2.0 x 0.2cm. to 4.0 x 3.0 x 0.2cm.). Each nest rested horizontally in a lidded 10 x 10 x 1.8cm. high petri dish (colonies 1-3) or a 17 x 11 x 4cm. high entomological box (colonies 4-6) containing a drinking water supply (water tube stoppered with damp cotton wool), a humidifier (gauze covered water-tray), artificial ant diet (Bhatkar and Whitcomb 1970), and (except in colony 1) fresh insect food (*Drosophila* larvae). Interior nest box walls were coated with the dry lubricant PTFE (polytetrafluoroethylene, "Fluon") to prevent escapes. Food and water were renewed every two to four days.

Colonies were maintained in laboratory incubators adjusted to simulate annual climatic cycles (adapted from Buschinger 1973b,1974b, pers. comm.). A complete cycle involved the following daily conditions of temperature (^{o}C) and photoperiod: 6-12 weeks at $10^{o}/0^{o}$ for 10h./14h. (hibernation), 2-4 weeks at $20^{o}/10^{o}$ for 12h./12h., 2 weeks at $25^{o}/15^{o}$ for 12h./12h., 8 weeks at $27^{o}/15^{o}$ for 14h./10h. (peak egg laying, sexual production), 2-4 weeks at $25^{o}/15^{o}$ for 12h./12h., and 2 weeks at $20^{o}/10^{o}$ for 12h./12h., with artificial daytime coinciding with the daily higher temperature period.

Marking method All slave-makers were individually marked. In colony 1 ants were marked with coloured paints (E.T. Marler Ltd., London). In colonies 2-6 ants were marked with 0.65 x 0.85mm. paper letters glued to the thorax. The letters were cut-outs of camera-reduced "Letrasett" transfers printed on photographic paper. The glue was "Araldite Rapid" Epoxy Resin (Ciba-Geigy, Cambridge). I abandoned paint marks because paper letters lasted longer (maximum recorded life 18 months) and, unlike paint, individually characterized ants on black and white video recordings.

Definitions of behaviours Colonies 1-4 were each observed over 3-5 weeks in a series of separate (approximately daily), standardized one hour observation bouts. In each bout I recorded every occurrence of dominance, aversion and trophallaxis involving slave-makers. These behaviours were defined as follows:

Dominance: Dominance took two forms. In the severe form a slave-maker bit and gripped another's appendage (e.g. leg, antenna) for a few seconds to several minutes. In the milder form a slave-maker rapidly approached and antennated another. Attacking ants frequently flexed their gasters towards those under attack as if to sting them, although they never actually protruded their stings. Ants under attack never defended themselves but typically withdrew their antennae and remained still. Attacks ended with the release of the attacked by the attacking ant.

Aversion: Aversion (or avoidance: Franks and Scovell 1983) occurred when a slave-maker recoiled violently from another (higher ranking) slave-maker following antennal contact.

Trophallaxis: As discussed in Ch.3, trophallaxis (solicitation of liquid food) from slaves or larvae is the sole means by which slave-makers obtain nourishment.

I also recorded the amount of time each slave-maker spent outside the nest in the nest box arena, and in colonies 1 (every 15 mins.) and 4 (every 5 mins.) the identity of the slave-maker nearest the egg-pile.

<u>Observation methods and conditions</u> In each observation bout I watched an entire colony through a Zeiss or Olympus binocular microscope. Each colony was illuminated by a cold light source and maintained at 25-27^{o}C by a heated stage, except colony 1 (unheated, average temperature 21.3^{o}C). All observations took place with colonies in their artificial summer phase (27^{o}/15^{o}, 14h./10h.) to coincide with egg-laying, except the first 15 hours observation of colony 1, which took place during artificial springtime (25^{o}/15^{o}, 12h./12h.). All observations were made in daytime, and at least 30 mins. acclimatization was allowed between transferring the colony to the microscope stage and starting observations.

Video recording To obtain a record of egg-laying, activity in colony 4 was video recorded between observation bouts. The colony was filmed with a Panasonic TV camera (Model WV 1850/B, Matsushita Communication Industrial Co. Ltd., Japan) mounted on a Zeiss binocular microscope, and recordings made with a Panasonic Time Lapse Video Recorder (Model NV-8050). Temperature and photoperiod matched those in incubators in the artificial summer phase, except that at night temperature was uncontrolled (room temperature was c. $18^{o}C$) and the colony was illuminated by an infra-red source, to permit night filming (infra-red light is invisible to ants). Colony 4 was videoed in 13 separate sessions for a total 229.8 hrs.

Experimental treatments Colony 1 (queenright) was observed for 30 hrs. over 3 weeks to determine basic social structure.

Colonies 2 and 3 (both queenless) were each observed for 36 hrs. over 5 weeks to determine social structure in orphaned colonies, and to study effects of removing the top-ranking worker. I therefore observed each colony for 12 hrs. with the top-ranking worker present, 12 hrs. with the worker removed, and 12 hrs. with the worker returned (control). In both colonies daily fluctuations in egg number were recorded to infer the identity of layers.

Colony 4 (queenright) was used to test for queen inhibition of worker fertility (queen control) in an experiment with the same design as the worker removal experiments (12 hrs. observation when queen present, 12 hrs. when removed, 12 hrs. when returned). Before the first observations the queen was isolated for 5 days in a dish containing the dye Fat Red 7B (Sigma Chemical Co., St.Louis), to stain her eggs. All other colony 4 slave-makers were simultaneously isolated in dye-less dishes to minimize effects of the queen's absence prior to the experiment. After observation bout 24, egg-laying in colony 4 was also video recorded (details above).

Colonies 5 and 6 (both queenright) were used to investigate individual differences between slave-makers in scouting (searching for slaves) and slave-raiding. Slave raids were induced following Winter's (1979) split arena technique. During their artificial summer the nest containing each colony was placed for 6-7 days in a large (48 x 48 x 8cm. high) arena separated by a removable barrier from a 48 x 24 x 8cm. high arena containing a colony of *Leptothorax acervorum* (slave species). Both arena floors were unevenly covered in sand, and additional orientation cues were provided by wooden strips lying in the arenas and a fixed, overhead polarized light source. PTFE on arena walls prevented escapes. Daytime temperatures were 22-28°C. Over 6-7 days before raiding, scouting by slave-makers was recorded in 6 daily 2h. bouts. A slave-maker was considered to be scouting on leaving a 10 x 10cm. area around the slave-maker nest (slave-makers do not forage: Ch.3). At c. 1400h. on the 6th or 7th day in the arena, a slave raid was induced by removing the barrier separating the slave-maker from the *L.acervorum* colony. The behaviour of individual slave-makers was continuously monitored for the duration of each raid.

Brood removal and colony size manipulation In all colonies except 1 and 2 slave-maker brood was removed before (or shortly after) the first observations and replaced with equivalent amounts of *L.acervorum* brood. This was to prevent new *H.sublaevis* females reaching adulthood during the study period. In some ants, the presence of conspecific brood has been shown to inhibit worker fertility (e.g. Dartigues and Passera 1979, Smeeton 1982a). However, such an effect did not account for the results of this study since, as will be described, workers in colony 1 exhibited ovary development even though the brood was not replaced in this colony, and conversely worker egg-laying activity only appeared in colony 4 after the queen's removal, and then ceased when she was returned, although the brood was replaced before the start of observations.

In colonies 5 and 6 alone the numbers of adult slave-maker females were artificially reduced before observations began, because previous numbers were too high to allow simultaneous observation of all ants, and suitable queenright colonies were otherwise unobtainable. In colony 5 the slave-maker population was reduced from 33 females to 20, in colony 6 from 41 to 23. Excluded females were arbitrarily chosen (except I ensured the colony queen remained in each colony). Removals were carried out c. 4 weeks before scouting recordings began.

Ovarian dissections and size measurements At the end of each experiment slave-makers from all colonies were dissected to determine their caste and reproductive status. The ovaries were removed in Ringer's solution with fine forceps, and the numbers of active ovarioles, oocytes, and corpora lutea were counted under a compound microscope (dissection method after Buschinger and Alloway 1978). Insect corpora lutea are ovariolar structures indicative of egg-laying activity (Imms 1977:297). Ergatoid queens (morphologically externally indistinguishable from workers) could be positively identified by the spermatheca (see Ch.3), visibly full of sperm if queens were mated. The maximum pronotal (thoracic) width of each slave-maker was simultaneously measured as an index of body size.

Note on virgin queens In colonies 3,4 and 5 dissections revealed that a small number of ants (4,4 and 6 respectively) were supernumerary, non-laying queens (Table 4.1). In nature young H.sublaevis queens leave the maternal nest in the year of production first to attract a mate by sexual calling and then to found new colonies (Buschinger 1968a, Buschinger and Winter 1978; Ch.3). Colonies 3-5 were all given the opportunity to release sexuals following capture, but for unknown reasons not all H.sublaevis queens exhibit sexual calling in laboratory conditions. The lingering presence of small numbers of young queens in colonies 3-5 was therefore unnatural. However, the presence of these queens did not appear to perturb colony organization, since they never exhibited dominance behaviour and high ranking workers treated (and dominated) them apparently like passive workers.

Results

Worker dominance hierarchy in a queenright H.sublaevis colony (colony 1) In colony 1 the results (Figs. 4.1 and 4.2) showed that a subset of 3 slave-maker workers behaved aggressively towards the remaining 11, non-aggressive slave-maker workers. The queen and the 3 aggressive workers formed a stable, linear dominance order headed by the queen (0.9% of aggressive interactions involved rank reversals). Rank was correlated with the following : (1) Ovarian development. All 3 aggressive workers had ovarian development, compared to only 1 of the 5 passive workers dissected (One tailed Fisher's exact test, p=0.07). (2) Frequency of trophallaxis. The aggressive workers solicited trophallaxis from slaves or larvae at a mean rate of 0.89 times/h., compared to 0.51 times/h. in passive workers (One tailed Mann-Whitney U-test, U=28, p=0.05). The queen had the greatest rate of trophallaxis (2.27 times/h.) and fed disproportionately more often from larvae than workers (0.24 of the queen's trophallaxis involved larvae, compared to 0.15 of the workers'). Trophallaxis between slave-makers was extremely rare (0.7% of all their trophallaxis), as was interference by slave-makers with other slave-makers' trophallaxis (0.9% of slave-maker/slave trophallaxis resulted from one slave-maker interrupting another). (3) Time outside the nest. Only the queen and the top-ranking aggressive worker never left the nest. Aggressive workers on average left the nest for 5.2 mins./h., whereas passive workers were outside 11.3 mins./h., although this difference was not significant (One tailed Mann-Whitney U-test, $U=26$, $p=0.1$).

The queen was the slave-maker nearest the eggs for 85% of all records (n records=96), far greater than the expectation based solely on the amount of time the queen spent in the nest relative to the other slave-makers (X^2 test, $X^2=746.7$, $p<0.001$).

Size did not appear to be a correlate of dominance. The mean pronotal widths of aggressive and passive workers were 0.61 and 0.60mm. respectively (t test, $t=0.840$, $p>0.1$).

These results suggested that in a queenright H.sublaevis colony potentially fertile H.sublaevis workers (1) inhibit their prospective rivals' ovarian development with aggressive dominance behaviour, (2) consume extra food for egg development, and possibly (3) protect their reproductive futures by avoiding risks outside the nest.

Worker dominance hierarchies in queenless H.sublaevis colonies, and effects of removing top ranking workers (colonies 2 and 3) The initial 12 hrs. observation of colonies 2 and 3 showed that in both these queenless colonies worker dominance hierarchies existed as in the queenright colony 1 (Figs. 4.3 and 4.4). The numbers of aggressive workers in the two colonies were 2 and 3 respectively. Correlates of dominance (ovarian development, frequency of trophallaxis, time outside the nest) were the same as in colony 1, except that in colony 3 for unknown reasons the passive workers had a higher rate of trophallaxis. Thus, in colony 2, the mean trophallaxis rate of aggressive workers was 2.81 times/h., compared to 0.93 times/h. in passive workers. The mean time outside the nest in aggressive workers was 0.35 mins./h., and in passive ones 7.7 mins./h. (Fig.4.3). In colony 3 the mean trophallaxis rates of aggressive and passive workers were 1.29 and 1.69 times/h. respectively, and the mean times outside the nest 2.5 and 27.0 mins./h. respectively (Fig.4.4). As in colony 1, all aggressive workers in colonies 2 and 3 were ovary-developed, and there was only one passive, ovary-developed worker per colony (Figs.4.3,4.4). Therefore, considering colonies 2 and 3 together, all 5 aggressive workers had ovarian development, compared to 2 out of 8 passive workers, indicating a significant association between worker dominance behaviour and ovarian development (One tailed Fisher's exact test, p=0.02). Finally, for unknown reasons, the level of aggression was far higher in colony 2 than in colony 1, but in colony 3 it was lower (the numbers of dominance acts per aggressive ant per hour were 0.47 in colony 1, 5.28 in colony 2, 0.36 in colony 3).

In both colonies 2 and 3 immediately after the top-ranking (alpha) worker was removed, the egg count stopped rising. Both alpha workers continued laying in isolation, suggesting they were initially the sole layers in their respective colonies. Within 5 (colony 2) and 6 (colony 3) days of the alpha worker's removal, the egg count in both colonies started rising again. Since in both colonies later dissection revealed the only other slave-maker apart from the alpha to possess corpora lutea was the second-ranking (beta) worker, the new egg layer following alpha's removal must have been beta in both cases. In colony 3 beta was in fact seen laying an egg 6 days after alpha's removal.

The results of returning the alpha worker differed in the two colonies. In colony 2 the newly-returned alpha attacked the former beta, and thereby resumed its top-ranking position. Beta ceased both dominance behaviour and egg-laying within a day of alpha's return. In colony 3 the newly-returned alpha was itself attacked by the former third-ranking (gamma) worker (risen to the beta position in alpha's absence). Over the following days alpha, like a passive ant, exhibited neither dominance behaviour nor laying. Beta, by contrast, continued to show dominance behaviour and was also observed egg-laying. These conclusions are summarized in Fig.4.5.

The fact that in both colonies the beta worker started laying eggs following alpha's removal, and that on alpha's return each beta ceased or continued laying according to whether alpha assumed a higher or lower rank, confirmed that in H.sublaevis workers dominance behaviour inhibits egg-laying in subordinates. The reason for the (instructive) failure of the alpha worker to regain its top-ranking position in colony 3 was unknown.

As in colony 1, large size was not a correlate of dominance in colonies 2 and 3. The mean pronotal widths of the aggressive and passive ants were respectively 0.55mm. (n=2) and 0.57mm. (n=4) in colony 2, and 0.51mm. (n=3) and 0.58mm. (n=4) in colony 3 (Figs. 4.3 and 4.4).

Effects of queen removal (colony 4) In the initial 12 hrs. observation of colony 4 worker (and queen) dominance behaviour was totally absent. All eggs laid were dyed, indicating the queen was sole layer at this time. When the queen was removed one worker (J) began to show dominance behaviour within 24 hrs. (Fig. 4.6). A second aggressive worker arose 12 days after the queen's removal. Within 8 days of the queen's removal J began egg-laying. Video recordings and later dissection showed J to be sole layer in the queen's absence. When the queen was returned, J ceased laying in about 2 days, but continued with dominance behaviour (Fig. 4.6).

Initially the queen in colony 4 occupied the position nearest the egg-pile for 82% of records. This figure was very close to that recorded for the queen of colony 1 (see above), and again far greater than expected on the sole basis of the relative amount of time the queen was in the nest (X^2 test, X^2=1383.1, n records=134, p<0.001). As in colony 1, the queen had the greatest frequency of trophallaxis (1.67 times/h. compared to the average worker trophallaxis frequency of 0.48 times/h.), and fed more often from larvae (0.65 of the queen's trophallaxis was from larvae, compared to 0.03 of the workers'). In the queen's absence the laying worker J adopted the position nearest the eggs for 55% of records, a figure far higher than the corresponding figures for the previous and following periods when the queen was present (J nearest eggs for 2% and 5% of records respectively) (X^2 test, X^2=146.1, n records=403, p<0.001). J's rate of trophallaxis increased from 0.26 times/h. to 1.75 times/h. on the queen's removal, and fell to 0.85 times/h. on the queen's return. J only conducted trophallaxis with larvae (0.21 of J's trophallaxis) during the queen's absence.

These results suggested that the H.sublaevis queen inhibits worker dominance behaviour and egg-laying. Queen control is presumably mediated pheromonally, since in colony 4 the queen was never aggressive, and in colony 1 she was only slightly aggressive (Fig.4.1). The results also showed that laying workers, like queens, characteristically remain close to the egg-pile, and furthermore confirmed that laying workers display an increased rate of trophallaxis. Trophallaxis with larvae appeared to be associated with egg-laying.

Individual differences between ovary-developed and non-ovary-developed workers in scouting and raiding (colonies 5 and 6) Six of the 13 slave-maker workers in colony 5, and ten of the 22 workers in colony 6, exhibited ovarian development. However, in both colonies the overall level of ovarian development was low, the mean oocyte number being 4.7 in colony 5 and 2.9 in colony 6 (Table 4.2), compared to 6.0, 8.3 and 9.2 in colonies 1, 2 and 3 respectively (Figs.4.1,4.3,4.4). Despite this, in each colony ovary-developed workers spent significantly less time scouting than workers without ovarian development (Table 4.2). This difference was greater in colony 5 than in colony 6, matching the greater ovarian development of colony 5 workers relative to colony 6 workers. Therefore, the prediction ovary-developed workers should avoid risks, possibly great in scouting since scouting is a solitary activity (Buschinger, Ehrhardt and Winter 1980, Franks and Scovell 1983), was fulfilled.

The two slave raids followed the sequence - discovery of Leptothorax nest, tandem recruitment, fighting, brood transport - typical for H.sublaevis (Winter 1979, Buschinger, Ehrhardt and Winter 1980) (Ch.3). In agreement with these authors' findings, both the relative duration of each phase, and total raid length (first recruitment to last brood transport, i.e. 3.7h. in colony 5 and 6.9h. in colony 6), differed greatly in the two colonies (Table 4.2).

In both colonies every slave-maker entered the *Leptothorax* arena during the raids except the two colony queens, who remained in their nests throughout, and in colony 6 a single non-ovary-developed worker, who left the slave-maker nest to fight with *Leptothorax* ants but did not leave the slave-maker arena. However, although all ovary-developed workers therefore took part in raiding, their degree of participation was on average less than that of non-ovary-developed workers, in the following ways. (1) Response time to recruitment. One measure of the level of participation of slave-makers in the raids, and of the risks they underwent, was the time between the start of each raid and the first entry of each slave-maker into the arena of hostile *Leptothorax* ants. In ants outside the slave-maker nest at the time of the first tandem recruitment, i.e. the intranidal excitation display and tandem "call" (see Ch.3) by the first slave-maker scout to discover the *Leptothorax* colony (following removal of the intervening barrier) and return to the slave-maker nest for recruits, time of first entry to the *Leptothorax* arena was largely determined by chance. This was because such ants, unless they had already happened to cross from the slave-maker to the *Leptothorax* arena, could not have "known" a raid had begun. Only slave-makers in the nest at the first recruitment could have been alerted to this. In both colonies, ovary-developed workers present in the slave-maker nest when the first recruitment occurred, on average entered the *Leptothorax* arena later than the non-ovary-developed workers also present in the nest at that time (Table 4.2). Although this difference was small in colony 6 and not significant in either colony, in colony 5 chiefly because of small worker numbers (One tailed Mann-Whitney U-tests, both p>0.1), this finding implied a reluctance to respond to recruitment to the raid target on the part of the ovary-developed workers.

Ovary-developed workers also differed in (2) Adoption of specialist roles. In H.sublaevis slave raids, specialist roles include those of tandem leader, and brood transporter (Buschinger, Ehrhardt and Winter 1980; Ch.3). Brood transport tended to be monopolized by one or two slave-makers. Thus, in colony 5, two workers transported 43% and 46% of all captured brood items, and in colony 6 just one worker performed 91% of all brood transport. Considering both colonies together, 12 of the combined population of 35 slave-maker workers (comprising 19 non-ovary-developed and 16 ovary-developed workers: Table 4.2), acted as tandem leaders or brood transporters. Eight were non-ovary-developed and 4 had ovarian development. Therefore, fewer ovary-developed workers adopted these specialist roles than expected on the basis of their relative abundance, although this difference was not significant ($X^2=0.76$, d.f.=1, p>0.1). (3) Involvement in fights. Of 56 separate fights between slave-makers and hostile Leptothorax recorded in the two raids, 34 involved non-ovary-developed workers and 22 workers with developed ovaries. Therefore ovary-developed workers took part in fewer fights than their relative abundance suggested, though again the difference was not significant ($X^2=0.93$, d.f.=1, p>0.1). A single slave-maker was killed by hostile Leptothorax in the raids, a non-ovary-developed worker from colony 6.

Although not individually conclusive, together these findings suggested a quantitative difference existed in the level of participation by slave-maker workers in slave raids, based on workers' ovarian development. Ovary-developed workers responded to recruitment to raiding later, and played lesser roles thereafter. To this extent, the hypothesis prospectively reproductive *H.sublaevis* workers should avoid the risks associated with slave-raiding was confirmed. A possible reason for the fact no ovary-developed worker failed to participate totally in raids was the low average level of ovarian development among such workers in both colonies (see above and Table 4.2). The reduction of slave-maker number prior to observations in both colonies (see Methods) could also have had a greater disruptive effect on colony organization than anticipated.

In agreement with previous results, size was not a correlate of ovarian development or scouting and raiding behaviour in colonies 5 and 6 (Table 4.2).

Discussion

Competitive worker dominance orders such as those reported in *H.sublaevis* in this chapter are relatively common among social wasps and bees (Wilson 1971) (see Ch.8), but in ants only two other cases have been described (*Leptothorax allardycei*, Cole 1981, 1986, *Harpagoxenus americanus*, Franks and Scovell 1983). The evident ability of *H.sublaevis* workers and queens to inhibit (behaviourally or pheromonally) egg-laying by subordinates is similarly matched by many other social insects (Wilson 1971, Brian 1983). This study also confirms that worker reproduction constrains the temporal division of labour (Wilson 1985) (see Ch.8).

An alternative to the conclusion that the social structure of *H.sublaevis* colonies reflects intra-colony reproductive competition is that the dominance system promotes a more (not less) efficient division of labour at colony level, as has been suggested is the case in wasp colonies with dominance orders (Wilson 1971:334). In H.sublaevis the system arguably serves as a mechanism for determining which slave-makers should raid, since it is probably disadvantageous from the colony's viewpoint for all slave-makers to raid (and risk death) simultaneously. However, several lines of evidence contradict the division of labour hypothesis. First, the hypothesis does not explain the apparent lack of dominance activity in some queenright colonies (e.g. colony 4). Second, the hypothesis arguably predicts that worker size should be correlated with division of labour, since larger workers presumably make better raiders. But no such correlation exists (Table 4.2). Third, dominance behaviour is costly to the colony because of the increased trophallaxis rate of the aggressive slave-makers. Assuming the mean trophallaxis rate of the 3 aggressive workers of colony 1 (0.89 times/h.) would drop to the mean rate of the 11 passive workers (0.51 times/h.) in the absence of the dominance system, I calculate (following Cole 1986) that dominance increases the food cost of maintaining the slave-maker workers by 16%. Although there is no clear evidence that worker dominance activity actually reduces colony productivity (see following chapter), these facts make it unlikely that the dominance system enhances efficiency.

An unknown factor in this study was worker age. Commonly, reproductive worker social insects lay eggs when young and switch to risky colony-beneficial tasks when old. In this way they change their reproductive strategy according to their diminishing chances of future survival as senescence approaches (Wilson 1985) (Ch.8). Reproductive H.sublaevis workers may undergo this change, since Buschinger, Ehrhardt and Winter (1980:251) found that H.sublaevis scouts were aged at least one year. However, it is almost certain that not all *H.sublaevis* workers are reproductive when young, because if they were a greater proportion of workers with corpora lutea but without ovarian development would occur than is found. In colonies 1-6 there were only two such workers (colony 5: Table 4.2). Nevertheless, this finding does not exclude the possibility that reproductive *H.sublaevis* workers lay eggs when young and raid when old.

The social structure of *H.sublaevis* colonies closely resembles the competitive dominance system in *H.americanus* uncovered by Franks and Scovell (1983). But since *H.americanus* is not a true congener of *H.sublaevis*, and almost certainly arose from a separate (free-living) leptothoracine stock (Buschinger 1981; Ch.3), both slave-making and dominance must have evolved convergently in the two species.

In colony 1 as well as aggression between slave-makers I also noted a low frequency of attacks by slave-makers on slaves (6.2% of all aggression involving slave-makers), slaves on slave-makers (3.1% of all aggression involving slave-makers), and slaves on other slaves (12 occurrences in 30 hrs.). In colonies of the slave-maker *Leptothorax duloticus* Wilson (1975b) also recorded attacks by slaves on other slaves and on slave-makers. All these attacks conceivably resulted from residual odour differences between ants, since slaves not only have a different species odour to slave-makers but possibly also differ in odour among themselves if, as is likely, they originate from different colonies.

Another intriguing kind of behaviour occurred in colony 3 when the beta worker, risen to alpha rank on the original alpha's removal, was seen to eat (sharing with a slave worker) an egg recently laid by another slave worker. The egg, the only one I saw laid by a slave in a slave-maker colony, was small and flaccid and presumably non-viable. Wesson (1940) and Wilson (1975b) both observed L.duloticus slave-maker queens eating slave worker-laid eggs. This incident in H.sublaevis may be connected with two additional observations in which the queen of colony 1 and the new alpha worker of colony 3 (as above) were each seen apparently eating an emission from the abdominal tip of a slave worker. In both cases the slave worker was bent double and its sting was exposed as in egg laying, strongly suggesting that both times a slave-maker was eating a slave's egg as the egg was being laid. Similar behaviour ("abdominal trophallaxis") has been observed in free-living ants, e.g. Myrmecia gulosa (Freeland 1958), Zacryptocerus varians (Wilson 1976), and Procryptocerus scabriscutus (Wheeler 1984). However, the H.sublaevis case does not resemble abdominal trophallaxis as reported by Stuart (1981) in H.americanus, which involved the transfer of substances from slave-maker to slave. If abdominal trophallaxis in H.sublaevis involves slave egg consumption, the two observed cases fundamentally resemble the original record of a slave-maker eating a slave's egg. Since the recipient in all these cases was a queen or alpha worker, it appears that slave egg consumption, like ordinary trophallaxis, was another correlate of dominance rank.

Clearly, worker reproduction in H.sublaevis strongly influences the colony's social structure, nutrient flow, and division of labour. But the level of intra-colony relatedness in H.sublaevis is maximal (all workers within a colony are full sisters: see following chapter), and consequently a high degree of worker "selfishness" is unexpected, assuming H.sublaevis workers are striving to follow Hamilton's sterile worker option (see Introduction). The question therefore arises as to why H.sublaevis workers are so "selfish". I suggest the following, two-step answer. First, I propose that worker reproduction was formerly even more prevalent in H.sublaevis because H.sublaevis workers, instead of adopting sterility, followed the reproductive Hamiltonian option of raising sisters and producing sons outlined in the Introduction. Second, I suggest that as a result H.sublaevis queens developed increasingly effective power to inhibit worker laying (which diverts resources from queen progeny), to the point worker reproduction is now absent in some queenright colonies. In other words, the best interpretation of the social structure of H.sublaevis described in this study is to view it as representing the current state of a queen-worker conflict over male parentage.

Since it would be advantageous to every H.sublaevis queen to inhibit queenright reproductive activity in her workers, reasons must exist for why this has so far failed to occur. I now propose to discuss three such reasons (not mutually exclusive), the first of which involves monogyny (the existence of one maternal queen per colony). In monogynous species colony orphanage through natural queen mortality is a likely event. The proportion of orphaned H.sublaevis colonies in the Bröms-Kristianopel population was c.30% (see next chapter). Orphanage evidently frees workers with reproductive capability from queen inhibition. In this study the highest level of worker dominance activity occurred in the queenless colony 2 (see Results). Further, data in the next chapter show that H.sublaevis workers are most commonly fertile in queenless colonies, and that such workers give rise to most worker-produced males. Therefore, in monogynous species, workers may have been selected to retain reproductive capability because of the high probability that orphanage will afford them a rich opportunity to produce male eggs free from queen interference (see also Ch.8). Some of the behaviour of ovary-developed workers in queenright H.sublaevis colonies could consequently be for maintaining dominance rank in anticipation of the queen's death, that is for future rather than present reproductive gain. However, my main point is that workers selected to be in a state of readiness for reproduction when the queen dies, will also be harder for queens to inhibit in queenright conditions.

A second reason for the high level of H.sublaevis worker reproductive activity arises from consideration of sex ratio. As stated in the Introduction, the sterile worker option in Hamilton's kinship theory involves workers raising a female-biased brood of the queen's sexual offspring. It was taken as corroboration of Hamilton's theory that in many free-living (i.e. non-parasitic) ant species, sterile workers raise such broods (Trivers and Hare 1976, Nonacs 1986a). It was also recognized that in slave-making species, workers - since they are not involved in brood care - almost certainly lack the practical power found in workers of free-living ants, to manipulate brood composition towards the female-biased sterile worker optimum in the face of opposition from queens (who prefer equal investment in their progeny) (Trivers and Hare 1976). This presumed lack of worker control over sex ratio in slave-making ants appears genuine, since earlier studies (Trivers and Hare 1976, Nonacs 1986a) and this one (see following chapter) all find approximately 1:1 sex investment ratios in slave-makers, including H.sublaevis. But what was not previously realized was that this situation arguably promotes worker reproduction in slave-makers. Rather than follow the (for them) suboptimal course of helping raise an evenly balanced brood of the queen's progeny, slave-maker workers should instead pursue the alternative course of individual male production. In other words, their inability to raise a female-biased queen-produced brood could explain the apparently strong selection on H.sublaevis workers to retain their reproductive option.

A third, more speculative, reason for reproduction by H.sublaevis workers is a historical one involving the evolutionary route to slavery advocated earlier (Ch.2). I argued that the evolution of slave-making began with non-independent colony foundation by single queens with polygynous antecedents. If this route is correct, the evolution of slave-making involved a re-evolution of monogyny. A queen heading a colony in which monogyny is recent and secondary may require time (in evolutionary terms) to acquire the power single-handedly to inhibit all worker reproduction.

H.sublaevis is only one of numerous social Hymenopteran species with reproductive workers (see Ch.8). It seems likely that many features of Hymenopteran eusociality are best explained by supposing, as in H.sublaevis, that within each species worker reproduction was formerly even commoner and that queen-worker conflict over worker reproduction is a major theme of each species' subsequent social evolution. These issues are further discussed in chapter 8. To sum up this chapter, the social structure of H.sublaevis points to the importance of kinship theory's reproductive alternative in the evolution of H.sublaevis society, and possibly of Hymenopteran societies in general.

Summary of Chapter 4

1. In a queenright *H.sublaevis* colony the queen and a small subset of workers formed a linear dominance order in which rank was correlated with ovarian development, frequency of trophallaxis with *Leptothorax* slaves, length of time spent inside the nest, but not body size. These findings suggest that reproductive competition leads potentially fertile *H.sublaevis* workers to inhibit their prospective rivals' ovarian development with aggressive dominance behaviour, consume extra food for egg development, and protect their reproductive futures by avoiding risks outside the nest.

2. Identical dominance orders occurred among workers in queenless colonies. When each egg-laying, top-ranking worker from two queenless colonies was experimentally removed, both former second ranking workers started to lay eggs, and then persisted or ceased egg-laying according to whether the original top-ranking worker failed or succeeded in regaining its former rank on its return, confirming that dominance in *H.sublaevis* workers inhibits egg-laying in subordinates.

3. Not all queenright colonies contained dominant workers. But the removal of the queen from such a colony resulted in the emergence of two dominant workers, one of which began laying eggs. Hence *H.sublaevis* queens inhibit dominance behaviour and egg-laying in workers. This inhibition appeared to be pheromonal, since queens were themselves rarely aggressive.

4. Ovary-developed *H.sublaevis* workers on average spent less time scouting for slaves, and tended to participate less in slave raids, than workers without ovarian development. These findings confirmed

that prospectively reproductive workers avoid the potentially fatal risks associated with leaving the nest.

5. In *H.sublaevis* worker reproduction therefore strongly influences the colony's social structure, nutrient flow, and division of labour. Worker reproduction could formerly have been even more prevalent in *H.sublaevis* assuming workers followed the strategy of raising sisters and producing sons predicted by kinship theory. The continued existence of worker reproduction in *H.sublaevis* despite queen opposition conceivably results from several causes, including selection on orphaned workers to reproduce and the inability of slave-maker workers to raise a female-biased brood. Hence the social organization of *H.sublaevis* colonies points strongly to the importance both of worker reproduction and of the concomitant queen-worker conflict over male parentage in the evolution of Hymenopteran societies.

Table 4.1 Adult composition of 6 experimental H.sublaevis colonies

Number of individuals

Colony no.	H.sublaevis maternal (mated,egg-laying) queen	H.sublaevis workers	H.sublaevis supernumerary queens	L.acervorum slaves
1	1	14	0	85
2	0	6	0	39
3	0	7	4	19
4	1	14	4	37
5	1	13	6	50
6	1	22	0	34

Notes: a. See Methods for explanation of the (unnatural) presence of H.sublaevis supernumerary queens in colonies 3-5.

b. In colonies 5 and 6, the size of the slave-maker population was artificially reduced to the levels shown here (see Methods).

Table 4.2 Individual differences between slave-maker workers in scouting and raiding (colonies 5 and 6)

Colony	No. workers: Without O.D.[a]	No. workers: With O.D. (Mean no. oocytes) (range)	Pronotal width (mm.) (S.D.): Workers without O.D.	Pronotal width (mm.) (S.D.): Workers with O.D.	Scouting results: Mean time (mins.)/h. scouting (S.D.): Workers without O.D.	Scouting results: Mean time (mins.)/h. scouting (S.D.): Workers with O.D.	Raids results: Duration (mins.) from start of raid of: (i) Time to first recruitment[b] (ii) Tandem recruitment (iii) Fighting in target nest (iv) Brood transport	Raids results: Mean time (mins.) from first recruitment to entry to target arena in workers present at first recruitment: Workers without O.D. (n)	Raids results: Mean time (mins.) from first recruitment to entry to target arena in workers present at first recruitment: Workers with O.D. (n)
Colony 5	7[c]	6(4.7)(1-9)	0.55[d](0.03)	0.55[d](0.01)	26.7[f](9.9)	5.6[f](4.8)	(i) 0-13 (ii) 13-233 (iii) 41-102 (iv) 122-233	20(2)	94(5)
Colony 6	12	10(2.9)(1-9)	0.55[e](0.02)	0.56[e](0.02)	14.2[g](11.8)	9.5[g](13.7)	(i) 0-14 (ii) 14-414 (iii) 43-59 (iv) 147-428	46(7)[h]	53(5)

Notes:
a. O.D = ovarian development (oocytes present).
b. Raid phases defined as follows: (i) Time from barrier removal to first tandem recruitment; (ii) First to last tandem recruitment; (iii) First entry into *Leptothorax* nest to expulsion of all occupants; (iv) First to last brood transport.
c. Included two workers with corpora lutea.
d. Not significantly different (t test, $t = 0.138$, $p>0.1$).
e. Not significantly different (t test, $t = 0.055$, $p>0.1$).
f. Significantly different (t test with pooled bout data [to homogenize variances], $t = 4.737$, $p<0.001$).
g. Significantly different (d test with unpooled bout data, $d = 2.102$, $p<0.05$).
h. Excluding single worker which did not enter *Leptothorax* arena (see text).

Figure 4.1 Worker dominance hierarchy and correlates of dominance in a queenright H.sublaevis colony (colony 1:30 hrs. observation).

		Subordinate															Total times dominating
		Q	RY	OP	OR	O	P	GR	PY	GP	GY	GO	PR	Y	G	R	
	Q	-		1			1									1	3
	RY		-	10			2	1	1		3		2	2	1	2	24
	OP		1	-	7	3	2	5	6	7	5	8	7	7	11	11	80
	OR				-	1				1		1		2	1	4	10
	O					-											0
Dominant	P						-										0
	GR							-									0
	PY								-								0
	GP									-							0
	GY										-						0
	GO											-					0
	PR												-				0
	Y													-			0
	G														-		0
	R															-	0
Total times dominated	:	0	1	11	7	4	5	6	7	8	8	9	9	11	13	18	117

Continued:

Figure 4.1 (continued)

		Q	RY	OP	OR	O	P	GR	PY	GP	GY	GO	PR	Y	G	R
Trophallaxis rate/h.	:	2.27	1.03	0.85	0.79	0.39	1.00	0.14	0.45	0.66	0.37	0.31	0.12	0.44	1.16	0.55
Mean time (mins.) outside nest/h.	:	0	0	3.5	12.0	14.0	2.2	16.2	18.6	14.7	6.0	15.3	10.0	14.8	10.2	2.0
Pronotal width (mm.)	:	0.65	0.60	0.60	0.62	0.57	-	0.62	-	0.57	0.60	0.62	0.59	-	0.60	-
No. active ovarioles	:	6	5	6	1	-	-	-	-	0	0	0	0	-	1	-
No. oocytes	:	17	10	12	1	-	-	-	-	0	0	0	0	-	1	-
No. corpora lutea	:	5	2	1	1	-	-	-	-	0	0	0	0	-	3	-

Notes: a. The upper part of the figure (see previous page) shows every instance of dominance between given pairs of ants, with results from both dominance forms pooled. The inseminated, colony queen (Q) ranks higher than the workers (RY to R) because the top-ranking worker (RY) averted from her (Fig.4.2).

b. - = information lacking due to ant's losing paint mark.

Figure 4.2 Worker dominance hierarchy in a queenright H.sublaevis colony (colony 1:30 hrs. observation): aversion behaviour

	Averting ant															Total times averted from:
Ant averted	Q	RY	OP	OR	O	P	GR	PY	GP	GY	GO	PR	Y	G	R	
Q	-	7	5	2		4				2						20
RY		-	27	3							1				1	32
OP			-	6			1				1					8
OR				-												0
O					-											0
P			1			-										1
GR							-									0
PY								-								0
GP									-							0
GY										-						0
GO						1					-					1
PR												-				0
Y													-			0
G														-		0
R															-	0
Total times averting :	0	7	33	11	0	5	1	0	0	2	2	0	0	0	1	62

Figure 4.3 Worker dominance hierarchy in a queenless colony (colony 2:12 hrs. observation)

		Subordinate (Averting ant)						Tot.x	Tot.x
		R	A	B	D	E	F	dominating	averted^b
	R	–	53(18)	17	21(1)	24(4)	25(1)	140	(24)
	A		–	17(1)	29	26	40(1)	112	(2)
Dominant (Ant averted)	B			–				0	(0)
	D				–			0	(0)
	E					–		0	(0)
	F						–	0	(0)
Tot.x dominated :		0	53	34	50	50	65	252	
Tot.x averting :		(0)	(18)	(1)	(1)	(4)	(2)		(26)
Troph. rate/h. :		2.92	2.70	1.20	0.52	1.76	0.25		
Mean time (mins.) outside nest/h. :		0	0.7	18.3	11.5	0.2	0.8		
Pronotal width (mm.):		0.57	0.52	0.62	0.56	0.61	0.47		
No. active ovarioles^a :		6	4	0	0	0	4		
Tot. no. oocytes ^{a} :		14	6	0	0	0	5		
No. corpora lutea^a :		3	4	0	0	0	0		

Notes: a. Ovarian dissections were performed after removal then replacement of top-ranking worker (R).

b. In this figure and Fig.4.4 (dominance hierarchy in colony 3), data on dominance interactions and aversive behaviour are combined, the number of aversions appearing in brackets after the number of dominance attacks for each pair of ants, e.g. R attacked A 53 times, and was avoided by A 18 times.

Figure 4.4 Worker dominance hierarchy in a queenless colony (colony 3 : 12 hrs. observation).

Subordinate (Averting ant)

		L	J	T	I	B	V^b	H^b	S^b	N	K^b	Z	Tot. x dominating	Tot. x averted
	L	-	3(14)	1(3)		1	1(1)	2	1	6(1)	9(3)	8	32	(22)
	J		-						1	2		1	4	(0)
	T			-								1	1	(0)
	I				-								0	(0)
	B					-							0	(0)
Dominant (Ant averted)	V						-						0	(0)
	H							-					0	(0)
	S								-				0	(0)
	N									-			0	(0)
	K										-		0	(0)
	Z											-	0	(0)
Tot. x dominated	:	0	3	1	0	1	1	2	2	8	9	10	37	
Tot. x averting	:	(0)	(14)	(3)	(0)	(0)	(1)	(0)	(0)	(1)	(3)	(0)		(22)

Continued:

<u>Figure 4.4</u> (continued)

		L	J	T	I	B	V^b	H^b	S^b	N	K^b	Z
Troph. rate/h.	:	1.0	1.27	1.59	1.71	1.29	0.34	0.72	1.28	1.65	0.85	2.11
Mean time (mins.) outside nest/h.	:	0	0.9	6.6	33.8	52.3	15.3	53.1	13.1	2.3	6.8	19.7
Pronotal width (mm.)	:	0.47	0.53	0.52	0.52	0.59	0.57	0.57	0.57	0.59	0.57	0.60
No. active ovarioles[a]	:	6	6	7	0	0	0	0	1	4	0	0
Tot. no. oocytes[a]	:	7	22	12	0	0	0	0	1	4	0	0
No. corpora lutea[a]	:	4	2	0	0	0	0	0	0	0	0	0

Notes: a. Ovarian dissections were performed after removal then replacement of top ranking worker (L) (except in the case of I, who died from unknown causes after Bout 4).

b. V,H,S and K were supernumerary, unmated queens (See Methods).

Figure 4.5 Effects of removing the top-ranking worker in queenless colonies (colonies 2 and 3: 36hrs. observation each).

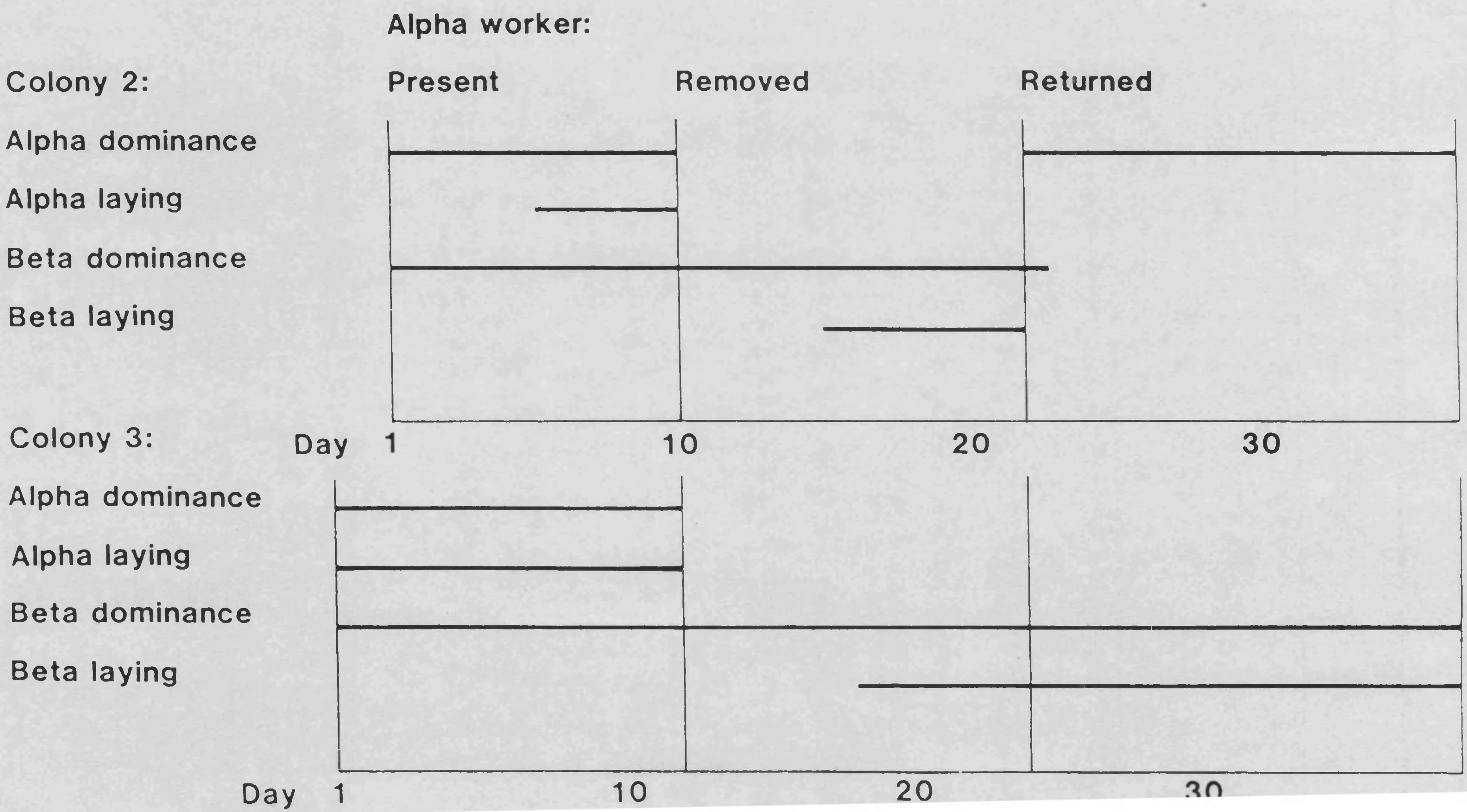
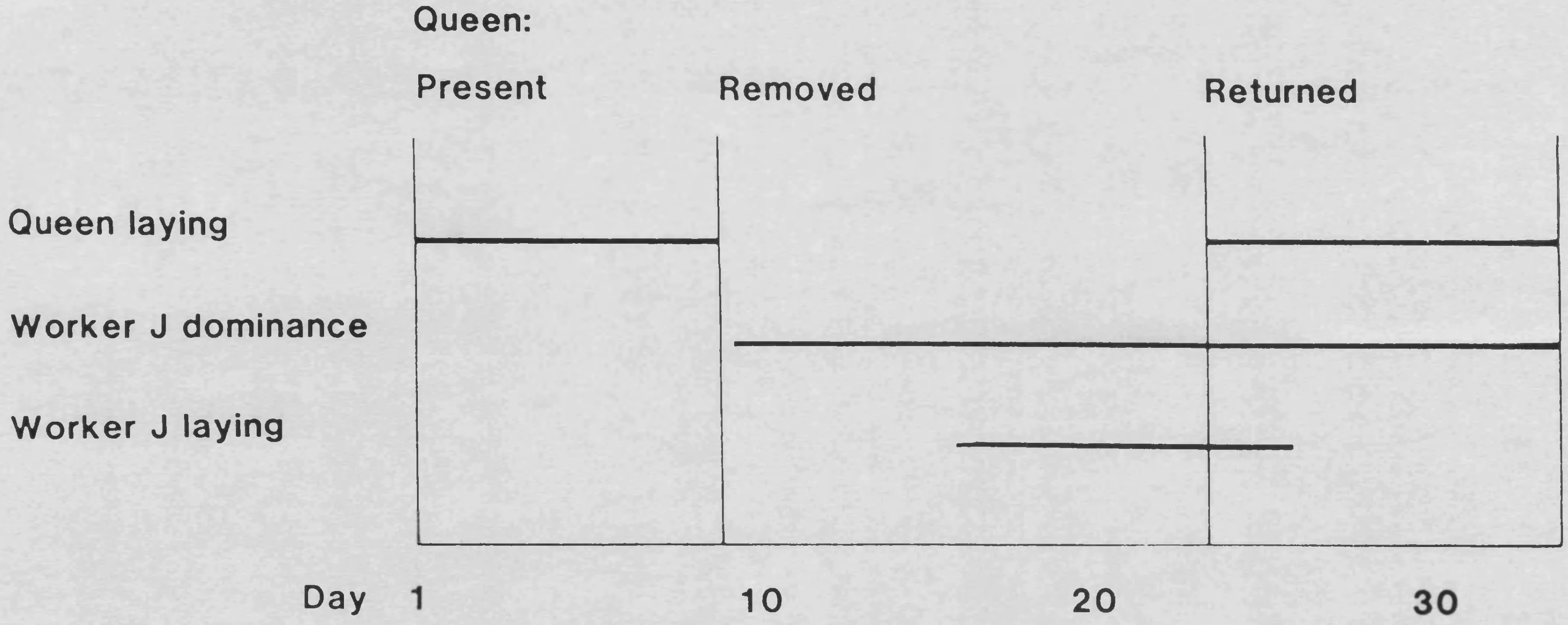


Figure 4.6 Effects of queen removal (colony 4: 36hrs. observation).



Chapter 5

Sex Investment, Colony Genetic Structure, Productivity, and Worker Reproduction in *Harpagoxenus sublaevis*

Introduction

Sex ratios have become important in social insect studies ever since Trivers and Hare (1976) showed that Hamilton's (1964,1972) kinship theory of Hymenopteran worker sterility lead to specific predictions concerning relative sex investment. Under kinship theory, the crucial influence on sex ratio is the level of genetic relatedness between colony members and reproductive brood. But since queens and workers are unequally related to brood, and therefore favour different sex ratios (i.e. there is queen-worker conflict over sex ratio as over male parentage: Ch.4), sex investment is also influenced by the relative ability of queens and workers to bias brood composition to their advantage. Trivers and Hare (1976) claimed that data from free-living ant species with single, once-mated queens and non-laying workers matched the 3:1 female:male sex investment ratio predicted by kinship theory and worker control over sex investment. However, Alexander and Sherman (1977) suggested that Hamilton's (1967) theory of local mate competition was a better explanation of female-biased sex investment in ants. This theory predicts that when relatives compete for mates with each other more than with unrelated individuals, investment in members of the competing sex should fall, because their reproductive value to their parents is reduced relative to that of members of the opposite sex. Typically, local mate competition occurs between males in populations with sib-mating (Hamilton 1967), so that the theory then predicts female-biased broods, e.g. in the extreme case where all reproductives in a brood derive from one female parent and brothers mate exclusively with sisters, production of maximum numbers of females (brood foundresses) and only sufficient males to fertilize them. Hence the demonstration of sib-mating (or inbreeding) in ants would strongly suggest a role for local mate competition in causing female-biased sex investment, though absence of inbreeding does not preclude this phenomenon (see Discussion).

Alexander and Sherman (1977) also criticized Trivers and Hare for failing to take account of possible widespread worker male production in interpreting ant sex ratios. Clearly, to discriminate between the hypotheses concerning sex ratio determination in particular species, it is necessary to measure several factors including the number of maternal queens (gyny), the number of queen matings (both affecting intra-colony relatedness), the proportion of worker-produced males, and levels of inbreeding (indicating local mate competition).

This chapter recounts my attempt to measure these factors and thereby explain patterns of sex investment in a population of Harpagoxenus sublaevis. Slave-making ants provide an especially powerful test of the genetic relatedness hypothesis of sex ratio (so designated by Nonacs 1986a), because their lack of brood care suggests slave-maker workers cannot favourably bias brood composition. Therefore, slave-maker queens should achieve their preferred, unbiased sex investment ratio, unlike queens of free-living species (Trivers and Hare 1976; Ch.4). Available data from slave-makers approximately fit 1:1 sex investment in agreement with this prediction (Trivers and Hare 1976, Nonacs 1986a). However, previous measures of slave-maker sex investment either have involved lumped population data, and so ignored between-colony and between-population variability, or through lack of information have made assumptions concerning number of queen matings and male parentage, or both. In addition, local mate competition has not been ruled out in slave-makers, despite speculation that socially parasitic ants may mate with nestmates rather than disperse for mating because of low colony densities associated with parasitism (Nonacs 1986a). H.sublaevis appears additionally prone to local mate competition because most queens lack wings (Buschinger and Winter 1975; Ch.3). Therefore a study of sex investment and relevant biological factors in H.sublaevis was desirable to test critically Trivers and Hare's (1976) prediction. In this chapter I also analyze data from other studies of sex investment in slave-makers where investment in individual colonies, in single populations, is reported.

In addition, this study examines colony genetic structure and productivity in H.sublaevis. Knowledge of genetic structures is important for assessing whether the high relatedness levels assumed by kinship theory actually occur (Hamilton 1964, Gadagkar 1985), and for investigating whether caste determination in ants has a genetic basis (Crozier 1980, Winter and Buschinger 1986; Ch.3). Colony production schedules are of interest following recent formulations of social insect life history theory (Oster and Wilson 1978, Brian, Clarke and Jones 1981).

Production data are also required to interpret sex investment. Several authors (e.g. Boomsma, van der Lee and van der Have 1982, Nonacs 1986b) have suggested that individual ant colony sex investment ratios are proximately influenced by resource levels. Boomsma et al. found that in the monogynous free-living Lasius niger, greater investment in queens was associated with high sexual productivity in an optimal, competitor-free habitat. The 3:1 kin-selected optimum was only found in a population from such a habitat. Further, within populations, colonies with low sexual productivity exhibited greater variance in sex ratio than productive colonies. In this chapter, I test for such effects in H.sublaevis by the methods of both Boomsma et al. (1982) and Nonacs (1986b).

Finally, this chapter investigates genetic and demographic aspects of worker reproduction in H.sublaevis. In chapter 4, I presented evidence that H.sublaevis worker reproduction strongly affects colony social structure and division of labour. This chapter examines issues raised by these findings. First, it determines whether H.sublaevis worker reproduction is promoted by low intra-colony relatedness, which reduces the genetic benefits of rearing brood. Second, it estimates levels of worker fertility and the proportion of males workers produce in a natural population. Such information is lacking for most ants. Buschinger and Winter (1978), in ovarian dissections of H.sublaevis in ten hibernating colonies from the Nuremburg Reichswald in West Germany, found fertile workers in four of the eight queenright colonies and both queenless colonies of their sample. Queenless colonies maintained in the laboratory produced males. Hence the level of H.sublaevis worker male production will depend on the amount of worker reproduction in colonies with a queen, and the frequency and productivity of orphaned colonies, all of which were investigated in the present study. Lastly, this chapter explores the hypothesis that H.sublaevis worker reproduction reduces colony productivity, following Cole's (1986) conclusion that worker reproduction only persists when its cost to "colony fitness" (see Discussion) is small.

I measured sex ratio and productivity in a population of H.sublaevis using colony censuses. Ovarian dissections were performed to confirm monogyny (Buschinger 1974b; Ch.3; Ch.4), to determine the frequency of fertile workers, and to measure the production of new queens (because in Swedish H.sublaevis all queens are wingless and externally worker-like: Ch.3). The number of queen matings, the level of intra-colony relatedness, male parentage, and possible inbreeding, were investigated with electrophoretic allozyme analysis. The electrophoretic part of this study was carried out jointly with Dr. T.M. van der Have, Department of Population and Evolutionary Biology, University of Utrecht, The Netherlands, and is reported here with his consent.

Methods

Field collections Forty-seven colonies were collected in June-July 1985 from a population of H.sublaevis in coastal pinewoods between Bröms and Kristianopel, Blekinge, S.E. Sweden. This was the same population from which five of the six colonies used in the behavioural studies in chapter 4 originated. All colonies came from a c. 500 x 500m. area (25 ha.) of the woods divided by a narrow, little-used road. The colonies, containing H.sublaevis and one or more of the three slave species Leptothorax acervorum, L.muscorum, and L.gredleri, were found nesting in dead twigs on the ground. Since each colony was monodomous (occupied a single twig), whole colonies were collected by fragmenting twigs and aspirating the ants and brood. All colonies found were collected, so that assuming all sizes and host classes of colony were found equally easily the 47 colonies represented an unbiased population sample.

In 23 colonies the completeness of each collection was checked by placing pitfall traps overnight at the exact site where each colony was found. Assuming all captures originated from collected colonies, the results indicated that on average 2.0% of slave-maker females and 9.1% of slaves were outside the nest at the time of collection.

Colony censuses, ovarian dissections, and size measurements

All colonies were censused on the day of collection and then maintained in artificial nests in laboratory conditions (see Ch.4, Methods section) until mid-August when all pupae had eclosed. Each colony was then censused again. At the second census all adult females from every colony were frozen at $-40^{\circ}C$ and subsequently dissected to record their degree of ovarian development and their caste (queens have a spermatheca which workers lack: Ch.3). Most colonies minus the adult slave-makers were then maintained in the laboratory and subjected to an artificial hibernation to allow the slaves to rear the 1986 generation of slave-makers from the brood.

Ovarian dissections were as described in chapter 4, Methods section. A fertile slave-maker worker was defined as a worker whose ovaries contained yolky eggs, or corpora lutea, or both. (All H.sublaevis workers possess ovaries but in most workers they are undeveloped: see Ch.3, Ch.4). Colony queens were identified by their elongated ovarioles, numerous yolky eggs, corpora lutea, and full spermatheca. All non-ovary-developed, unmated queens in the 1985 adult sample must have been 1985 production, because dissections by Buschinger and Winter (1978) of hibernating H.sublaevis from the field showed young queens do not overwinter in the maternal colony but disperse in the year of eclosion. In 11 colonies from the 1985 sample the head and thorax of each slave-maker female, following removal of the gaster for dissection, were used for electrophoretic allozyme analysis (see below).

Total female production was estimated as the difference between female counts in the first and second censuses. At the time of the first census in some colonies, newly-produced females had already eclosed (such "callow" females were recognizable from their unpigmented appearance). These were also added to the figure for total female production. Since all non-ovary-developed, unmated queens represented new queen production (see above), new worker production equalled the estimate of total female production minus the number of such queens. However, in some colonies a problem arose over this calculation, because the number of new queens exceeded the estimate of total female production. This meant that some queens must have eclosed and outgrown the callow stage prior to collection. Therefore in these colonies (marked in Table 5.1) it was impossible to estimate production of new workers other than to assume it to be zero, and correspondingly to assume all workers in the colony originated from previous years. This was arguably not too inaccurate an assumption, since colonies producing queens early were presumably the most queen-productive.

In 38 colonies representative of each host class the maximum pronotal (thoracic) width of every slave-maker female was recorded. In addition, dry weights were measured from 40 queens (identified by their sexual calling behaviour: see Ch.3) and 60 males taken from the Bröms-Kristianopel population in 1984 and 1985. These provided a measure of the relative unit cost of males and queens for calculating investment. They were also used to establish a relation between female pronotal width and dry weight, so that the weight of the average worker and queen reared by each host class could be estimated from the pronotal width data. These estimates were in turn used to calculate production in terms of biomass. In addition, pronotal width measurements were used to compare the sizes of sterile and fertile workers.

Electrophoresis Electrophoretic allozyme analysis was conducted on 1009 female and 256 male H.sublaevis from 49 colonies collected between 1983 and 1986 from the Bröms-Kristianopel population. With some exceptions ants for electrophoresis came not directly from the field but instead from slave-maker brood reared by slaves after colonies had undergone one or more laboratory hibernations following collection. Exceptions included 3 colonies collected in 1986 and 11 collected in 1985 (marked in Table 5.4), whose members were used for electrophoresis in the year of collection. Only these colonies could be used to investigate the allocation of male parentage in the field. Since colonies were not systematically chosen for electrophoresis, the complete sample of 49 colonies represents an unbiased sample of female genotypes.

In 18 colonies of the electrophoretic sample each female's gaster, removed prior to electrophoresis of the head and thorax, was used for ovarian dissection. These 18 colonies included the eleven 1985 colonies subjected to electrophoresis in the year of their collection (see above). The remaining 7 consisted of arbitrarily selected 1984-1986 colonies. By combining the electrophoretic data on genotype and the dissection data on caste from all 18 colonies it was possible (1) to identify the genotype of colony (maternal) queens, where present; (2) to seek evidence for a possible genetic influence on caste in the form of genetic differentiation within colonies between worker and queen siblings. The dissection data from the eleven 1985 colonies were further used in the determination of the frequency of fertile workers, and of new queen production, since as earlier mentioned these colonies formed part of the 1985 population sample.

Electrophoresis was conducted on horizontal starch gels. Gels were prepared by dissolving 42g. Connaught hydrolysed starch in 350ml. gel buffer (0.008 M tris and 0.003 M citric acid, pH 6.7: Menken 1980). Ants (adults minus their gasters, or pupae) were crushed individually over ice in 10μl. demineralized water, and the resulting homogenate was applied to strips of Whatman 3MM chromatography paper which were then inserted in the gel. Gels were maintained at 5°C and run for 4-4.5h. at 120V and 100mA. The electrode buffer was 0.233 M tris and 0.086 M citric acid, pH 6.3 (Menken 1980).

In H.sublaevis the two loci encoding malic enzyme (Me) and cathodal malate dehydrogenase (Mdh-2) respectively are polymorphic and were used as genetic markers in this study. Gels were stained for these enzymes using staining solutions composed as follows: Me - 30ml. tris-HCl buffer (pH 8.4), 55mg. malic acid, 4mg. NADP, 5mg. MTT, 25mg. $MgCl_2$, 2mg. PMS; Mdh-2 - 30ml. tris-HCl buffer (pH 8.4), 100mg. malic acid, 4mg. NAD, 5mg. MTT, 2mg. PMS.

Simple Mendelian genetic control of allozyme variation at the two enzyme loci (triallelic in the Me locus, biallelic in Mdh-2) was confirmed by (1) the presence of single, narrow staining bands in samples from haploid males, (2) patterns of variation consistent with Mendelian inheritance in colonies where colony queens and their female and male progeny were all analyzed.

Analysis of electrophoretic results Following Pamilo (1982a), population mating structure was analyzed by calculating the fixation index or coefficient of inbreeding

$$F = 1 - H_o/H_e$$

where H_o= observed heterozygosity (proportion of heterozygotes), and H_e= expected heterozygosity assuming Hardy-Weinberg equilibrium, i.e.

H_e= 2pq where p and q are biallelic allele frequencies. If H_o=H_e (i.e. F=0) the population mating structure is panmictic (outbreeding). If H_o<H_e (i.e. $0<F\leq 1$) then there is an excess of homozygotes in the population, implying inbreeding. The significance of the deviation of F from zero can be tested by $X^2=NF^2$ with 1 degree of freedom, where N = half the total number of independent haploid genomes sampled. The number of such genomes equals three in a colony with a single, once-mated queen (Pamilo 1983), so in a population of similar colonies N = 1.5 x the number of colonies. In calculating F, the quantities H_o, p and q were all calculated as average values per colony so that all colonies were weighted equally, ensuring that colonies from which many individuals were electrophoretically analyzed did not disproportionately influence the results (Pamilo 1982a).

To estimate intra-colony relatedness from the allozyme data the method of Pamilo and Crozier (1982) was used to calculate a within-group regression coefficient of relatedness, b, which measures the average proportion of identical genes shared by two group members. This method includes corrections for small group (colony) sample sizes, weights colonies equally, and assigns standard errors to each regression estimate. The significance of any difference between b and the coefficient of relatedness expected on the basis of inferred pedigree (r) was tested by dividing the deviation by the standard error of b and equating to d, the standardized normal deviate.

Since the calculations of both F and b require biallelic data, for the purposes of analysis the rare Me allele 104 (found in only one colony - S 85 60, frequency 0.006) was grouped with Me allele 96, the less common of the two main Me alleles. All calculations assumed selective neutrality of the marker alleles.

Results

Sex investment ratio

Table 5.1 gives data on colony composition, production, worker fertility, and sex ratio for the 47 colonies of the 1985 population sample. Of 46 colonies yielding production data, 37(80%) produced one or both kinds of sexuals and 9(20%) produced no sexuals. The analysis of sex investment ratio for the 37 sexual producing colonies is in Table 5.2. Relative sex investment was expressed as the dry weight biomass of queens divided by the dry weight biomass of all sexuals, averaged over all colonies. Queenright and queenless colonies did not differ significantly in sex investment (Table 5.2), and neither did colonies from different host classes. The population mean per colony proportionate investment in queens (95% confidence limits) was 0.540(0.384-0.691). This was not significantly different from 0.5(1:1 investment), but did differ significantly from 0.75(3:1 investment) (Table 5.2). The result was therefore consistent with the genetic relatedness hypothesis.

As indicated above, in calculating sex investment ratio the relative cost of raising one member of each sex (the cost ratio) was estimated by Trivers and Hare's (1976) method of comparing mean male and female dry weights. Boomsma and Isaaks (1985) have criticized the dry weight cost ratio on the grounds it does not correctly estimate the relative energetic cost of raising a member of each sex, as Trivers and Hare assumed. Boomsma and Isaaks calculated that instead the dry weight cost ratio underestimates the relative cost of males. This is because in ants the smaller males have a higher metabolic rate than queens, and because - unlike queens - they lack non-metabolizing fat reserves. Consequently male tissue consumes more energy (investment) per unit weight than queen tissue. Boomsma and Isaaks recommended that in species (like H.sublaevis) with relatively little sexual size dimorphy, the female:male energetic cost ratio should be estimated by reducing the female:male dry weight cost ratio to 70-75% of its original value. When a midway correction of 72.5% was applied to the H.sublaevis dry weight cost ratio, the population mean proportionate investment in queens (95% confidence limits) became 0.487(0.336-0.639), closer to the 0.5 expectation than the original estimate.

The distribution of sex investment ratios in individual H.sublaevis colonies is illustrated in Fig.5.1 (cf. Fig.4 in Nonacs 1986a). The variance in sex investment appeared notably large. However, the figure shows that much of this variance was due to sex ratio values from 7 colonies which produced 4 sexuals or less, in which investment in queens tended to be 0 or 1 on chance grounds. Excluding these 7 colonies from the sample resulted in estimates of the population mean proportionate investment in queens (95% confidence limits) of 0.626(0.507-0.738) (based on the dry weight cost ratio), and 0.563(0.439-0.681) (based on the estimated energetic cost ratio). The first of these fell between the expected values of 0.5 or 0.75, and differed significantly from both (both $t>2.15$, both $p<0.05$). However, the second estimate was not significantly different from 0.5 ($t=1.039$, $p>0.1$), but did differ significantly from 0.75 ($t=3.315$, $p<0.01$). This analysis confirmed that relative investment in queens did not reach 0.75 in H.sublaevis, and that the sex investment ratio instead approximated unity, assuming the estimated energetic cost ratio measured relative unit investment more accurately than the dry weight cost ratio.

Fig.5.2 plots relative queen investment in H.sublaevis against total sexual production (cf. Fig.6 in Boomsma, van der Lee and van der Have 1982). There was no significant correlation between these two quantities ($r=0.146$, d.f.=35, $p>0.1$). Further, there was no pronounced reduction in the variance of queen investment as sexual productivity increased, contrary to the findings of Boomsma et al. in Lasius niger (see Introduction). The apparently large variation in queen investment in unproductive H.sublaevis colonies (Fig.5.2) was again due to extreme investment values (0 or 1) in colonies producing very few sexuals (≤ 4). In the Discussion I further examine the possible influence of productivity and resource availability on sex ratio in H.sublaevis, following Nonacs' (1986b) method of analysis.

Proportion of queenless colonies and fertile workers

Of the 47 colonies in Table 5.1, 3 were newly-founded or incipient (on collection contained a single, mated H.sublaevis queen and Leptothorax slaves and brood), 30 non-incipient and queenright, and 14 queenless. Hence the proportion of queenless colonies was 14/47 = 29.8%.

Data on worker fertility exist for 38 colonies (26 queenright and 12 queenless) (Table 5.1). One or more fertile workers occurred in all 12 queenless colonies but in only 15 (58%) of the 26 queenright colonies, the remainder having no fertile workers. Thus there was a significant association between worker fertility and queenlessness (One tailed Fisher's exact test, $p=0.006$). Further, in the 15 queenright colonies with fertile workers, the mean per colony percentage (95% confidence limits) of fertile workers was 9.8% (5.7%-14.8%), but in the 12 queenless colonies it was higher at 19.2% (12.0%-27.5%). This difference was significant (t test with angular transformed data, t=2.440, $p<0.05$). Therefore the results confirmed that queens inhibit worker fertility in H.sublaevis (see Ch.4).

Size comparison of fertile and sterile workers

In 18 colonies containing both worker types from the two principal host classes in which fertile workers occurred, fertile and sterile workers did not differ significantly in pronotal width (Table 5.3). This finding matched the conclusion from chapter 4 that in H.sublaevis worker dominance is not associated with large body size.

Genetic evidence for single mating in H.sublaevis

Table 5.4 gives the electrophoretic results. H.sublaevis is monogynous and there is simple genetic control of allozyme variation. Therefore, if H.sublaevis queens mate once, no more than two genotypes of female offspring should occur per colony (because the only possible mating types are FFxF, FFxS, FSxF, FSxS, SSxF, SSxS, where F and S denote alleles). This expectation was met for both the Me and Mdh-2 loci. In addition, if there is single mating, when colonies contain two female offspring genotypes one should be heterozygote and the ratio homozygotes: heterozygotes should be 1:1 (mating types FSxF, FSxS). Heterozygotes were always present in colonies with two female genotypes, for both loci. However, the number of individuals sampled per colony was in some cases too small to test the deviation of the homozygote: heterozygote ratio from 1:1 without pooling results from different colonies. But the two largest deviations from 1:1 clearly appeared in colonies S 85 96 (Me data) and S 84 73 (Mdh-2 data) (Table 5.4). In S 85 96 the Me genotype of the maternal queen was 100/100. Therefore the single 100/100 ant in the progeny sample (otherwise entirely heterozygous) must have been the offspring of a second mating (with a 100 male), if it was the queen's daughter. But the unlikelihood of such a second mating producing only one offspring out of 72 suggests that the 100/100 individual in the progeny sample was not the queen's daughter but a contaminant. This individual could have been accidentally transferred from another captive colony, or possibly naturally acquired on a raid against another slave-maker colony (see Ch.6). Similarly, the 3 heterozygotes in the S 84 73 Mdh-2 progeny sample were also probably contaminants. Therefore I omitted colonies S 85 96 and S 84 73 from all genetic calculations to follow.

In all colonies with two female genotypes except S 85 96 and S 84 73, the pooled totals of homozygotes and heterozygotes were not significantly different (Me: n colonies=19, n homozygotes=225, n heterozygotes=236, X^2 test, X^2=0.262, $p>0.5$; Mdh-2 : n colonies=8, n homozygotes=65, n heterozygotes=77, X^2=1.014, $p>0.1$). Therefore the genetic evidence indicates *H.sublaevis* queens mate singly.

Intra-colony relatedness

For the Me locus, the regression coefficient of relatedness (±S.E.) among female offspring within colonies was 0.73±0.07 (n colonies=47). This did not differ significantly from the pedigree coefficient of relatedness 0.75 expected in a colony with a single, once-mated outbred queen (d=0.309, $p>0.1$). For the Mdh-2 locus, the regression coefficient of relatedness was 0.60±0.08. This also did not differ significantly from 0.75(d=1.913, $p>0.05$). The low value of this estimate seems to have resulted from the fact the regression method loses accuracy in loci (like Mdh-2 : see below) where the allele frequencies are very high or low (Pamilo and Crozier 1982, Wilkinson and McCracken 1985).

Evidence for random mating in H. sublaevis

The mean frequency of the Me allele 100 was 0.76, and the mean frequency of Mdh-2 100 was 0.94 (Table 5.4; see also Table 6.9). Table 5.5 gives the fixation indices for both loci for sets of colonies grouped by *Leptothorax* host class and by area. Colonies were classified by host class because it was possible *H.sublaevis* with different hosts constituted genetic subpopulations. (This possibility is fully discussed in chapter 6, where I conclude the apparent genetic differences between host classes - e.g. the lack of Mdh-2 97 in *H.sublaevis* with *L.muscorum* slaves: see Table 5.4 - resulted from chance sampling variation. In any event, the possibility of genetic differentiation by host class, even if true, does not affect present calculations regarding single mating and relatedness). The area classification in Table 5.5 divides colonies into those collected on the left (Area L) of the road dividing the study area (see Methods), and those from its right (Area R). This road was the only obvious potential gene flow barrier (to queens, not males) in an otherwise uniform open woodland habitat.

None of the F values in Table 5.5 suggests that inbreeding occurred in the *H.sublaevis* population or hypothetical subpopulations. There is no evidence that deviations from panmixia occurred. In this population, competition for mates was therefore almost certainly population-wide.

Evidence against queen-worker genetic differentiation

The electrophoretic results (Me locus) from the 18 colonies in the electrophoretic sample in which ovarian dissections permitted classification of ants into workers or queens (see Methods) are given in Table 5.6. They show that although queens had greater heterozygosity than workers, the genotype frequencies of queens and workers were not significantly different. Therefore this analysis provided no firm evidence for genetic differentiation by caste in H.sublaevis. This result is important since in most colonies in Table 5.4 the female sample included workers and queens in unknown proportions. However, the possibility of a genetic influence on caste in H.sublaevis cannot be ruled out by this result (see Discussion).

Male parentage

(a) Genetic evidence

Eleven 1985 colonies of those from the electrophoretic sample whose members could be assigned to their caste (including the colony queen, where present) were all subjected to electrophoresis shortly after collection (see Methods) and contained males. Therefore these 11 colonies were used for investigating the natural allocation of male parentage. That is, from knowledge of the genotypes of female colony members, it was possible to infer the mating type for each colony and then assess whether the observed male genotype frequencies were consistent with queen or worker male production.

Eight of the 11 colonies were queenright and 3 queenless. Of the 8 queenright colonies, analysis of the Me data suggested that in two colonies (S 85 9 and 96) the queen almost certainly produced all males. All males were of the single genotype consistent with production by the queen alone (Table 5.4). In another two colonies (S 85 23 and 77) the queen probably produced all males. The males were of two genotypes in a ratio more consistent with sole queen production than sole worker production, or mixed production (Table 5.4). In the remaining four colonies (S 85 27,70,75 and 107) male parentage could not be deduced, either because all colony members were homozygotes (queen- and worker-produced males are genetically indistinguishable), or because male sample size was too small. For the Mdh-2 data, only one colony with sufficient males was not 100% homozygous. The two male genotypes in this colony (S 85 23) were in a ratio statistically indistinguishable from that expected assuming either all queen or all worker male production.

Therefore in four queenright colonies yielding genetic information concerning male parentage, the queen probably produced all males. But since this conclusion is uncertain, and four colonies represent a small sample, worker male production in queenright colonies cannot be ruled out.

Only one of the three queenless colonies (S 85 17, 101 and 106) yielded information on male parentage (S 85 101, Me data). In this colony worker male production was indicated. The four female colony members were all heterozygous (Table 5.4), suggesting the queen was homozygous. If so, some or all of the males must have been worker-produced, because the males were of two genotypes. The fact S 85 101 contained a fertile worker and produced only haploid brood (Table 5.1) supported this conclusion.

(b) Census evidence

The 1985 colony censuses also provided information concerning male parentage in queenless colonies. The total number of males produced by the 47 colonies of the 1985 sample was 961, of which 208 came from queenless colonies (Table 5.1). Only one queenless colony (S 85 101) had an all male brood, of 42 males. Therefore the proportion of males coming from queenless workers lay between 42/961 (assuming only males from queenless colonies raising haploid brood alone were worker-produced) and 208/961 (assuming males from all queenless colonies were worker-produced), i.e. between 4.4% and 21.6%. Hence the total proportion of worker-produced males was this estimated range plus the contribution of workers in queenright colonies, if any.

Production

Table 5.1 gives production data for the forty-seven 1985 colonies. There was a significant linear relationship between the log. transformed number of slaves in a colony and the log. transformed number of slave-maker workers present on collection (regression analysis, $F=13.5$, $v_1=1$, $v_2=39$, $p<0.01$) (Fig.5.3). Retransformed from logs., these two quantities fitted the equation, no. slaves = 17.42 (no. slave-maker workers)$^{0.6222}$. The fact the exponent in this equation fell below unity indicates that the number of slaves per slave-maker worker decreased as the number of slave-makers increased.

Table 5.7 gives the estimated dry weight of the average slave-maker queen, worker and male produced by each host class. These estimates were used to express colony production in terms of biomass : a full analysis of the influence of host type on slave-maker size evident in this table is deferred until the following chapter. There was a significant linear relationship between the log. transformed total biomass (mg. dry weight) of slave-makers produced by individual colonies and the log. transformed number of slaves they contained (regression analysis, $F=51.6$, $v_1=1$, $v_2=41$, $p<0.001$) (Fig.5.4). The equation describing the relation between the two quantities was: dry weight (mg.) of slave-makers produced = 0.5669 (no. slaves)$^{0.7731}$, indicating decreasing per capita production with increasing slave number.

Workers of the three Leptothorax slave species of H.sublaevis differ in size (see following chapter), suggesting they could also differ in work efficiency. However, in Fig.5.4 slave-number was reckoned disregarding slave species because it was unclear how to quantify such differences. This procedure was arguably justified, since colonies with slave populations of similar size appeared to produce roughly equivalent biomasses whatever their species composition (Fig.5.4).

In addition, analysis in the next chapter suggests that within host classes colonies with numerous slaves produced larger individual slave-makers than those with fewer slaves (e.g. Fig.6.2). However, in the present analysis such variance between colonies was ignored in converting colony slave-maker production into biomass. This was because pronotal width measurements were not made for all colonies in the 1985 sample, necessitating the use of mean host class figures for slave-maker size in this conversion. Consequently, in Fig.5.4, biomass produced by large colonies will tend to be underestimated, and biomass produced by small colonies will tend to be overestimated.

In queenright colonies the average per colony proportion of production (biomass) devoted to reproductives was 0.52. This proportion rose abruptly as the number of slave-maker workers increased among individual colonies (Fig.5.5a). However, the 6 queenright colonies which according to Fig.5.5a produced 100% reproductives were all colonies in which worker production was assumed to be zero because observed queen production exceeded estimated total production of females (see Methods). Since these colonies probably produced a high proportion of sexuals rather than nothing but sexuals, the curve in Fig.5.5a should probably reach the asymptote less steeply. Nevertheless, the transition from worker to sexual production was clearly sharp, not graded.

Finally, there was no clear evidence that worker reproduction reduced colony productivity. Fig.5.6 plots log. transformed slave number against log. transformed total biomass produced (cf. Fig.5.4) in queenright colonies with and without fertile slave-maker workers. The slopes of the two regression lines (b±S.E.) were 0.33±0.24 (14 colonies with fertile workers) and 0.81±0.19 (11 colonies without fertile workers), but these two slopes were not significantly different ($F=1.79$, $v_1=1$, $v_2=21$, $p>0.1$).

Discussion

Sex investment ratio in the Bröms-Kristianopel H.sublaevis population

The data from this study support the genetic relatedness hypothesis of sex ratio. Under conditions of monogyny, single mating of queens, population-wide competition for mates, non-conspecific brood care (implying queen control of sex ratio), and low levels of worker male production (whose influence I discuss later), the hypothesis predicts the sex investment ratio should be 0.5 (Trivers and Hare 1976, Nonacs 1986a). This study reports in H.sublaevis estimates of the population mean proportionate queen investment ranging from 0.487 to 0.626 (see Results). The conventional estimate (including all colonies, utilizing dry weight cost ratio) was 0.540. Arguably the most accurate estimate (excluding colonies with very low sexual output, utilizing estimated energetic cost ratio) was 0.563. All estimates differed significantly from 0.75, and all except the highest were not significantly different from 0.5.

The variance in queen investment among individual H.sublaevis colonies was relatively small when colonies producing 4 sexuals or less were excluded from the analysis (Fig.5.1). By contrast, reviewing data from numerous free-living ant species, Nonacs (1986a) commonly found high variances in colony sex ratios within species. In fact colony sex ratios tended to be distributed bimodally, with colonies producing either mostly males or females. H.sublaevis appears to be unusual in lacking this single sex specialization. Two main explanations have been advanced to account for the generally observed intra-specific variability of sex ratio, neither of which is mutually exclusive or incompatible with the genetic relatedness hypothesis (Pamilo and Rosengren 1983, Nonacs 1986a). First, various models (e.g. allele frequency simulations) indicate the genetic relatedness hypothesis predicts a population equilibrium sex ratio without prohibiting colonies from following dissimilar investment strategies. Second, evidence exists that the availability of resources in the environment proximately influences sex investment, such that female investment rises as resource levels increase, and kin-selected optima are only achieved by the most productive colonies in high quality habitats (Boomsma, van der Lee and van der Have 1982, Nonacs 1986a). The reason why in many species queen investment should be less when resources are poor is unclear, but could result from affected colonies channelling female larvae into becoming workers not queens (Nonacs 1986a). However, as shown in the Results, neither the level nor the (low) variance of colony sex investment in H.sublaevis appeared to be associated with resource availability (as indicated by sexual productivity) (Fig.5.2).

In free-living ant species, kinship theory suggests that as number of workers relative to queens in a colony increases, investment in females should also increase, because in the queen-worker conflict over sex ratio the expanding workforce controls investment increasingly easily. Therefore Nonacs (1986b) argued that the rise in female investment with increasing sexual productivity observed in many species could result either from the proximate effects of resource levels (see above), or from kin-selected queen-worker conflict over sex ratio (since colonies with large workforces are more productive). Nonacs attempted to discriminate between these two hypotheses with data from 24 ant species, by computing partial correlation coefficients between the three variables relative sex investment (which Nonacs expressed as proportionate investment in males, so increase in female investment now becomes decrease in male investment), number of workers, and total sexual biomass produced.

Partial correlation coefficients indicate the correlation between pairs of variables with the effects of additional, related variables held constant (Snedecor and Cochran 1980). Under the queen-worker conflict hypothesis, there should be no significant relation between male investment and total sexual biomass with worker number controlled, but the partial correlation between male investment and worker number (total sexual biomass controlled) should be significantly negative. The resource availability hypothesis reverses these predictions (significantly negative partial correlation between male investment and total sexual biomass, no relation between male investment and worker number with total sexual biomass controlled). Nonacs found that the data supported the resource availability hypothesis. However, the results of computing the relevant partial correlation coefficients for the present H.sublaevis data (Table 5.8) did not match this hypothesis. No significant negative partial correlation was found between male investment and total sexual biomass (Table 5.8). This confirmed the earlier conclusion that H.sublaevis sex ratios were not noticeably influenced by resource levels (see above). But this finding does not alter Nonacs' conclusion, which was based on cross-species trends. Neither did the results in H.sublaevis support the queen-worker conflict hypothesis, since there was no significant negative partial correlation between male investment and number of workers (Table 5.8). This was not unexpected, considering all indications are that H.sublaevis workers lack control over sex investment.

Queenless H.sublaevis colonies produced a similar sex ratio to colonies with queens. The reason queenless colonies did not all rear worker-derived males exclusively almost certainly stemmed from the two year larval developmental time of H.sublaevis queens (Winter and Buschinger 1986; Ch.3). This means queen larvae persist in the colony for two years after the colony queen's death. Orphaned workers should only produce all male broods after all such queen larvae (to which they are maximally related) have been reared, i.e. in the third and final year of orphanage (the estimated longevity of orphaned colonies is 3 years: see below and Ch.3). In fact in the 1985 sample only one of 14 queenless colonies produced males alone (S 85 101: Table 5.1), probably not significantly less than the small number of such colonies expected in a sample of this size assuming all orphan colonies survive fully 3 years. Evidence that the proportion of male-only broods rises with orphan colony age came from the results of raising the 1986 generation of slave-maker brood in the laboratory (see Methods), which showed that 4 of 10 queenless colonies whose brood was raised produced only males (Table 5.1).

Queenless H.sublaevis workers produced 4.4-21.6% of all males, and queenright workers an unknown (probably low) percentage. The question arises whether the population equilibrium sex ratio should have been perturbed by this level of worker male production. One model dealing with such questions is Taylor's (1981) model of sex ratio compensation. This assumed (as in slave-makers) queen control over investment, and found that where β =biomass of worker-produced males in orphaned colonies : biomass of queen-produced sexuals, the overall proportion of investment in queens = $(2+\beta)/(4+4\beta)$, and the queen-produced proportion of investment in queens = $1/2 + \beta/4$.

Overall queen investment falls below 0.5 for all $\beta > 0$. Hence Taylor argued that when workers produce extra males, queens should compensate by producing extra females, but not sufficiently to re-establish equal investment. The model may not fully apply to H.sublaevis because it assumed orphaned colonies produce only males. However, from the model β must clearly be quite large to alter overall investment substantially away from 0.5. Assuming all males from queenless H.sublaevis colonies were worker-derived, and none came from queenright workers, then β (calculated by pooling colony totals and using the dry weight cost ratio) = 0.098. So the predicted overall queen investment = 0.478, close both to predicted queen-produced investment in queens (0.525) and to 0.5. Therefore for small β the predictions of the model and expectations assuming zero worker male production are very similar. It seems the estimated level of H.sublaevis worker male production was not large enough to have altered the equilibrium sex investment ratio appreciably.

To conclude this discussion of sex investment in the Swedish H.sublaevis I note that, though confirming the influence of genetic relatedness, this study did not test the local mate competition hypothesis of sex ratio. This was because the genetic data showed conditions for local mate competition were absent in the study population. (I discuss the reliability of this conclusion below). However, in his 1986a survey, Nonacs concluded that in most ants local mate competition did not explain patterns of sex investment as well as the genetic relatedness hypothesis.

Sex investment ratio in slave-makers : analysis of published data

Previous studies of sex investment in obligate slave-makers on a colony-by-colony basis are few, and include those of Wesson (1939) on Harpagoxenus americanus, Buschinger, Frenz and Wunderlich (1975) on W.German H.sublaevis, and Winter and Buschinger (1983) on Epimyrma ravouxi. Wesson's study involved colonies from several different populations, with small numbers from each. In addition, some of his colonies could have been colony fragments because H.americanus, unlike H.sublaevis, is polydomous (colonies occupy multiple nests) (Buschinger and Alloway 1977, Del Rio Pesado and Alloway 1983; see also Ch.6). By contrast, the data of Buschinger et al. (1975) and Winter and Buschinger (1983) derived from single, well-represented populations - H.sublaevis from the Nuremburg Reichswald and E.ravouxi from near Würzburg, W.Germany. Also, like H.sublaevis, E.ravouxi is monodomous (Buschinger and Winter 1983). Therefore these data can be analyzed for comparison with the present results. Except for that of Buschinger et al. in outline form, these two studies have not figured in previous surveys of sex ratio in slave-makers or ants in general (e.g. Nonacs 1986a).

I assume (following results from this study) that in the German H.sublaevis there was single queen mating, population-wide mate competition, and relatively little worker male production. Table 5.9 presents the analysis of sex investment from the 33 colonies in Table

1b of Buschinger et.al.. Queenright and queenless colonies were not separately classified. Overall mean queen investment in the German H.sublaevis population (0.676) was greater than in the Swedish population. In fact, unlike the Swedish mean, the German mean differed significantly from 0.5, but not from 0.75. But the adjusted German mean (utilizing the estimated energetic cost ratio) was not significantly different from either 0.5 or 0.75 (Table 5.9). This estimate was therefore not inconsistent with the genetic relatedness hypothesis, though not discriminating between the predictions deriving from worker or queen control over investment. However, the female-bias in the German H.sublaevis requires explaining. Since it is unlikely there was worker control of sex ratio in Germany but not in Sweden, it may be the assumption local mate competition was absent in the German population was wrong. But data on this point are lacking at present. Another possible reason for greater female investment in German compared to Swedish H.sublaevis is the queen-biasing allele e, which is present in German but not in Swedish populations (Winter and Buschinger 1986; Ch.3). Genetically mediated caste determination in the German ants could constrain the degree to which sex ratio is subject to adaptive alteration.

In the following analysis of sex ratio in Epimyrma ravouxi from the data of Winter and Buschinger (1983), I also assume single mating of queens and unrestricted mate competition. However, as will become evident, in E.ravouxi worker male production was relatively high. The results of the E.ravouxi analysis are in Table 5.10. Estimates of mean queen investment (0.432 and 0.385) employing the dry weight and the energetic cost ratios respectively were each significantly different from 0.75, but not from 0.5. Therefore the E.ravouxi results supported the genetic relatedness hypothesis.

However, unlike the Swedish H.sublaevis, E.ravouxi queenright and queenless colonies produced significantly different sex investment ratios. This was almost certainly the consequence of greater male production by orphaned E.ravouxi workers. Queenless E.ravouxi colonies produced notably male biased broods (Table 5.10), which Winter and Buschinger (1983) attributed largely to laying workers. The estimated proportion of males from orphaned workers was 21.1-26.8% (404 of a total 1915 males came from queenless colonies producing male-only broods, and all queenless colonies together produced 513 males: Winter and Buschinger 1983). In Swedish H.sublaevis this range was 4.4-21.6% (see Results). Therefore Taylor's (1981) sex ratio compensation model (see above) predicted a greater difference in sex investment between queenright and queenless colonies in E.ravouxi than in H.sublaevis. As already indicated this prediction was met, though queenright E.ravouxi colonies produced a more female-biased sex ratio, and queenless colonies exhibited more male bias, than expected from Taylor's model (Taylor's β=0.147 assuming the maximal level of estimated worker male production). Taylor's model also predicted male bias in the overall sex investment ratio in E.ravouxi, and this too occurred. Therefore, sex ratio compensation was clearly a significant factor in the determination of sex investment trends in E.ravouxi, owing to a relatively high level of worker male production.

The mean queen investment for all three slave-maker populations discussed in this chapter - Swedish H.sublaevis, German H.sublaevis, and E.ravouxi - was 0.551. In summary, I conclude the balance of evidence supports the genetic relatedness hypothesis of sex ratio in slave-making ants. However, local mate competition cannot be conclusively eliminated in German H.sublaevis. Further, as evidenced by E.ravouxi, the genetic relatedness hypothesis should not be evaluated without considering worker male production. Hence this chapter highlights the need for detailed studies of single populations to test critically hypotheses concerning sex investment ratio.

H.sublaevis colony orphanage and the proportion of fertile workers

The proportion of queenless H.sublaevis colonies in the Bröms-Kristianopel population was 14/47 or 29.8% (see Results). Since H.sublaevis queens and workers live up to 10 and 3 years respectively (Buschinger 1974b, pers. comm.; Ch.3), these periods represent the respective estimated lifespans of queenright and orphaned colonies. So in a population at steady state in which mortality is concentrated in old colonies, the expected proportion of orphaned colonies = 3/13=23.1%. The observed proportion of queenless colonies was not significantly different from this expectation (X^2 test, $X^2=1.068$, d.f.=1, $p>0.1$). Therefore the apparently high proportion of queenless H.sublaevis colonies was no greater than that expected on simple demographic grounds concerning orphanage. The observed could have slightly exceeded the expected proportion because some colonies were falsely classified as queenless, e.g. because the queen escaped collection.

The greater frequency of fertile workers in queenless colonies confirmed that queens inhibit worker fertility in H.sublaevis, as experimentally demonstrated in chapter 4. The presence of fertile workers in only some queenright colonies but in all queenless colonies also matched the finding in chapter 4 that worker dominance behaviour was not apparent in every queenright colony, but did occur in both queenless colonies examined. Later I discuss one reason why some queenright colonies contained fertile workers but others did not, attributing this difference to presence or absence of energy surpluses.

Genetic colony and population structure in H.sublaevis

The conclusion from the genetic evidence that H.sublaevis queens mate singly matched results of laboratory observation. Captive H.sublaevis queens rarely exhibit sexual calling behaviour after their first mating (Buschinger 1974b, pers. comm., pers. observation). But occasionally they do call again, even when later dissection shows the spermatheca contained sperm. This behaviour presumably occurs rarely in nature, if ever, since the genetic data gave no unequivocal evidence of double mating.

Intra-colony relatedness in H.sublaevis reached the theoretical maximum ($r=0.75$) for outbred social Hymenoptera. Although many models of eusocial evolution have assumed 3/4 relatedness between females, this level has not often been found in nature. In a survey of reported relatedness values from 20 ant populations (10 species), Gadagkar (1985) found levels between 0.7 and 0.8 in only two cases. Additional examples of full relatedness include the monogynous form of Solenopsis invicta (Ross and Fletcher 1985) and probably Harpagoxenus canadensis (since this species is monogynous and, as in H.sublaevis, behavioural observations suggest queens mate singly: Buschinger and Alloway 1978, 1979). Gadagkar (1985) describes reasons that have been advanced to reconcile low observed relatedness with Hamilton's (1964) "3/4 relatedness hypothesis" of eusociality (e.g. temporary elevation of relatedness levels by non-random sperm usage in multiply-mated queens, or worker recognition and preferential treatment of closely related brood). In H.sublaevis the problem is reversed, and is to explain why high relatedness is accompanied by a relatively low degree of worker co-operation. Possible reasons (e.g. historical prevalence of the Hamiltonian reproductive alternative in workers, selection on orphaned workers to reproduce, inability of slave-makers to raise female-biased brood) were discussed in chapter 4, and will be further discussed in chapter 8.

Since the genetic evidence indicated local inbreeding did not occur in the *H.sublaevis* study population, *H.sublaevis* queens presumably disperse widely on foot before mating (see also Ch.6), or tend to reject related males (as suggested by Adlerz, in Wheeler 1910; see Ch.3). In addition, *H.sublaevis* males may fly far in search of queens. The lack of inbreeding in this population was not surprising in view of any supposed low colony density, because in the Bröms-Kristianopel woods *H.sublaevis* colonies occurred relatively close together (mean maximum nearest neighbour distance in one section = 6.6m., n=11; see Ch.6). Apart from some inquilines with known sib-mating (e.g. *Epimyrma kraussei*, Winter and Buschinger 1983), both observations of mating behaviour (especially of nuptial flights, Wilson 1971) and previous allozyme studies (reviewed by Crozier 1980) suggest most ants are outbred. However, lack of inbreeding - though a strong counter-indication - does not prove local mate competition is absent (see Introduction). Such competition could still occur if, for example, individual colonies released one sex of reproductives at a time, which then competed with each other for unrelated mates (Crozier 1980). But *H.sublaevis* colonies simultaneously contain adult males and virgin queens, and there is every indication they are released together (Buschinger 1982, pers. observation). Therefore, in the absence of contrary evidence, the conclusion remains that competition for mates in *H.sublaevis* is population-wide.

The lack of queen-worker genetic differentiation suggested by Table 5.6 was consistent with findings that queens and workers do not differ significantly in heterozygosity in other ant species (Crozier 1980). It was also consistent with the inferred absence of the queen-biasing allele (e) in Swedish H.sublaevis (see Ch.3, Ch.6). However, this result did not totally exclude a system such as the E/e system influencing caste, because the Me locus might not be linked to any caste-biasing locus present.

H.sublaevis male parentage

Workers in many ant species are known to be capable of male production, and in some workers probably produce all males (see Ch.8). But in the more common case involving mixed male parentage, few previous studies have estimated the natural proportion of worker-produced males. These include the investigations of Elmes 1974 (44.8% of males from workers in Myrmica sulcinodis), Forsyth 1981 (42.5% in Apterostigma dentigerum), and Winter and Buschinger 1983 (up to 26.8% in Epimyrma ravouxi) (see above, and Table 8.1). All these cases involve monogynous species in which nearly all worker-produced males came from orphaned colonies. In H.sublaevis orphaned workers gave rise to 4.4-21.6% of males (see above). No male production by queenright H.sublaevis workers was detected in this study, but it was not ruled out either. The occurrence of some queenright worker reproduction was indirectly suggested by the finding of fertile workers in over half the queenright colonies (Table 5.1; see also Buschinger and Winter 1978), and by dominance behaviour in such workers (Ch.4).

Production in H.sublaevis

The productivity of H.sublaevis colonies was, unsurprisingly, largely determined by the size of the slave force (Fig.5.4). Slave number was in turn positively associated with the number of slave-maker workers (Fig.5.3), as expected since slave-maker workers acquire slaves on raids. The decline in per capita production with increasing workforce size, and in the number of slaves per slave-maker with increasing slave-maker number, was also expected, since decreasing returns to scale are a common feature of biological systems, including insect colonies (Oster and Wilson 1978).

The most notable feature of the production schedule of H.sublaevis colonies was the abrupt switch from worker to sexual production at a colony size of 10-15 slave-maker workers (Fig.5.5a). Therefore H.sublaevis colonies appeared to conform to the predictions of Oster and Wilson (1978), who found from optimization models that to maximize fitness social insect colonies should follow a "bang-bang" life history policy, i.e. all worker followed in a stepwise transition by all sexual production. This interpretation of Fig.5.5a assumes the x axis (colony size) corresponds to colony age. However, the number of slave-maker workers cannot be entirely proportional to age if indeed older colonies curtail worker production. Instead, ageing colonies should decline in size. Such a decline would explain the distribution of points in Fig.5.5b, which shows orphaned colonies (older than queenright colonies assuming most colony queens die old) tended to be smaller than queenright colonies and, as expected, produced mostly sexuals.

Oster and Wilson's (1978) life history model referred to above was for annual colonies, so cannot fully describe the situation in H.sublaevis. A full understanding of the productivity schedule in H.sublaevis awaits the development of a perennial life history model for this species. In the concluding chapter I discuss further the aims and characteristics of such a model.

Does H.sublaevis worker reproduction reduce colony fitness?

I end this chapter with a discussion of the relation between worker reproduction and colony productivity in H.sublaevis. Cole (1986) presented a model exploring the conditions for the spread of a rare allele for worker male production assuming worker reproduction imposes a cost on colony fitness. (Colony fitness is a term of uncertain meaning by which Cole appeared to mean total colony output of reproductives; but despite its notional quality, colony fitness remains a currently necessary concept in this and other kinds of social insect strategic model, as I discuss in chapter 9). Cole found the allele would spread if the cost did not exceed a critical value of 17-22%. Reductions in colony efficiency (an indirect measure of colony fitness) due to worker reproduction in the ant Leptothorax allardycei matched this prediction (Cole 1986). In H.sublaevis behavioural studies (Ch.4) have already suggested that dominant workers impose costs on the colony by consuming extra food, or by failing to slave raid. In this chapter, using total biomass produced to indicate colony fitness, I attempted to detect such costs by comparing the difference in production between H.sublaevis colonies with and without fertile slave-maker workers (Fig.5.6).

The production curve for colonies with fertile workers was shallower than the curve for colonies without them, but the two slopes did not differ significantly (see Results). Also, the difference between the curves could have been an artefact of the smaller range in log. transformed slave number in colonies with fertile workers (Fig.5.6). Hence there was no clear evidence that fertile H.sublaevis workers imposed a cost on colony production. However, given the variation in the data, it seems unlikely even a 22% reduction in productivity would have been detectable. Nevertheless, the conclusion fertile workers did not affect productivity was not surprising given the small fraction of fertile workers in queenright colonies containing them (9.8%), their (inferred) low reproductive output, and the fact slave-maker workers are not producers in the sense of foraging, or rearing brood.

Fig.5.6 also shows that queenright fertile slave-maker workers occurred in colonies with above-average numbers of slaves. The mean numbers (±S.D.) of slaves in queenright colonies with and without fertile slave-maker workers were 193±93 and 73±50 respectively. These figures were significantly different (t test, $t=3.87$, $p<0.001$). This finding suggests fertile slave-maker workers may only have arisen in colonies where they purposefully would not have imposed any cost on colony productivity. Consideration of the economics of scale suggests large insect colonies, unlike small ones, are characterized by energetic surpluses (Oster and Wilson 1978). Therefore, notwithstanding Cole's (1986) model, worker reproduction might only be favoured in colonies where fertile workers can exploit such surpluses for egg-laying, and consequently increase their personal fitness without detracting from colony productivity, on which the kin-mediated component of their inclusive fitness must largely depend (Oster and Wilson 1978:95). This would explain the predominance of fertile H.sublaevis workers in colonies with many slaves, and also why workers in large but not small colonies are thereby apparently selected to resist queen inhibition of their fertility (see Ch.4).

To conclude this Discussion as it concerns H.sublaevis worker reproduction. The results indicate that in H.sublaevis the proportion of worker-produced males was too low to perturb the equilibrium sex ratio substantially. In addition, the presence of reproductive slave-maker workers in queenright colonies did not appear to reduce colony production. Hence H.sublaevis worker reproduction appears to have large effects at the level of individual behaviour (and by implication fitness), but less impact at colony or population level.

Summary of Chapter 5

1. In a population of Harpagoxenus sublaevis in S.E. Sweden the mean per colony proportionate dry weight investment in queens (95% confidence limits) was 0.540(0.384-0.691). This result differed significantly from 0.75(3:1 investment) but not from 0.5(1:1 investment). It therefore matched the prediction from the genetic relatedness hypothesis of sex ratio applied to slave-makers, given (as confirmed by this study) monogyny, single mating of queens, population-wide mate competition, and relatively low worker male production. Resource levels were not found to influence sex investment in individual H.sublaevis colonies.

2. The genetic relatedness hypothesis (assuming relatively high worker male production) also correctly predicted sex investment ratio in the slave-maker Epimyrma ravouxi (data of Winter and Buschinger 1983), but in a German H.sublaevis population (Buschinger, Frenz and Wunderlich 1975) investment was female-biased, implying local mate competition could not be ruled out.

3. The proportion of queenless colonies in the Swedish H.sublaevis population sample of 47 colonies was 29.8%, not significantly different from that expected on the basis of the predicted frequency of colony orphanage.

4. Fertile slave-maker workers occurred in 58% of queenright and all queenless colonies. In queenright colonies with fertile workers the mean percentage of fertile workers was 9.8%, whereas in queenless colonies it was significantly higher at 19.2%, confirming that queens inhibit H.sublaevis worker fertility. Fertile and sterile workers did not differ significantly in size.

5. Electrophoretic analysis of allozyme variation at two loci in ants from 49 H.sublaevis colonies from the Swedish population suggested queens mate singly. The regression coefficient of relatedness (±S.E.) between females within colonies was 0.73±0.07 (Me locus) and 0.60±0.08 (Mdh-2 locus), consistent with monogyny and single queen mating.

6. Electrophoretic allozyme analysis further indicated that deviations from panmixia did not occur in the Swedish H.sublaevis population. Hence competition for mates appeared to be population - wide. Queen and worker siblings within individual colonies were not genetically differentiated, but a genetic influence on caste determination in H.sublaevis could nevertheless exist.

7. In 4 queenright colonies yielding genetic information on male parentage, queens probably produced all males. But this result did not rule out queenright worker male production. Workers in queenless colonies produced 4.4-21.6% of all males in the population. This level appeared too low to perturb the predicted equilibrium sex ratio appreciably.

8. Productivity in H.sublaevis colonies was largely determined by the size of the slave force, which in turn was positively correlated with the number of slave-maker workers. There was an abrupt switch from all worker to all sexual production at a colony size of 10-15 slave-maker workers, in agreement with the hypothesis that social insect colonies should adopt a life history policy involving discrete bursts of worker and sexual production.

9. There was no clear evidence that the presence of fertile slave-maker workers reduced colony productivity. In queenright colonies, such

workers occurred in colonies containing above-average numbers of slaves. Hence fertile workers may deliberately exploit energetic surpluses in larger colonies. Worker reproduction in H.sublaevis therefore appears to have a greater influence at the level of individual behaviour than at colony or population level.

Table 5.1 Composition, production, sex ratio in 47 Harpagoxenus sublaevis colonies

Host class[a]	Colony no.[b]	Colony queen	Original no. HS workers	Original no. slaves[c] LA	LM	No. fertile HS workers d	Production (HS) No. queens	No. workers[e]	No.males	Brood type in 2nd generation[f]
HS+LA n=30	S85 1	1	54	252		8	71	0*	7	D
	4	0	66	34		4	21	0*	2	D
	15	1	4	79		1	4	40	14	D
	26	0	10	16		2	0	8	0	D
	29	0	11	23		3	7	0*	3	-
	41	0	8	98		3	34	0*	25	D
	42	0	15	31		3	0	13	4	D
	i49	1	0	3		0	0	2	0	-
	83	1	15	187		4	49	1	29	D
	84	0	4	47		4	0	16	0	D
	9	1	13	436		2	29	21	42	-
	11	1	34	224		2	28	0*	55	-
	17	0	18	108		2	28	0*	92	-
	18	1	21	198		3	55	0*	56	-
	22	1	5	87		0	8	0*	16	-
	23	1	40	165		5	17	0*	74	-
	27	1	25	153		3	38	0*	72	-
	70	1	28	140		0	55	8	69	-
	75	1	19	263		9	33	6	129	-
	96	1	17	115		3	44	17	68	-
	101	0	4	106		1	0	0	42	-
	106	0	5	29		2	7	0*	25	-
	10	1	6	85		-	0	51	3	D
	37	1	8	74		-	3	2	0	D
	40	1	2	32		-	0	17	0	D
	48	1	10	24		-	0	6	0	D
	71	0	34	79		-	28	37	6	D

(Contd.)

Table 5.1 (contd.)

Host class[a]	Colony no.[b]	Colony queen	Original no. HS workers	Original no. slaves[c] LA	LM	No. fertile HS workers[d]	Production (HS) No. queens	No. workers[e]	No. males	Brood type in 2nd generation[f]
	74	1	1	38		-	0	25	3	D
	82	0	18	122		-	18	0*	3	H
	97	1	11	37		-	21	5	22	D
HS+LA	S85 2	0	13	39	35	2	2	0*	0	H
+LM	24	1	7	---86---		4	2	37	12	D
n=9	47	0	9	5	7	1	0	3	2	H
	54	1	9	2	22	0	6	15	2	D
	60	1	12	93	153	1	9	24	0	D
	62	1	21	40	157	1	38	25	8	D
	68	1	8	95	7	4	2	25	6	D
	77	1	12	--159---		0	48	31	45	-
	107	1	22	10	80	0	17	11	13	-
HS+LM	S85 12	1	19		81	0	0	41	1	D
	i13	1	0		59	0	0	8	0	D
n=7	16	1	4		48	0	0	15	0	D
	38	1	8		12	0	0	13	0	D
	53	1	22		96	0	6	11	7	D
	61	1	29		82	1	-	-	-	D
	i72	1	0		3	-	0	0	0	-
HS+LM +LG n=1	S85 14	0	28	275 LM+LG		9	25	0*	4	H

(Contd.)

Table 5.1 (Contd.)

Notes: a. HS = *Harpagoxenus sublaevis*; LA = *Leptothorax acervorum*; LM =*L.muscorum*, LG = *L.gredleri*

b. i = incipient (newly-founded) colony, i.e. containing only HS queen and slaves on collection.

c. Original no. slaves = no. slaves in nest at first count, excluding callows. Slaves consist of both *Leptothorax* workers and dealate queens (2.8% of all slaves are dealate queens). Neither workers or queen slaves are ever reproductive in *H.sublaevis* colonies (Buschinger and Winter 1978) (See Ch.6).

d. No. fertile HS workers = no. fertile HS workers out of the total no. HS workers (i.e. original no. + no. produced), since workers cannot be aged.

e. Where zero worker production is indicated and marked with *, worker~~s~~ production was assumed to be zero because the number of queens produced exceeded the total estimated number of females produced (See Methods).

f. D = diploid or diploid and haploid brood; H = haploid (male) brood alone. Results come from rearing the brood of the 1985 colonies in the laboratory (See Methods).

Table 5.2 Sex ratio analysis of Harpagoxenus sublaevis from the Bröms/Kristanopel woods, Sweden

	Colony class	n colonies	Mean per colony sex ratio (proportion of queens)(95% confidence limits) [a]	Investment ratios tested against:	
				1:1 hypothesis	3:1 hypothesis
Numerical	Queenright	25	0.425 (0.265-0.594)		
	Queenless	12	0.481 (0.165-0.807)		
	All colonies	37	0.443 (0.303-0.589)		
Investment [b]	Queenright	25	0.531 (0.362-0.697)[c]	$t = 0.381$, NS	$t = 2.777$, $p<0.05$
	Queenless	12	0.556 (0.209-0.873)[c]	$t = 0.335$, NS	$t = 1.241$, NS
	All colonies	37	0.540 (0.384-0.691)	$d = 0.516$, NS	$d = 2.909$, $p<0.01$

Notes: a. All statistical analysis was carried out with angular transformed proportions (Sokal and Rohlf 1969).

b. Calculated on basis of dry weight cost ratio : F = 0.58mg. (n = 40), M = 0.32mg. (n = 60).

c. Queenright and queenless ratios are not significantly different ($t = 0.146$, $p>0.1$)

Table 5.3 Comparison of pronotal widths of sterile and fertile H.sublaevis workers in two host classes

Pronotal width

Host class:	HS+HA (n colonies = 12)			HS+HA+HM (n colonies = 6)		
	n indivs.	Mean	S.D.	n indivs.	Mean	S.D.
Fertile workers	32	1.958	0.145	12	1.838	0.125
		0.576	0.043		0.540	0.037
Sterile workers	250	1.961	0.142	150	1.880	0.134
		0.577	0.042		0.553	0.039
		d = 0.111	NS $p > 0.1$		d = 1.114	NS $p > 0.1$

Notes: a. Only workers from colonies containing both sterile and fertile workers were included in the comparison (see Tables 5.1, 6.5).

b. For each category of worker, the upper row gives the mean and standard deviation of pronotal width in graticule units, the lower row the same in millimetres.

1 graticule unit = 0.294 mm. Measurements were made to the nearest 0.05 graticule unit (0.015 mm.).

Table 5.4 Numbers of Me and Mdh-2 genotypes in 49 Harpagoxenus sublaevis colonies

Host class	Colony no.^{a,b}	Me							Mdh-2				Area^{c}
		Females				Males			Females		Males		
		96/96	96/100	100/100	100/104	96	100	104	97/100	100/100	97	100	
HS+	S83 22		8	8					9	2			1
LA	54	6	7							6			1
n=33	S84 38	*	10							10*			1/2
	73		60						3	57			2
	95			13						13			1/2
	99			9					4	5			1/2
	114		5	7						12			1/2
	S85 1	6	5				2			11		2	6
	4		6	9						15			3B
	9+		44	*			10			44*		10	3A
	10			7						7			3B
	15			9			7		7	2	4	3	3B
	17+		6	6		2	1			12		3	3B
	18			21			8		9	12	5	3	3B
	22	10	9			10	7			19		17	3A
	23+		19*	20		10	3		18*	21	4	9	3A
	26	1	6						7				3A
	27+		27*	20		3	3		23*	11	4	2	3A
	37			8						8			2
	40			19						19			2
	42		6							6			2
	48			10						10			4
	70+	46	37*			4	3			83*		7	4
	71		11						4	7			4
	74			9						9			4
	75+			26*			36			26*		36	5

(Contd.)

Table 5.4 (contd.)

Host class	Colony no.[a,b]	Me Females 96/96	Me Females 96/100	Me Females 100/100	Me Females 100/104	Me Males 96	Me Males 100	Me Males 104	Mdh-2 Females 97/100	Mdh-2 Females 100/100	Mdh-2 Males 97	Mdh-2 Males 100	Area[c]
	83			7						7			5
	84			17						17			5
	96+		71	1*			48			72*		48	5
	97	6	7							13			5
	101+		4			7	16			4		23	5
	106+			10			20			10		20	1
	S86 19			25						25			5
HS+	S85 24			8			4		3	5	2	2	3A
LA+	54		14	15		6	4			29		10	4
LM	60			11	15		1	6		26		7	4
n=7	62			10						10			4
	68			25			9			25		9	4
	77+		44*	32		15	4			76*		19	5
	107+			14*			3		-	-		-	1
HS+	S84 25		5*	8						13			1/2
LM	S85 12	7	10			1				17		1	3B
	13			13			1			13		1	3B
n=9	16			24						24			3B
	38		3	2						5			2
	53			19			2			19		2	4
	61		8							8			4
	S86 5		20							20			5
	17		3*	5						8*			5

(Contd.)

Table 5.4 (contd.)

Notes: a. + = 1985 colony subjected to electrophoresis shortly after collection and containing males (See Methods).

b. * = genotype of colony queen, in the 10 colonies where known; in these colonies genotype numbers do not include the colony queen.

c. Collecting area: The woods were divided into 7 adjoining collecting areas. These were arbitrary, except that areas 1,2 and 4 lay on the left of the road through the woods (See Methods), whereas areas 3A, 3B and 5 lay on its right. Area 6, where colony S 85 1 was collected, lay slightly away from the main collecting areas. Colonies designated 1/2 came from either area 1 or 2.

Table 5.5 Fixation indices (F) in a Harpagoxenus sublaevis population

Locus	Host class	All areas				Area L				Area R			
		n colonies	F	X^2	Significance	n colonies	F	X^2	Significance	n colonies	F	X^2	Significance
Me	HS+LA	31	0.079	0.290	NS	14	0.060	0.076	NS	16	0.036	0.031	NS
	HS+LA+LM	7	-0.133	0.186	NS	5	-0.119	0.106	NS	2	-0.169	0.086	NS
	HS+LM	9	-0.126	0.214	NS	4	-0.330	0.653	NS	5	0.023	0.004	NS
	All colonies	47	0.034	0.081	NS	23	-0.017	0.010	NS	23	0.027	0.025	NS
Mdh-2	HS+LA	31	-0.087	0.352	NS	14	-0.062	0.081	NS	16	-0.117	0.329	NS
	HS+LA+LM	6	-0.032	0.009	NS	4	-	-	-	2	-0.103	0.032	NS
	HS+LM	9	-	-	-	4	-	-	-	5	-	-	-
	All colonies	46	-0.062	0.265	NS	22	-0.038	0.048	NS	23	-0.088	0.267	NS

Notes: a. - = F value not given because all colonies in subpopulation consist exclusively of homozygotes and hence F cannot discriminate between panmixia and total inbreeding.

b. Area L = areas 1,2 and 4 in Table 5.4; Area R = areas 3A, 3B and 5.

c. $X^2 = NF^2$ with 1 degree of freedom, where N = 1.5 x number of colonies (see Methods).

Table 5.6 Me genotypes of worker and queen siblings in 18 *Harpagoxenus sublaevis* colonies

Colony no.	Workers			Queens		
	96/96	96/100	100/100	96/96	96/100	100/100
S 84 25		1	3		4	5
38		9			2	
99			4			3
114		1	2		4	4
S 85 9		23			20	
17			1		6	5
23		11	13		6	5
27		9	7		15	12
70	18	11		23	24	
71		3			8	
75			11			14
77		16	18		23	14
96		27	1		36	
97	2			4	7	
101		4				
106			4			6
107			8			5
S 86 17		2	4			
Totals	20	90	75	27	119	73

X^2 workers v.queens = 2.370 NS ($p>0.1$)

Note: S 85 96 is omitted from totals and X^2 test because it possibly contained ants from other colonies (see text).

Table 5.7 Estimated mean dry weights of Harpagoxenus sublaevis reared by different hosts

	Estimated mean dry weight (mg)[a]		
Host class	Queens	Workers	Males
HS+LA	0.58(280)[b]	0.53(435)	0.32[c]
HS+LA+LM	0.60(57)	0.49(184)	0.33
HS+LM	0.55(5)	0.42(164)	0.30
HS+LM+LG	0.62(24)	0.54(27)	0.34

Notes: a. Dry weights estimated from mean pronotal width on basis of relationship (in 40 HS queens) : $\log_{10}$ dry weight (mg.) = 2.0859 $\log_{10}$ pronotal width (mm.) + 0.2315 (Regression analysis, $F = 53.1$, $p<0.001$).

b. Numbers in brackets = no. individuals whose pronotal widths measured (see Table 6.6).

c. LA-reared HS male dry weight was measured in a sample of 60 HS males from the Bröms/Kristianopel population. Dry weights of the remaining categories of HS male were estimated assuming the same female:male dry weight ratio as was found within the HS and LA host class.

Table 5.8 Partial correlation coefficients between male investment, total sexual biomass produced, and worker number in Harpagoxenus sublaevis

n colonies	R and TOTAL[a]	R and no. workers	TOTAL and no. workers
37	-0.0157[b]	-0.3270[b]	+0.3478[c]

Notes: a. The abbreviations are those of Nonacs (1986 b):

R = proportion of investment in males (angular transformed)

TOTAL = total biomass of sexuals produced (mg.)

no. workers = no. (slave-maker) workers in colony.

b. Not significant.

c. Significant at 5% level.

Table 5.9 Sex ratio analysis of Harpagoxenus sublaevis data of Buschinger, Frenz and Wunderlich (1975)[a]

	n colonies[b]	Mean per colony sex ratio (proportion of queens)(95% confidence limits)[c]	Investment ratios tested against:	
			1:1 hypothesis	3:1 hypothesis
Numerical	33	0.587(0.441-0.724)		
Investment[d]	33	0.676(0.553-0.805)	d=2.494,$p<0.05$	d=1.131, NS
Corrected investment[e]	33	0.625(0.476-0.762)	d=1.719, NS	d=1.848, NS

Notes:
- a. The analysis involves the 33 colonies in Table 1b of Buschinger et al. (1975). I exclude from the analysis the sex ratio for 25 colonies in Table 1a of these authors, because (1) sexuals from these colonies, unlike the Table 1b sexuals, were raised without undergoing a natural hibernation; (2) the sex ratio of these colonies is extremely male biased, suggesting the two sets of data should not be pooled.
- b. Colonies were not classified as queenright or queenless by Buschinger et al..
- c. All analysis carried out with angular transformed data.
- d. Calculated on basis of dry weight cost ratio: F = 0.59 mg. (n=30), M = 0.34 mg. (n=30)(measured by Trivers and Hare [1976] using ants from the German population).
- e. Calculated on the basis of the dry weight cost ratio with 72.5% correction, following Boomsma and Isaaks (1985)(see text).

Table 5.10 Sex ratio analysis of Epimyrma ravouxi data of Winter and Buschinger (1983)

	Colony class	n colonies	Mean per colony sex ratio (proportion of queens) (95% confidence limits)[a]	Investment ratios tested against:	
				1:1 hypothesis	3:1 hypothesis
Numerical	Queenright	18	0.533(0.355-0.706)		
	Queenless	12	0.133(0.004-0.402)		
	All colonies	30	0.357(0.202-0.529)		
Investment[b]	Queenright	18	0.630(0.453-0.790)[d]	t=1.552 NS	t=1.545 NS
	Queenless	12	0.165(0.005-0.477)[d]		
	All colonies	30	0.432(0.256-0.617)	t=0.750 NS	t=3.624 $p<0.01$
Corrected investment[c]	Queenright	18	0.569(0.391-0.739)[e]	t=0.817 NS	t=2.258 $p<0.05$
	Queenless	12	0.145(0.004-0.431)[e]		
	All colonies	30	0.385(0.222-0.562)	t=1.329 NS	t=4.324 $p<0.001$

Notes:

a. All analysis carried out with angular transformed data

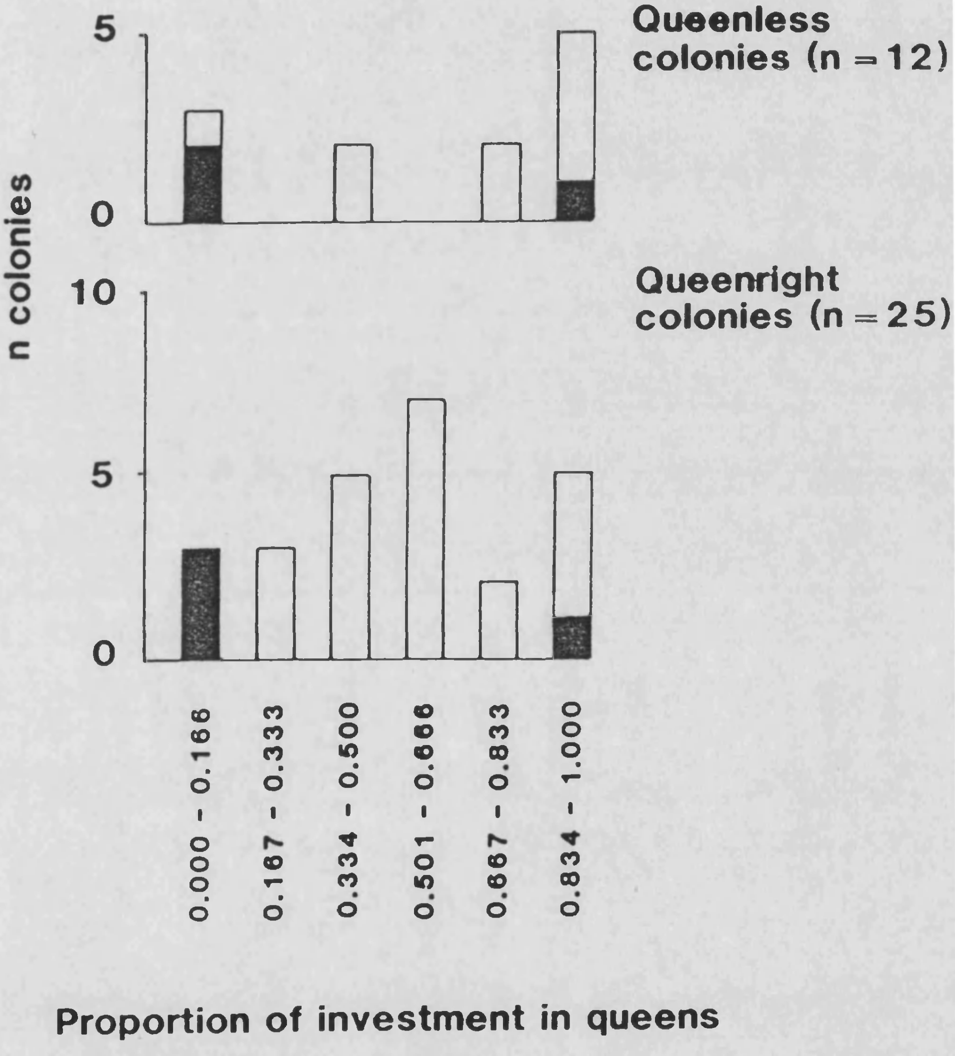
b. Calculated on basis of dry weight cost ratio: F = 0.35 mg. (n=4), M = 0.21mg. (n=12)(Winter and Buschinger 1983 Table 4).

c. Calculated on basis of dry weight cost ratio with 72.5% correction, following Boomsma and Isaaks (1985)(see text)

d. Queenright and queenless ratios are significantly different : t = 3.040, $p<0.01$

e. Queenright and queenless ratios are significantly different : t = 2.921, $p<0.01$

Figure 5.1 Distribution of sex investment ratios in colonies of H. sublaevis from the Bröms-Kristianopel population.



Shading represents colonies producing 4 sexuals or less (i.e. colonies S 85 2, 10, 12, 37, 42, 47, 74)(Table 5.1).

Figure 5.2 Relation between sex investment ratio and sexual productivity in H. sublaevis colonies.

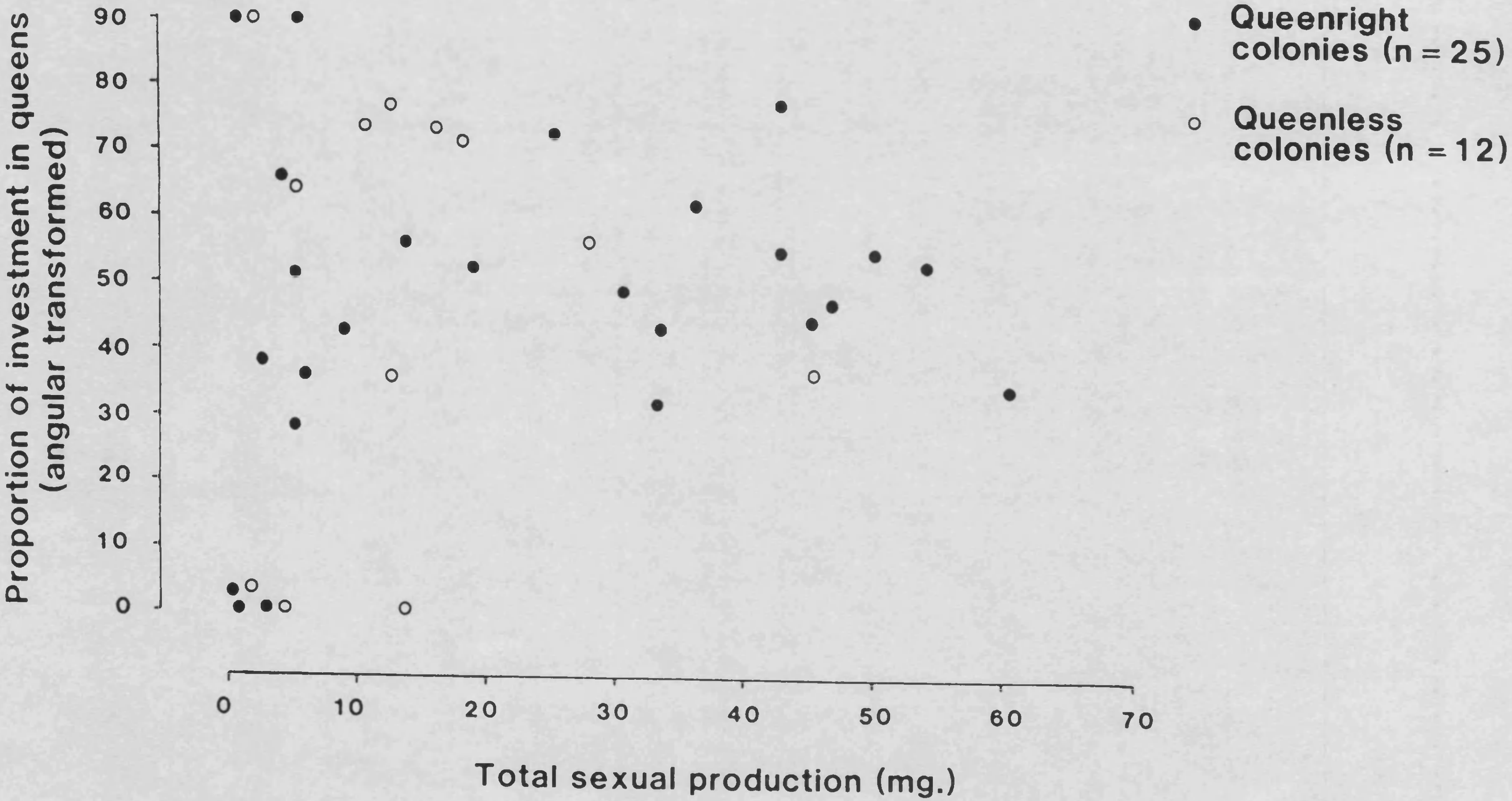
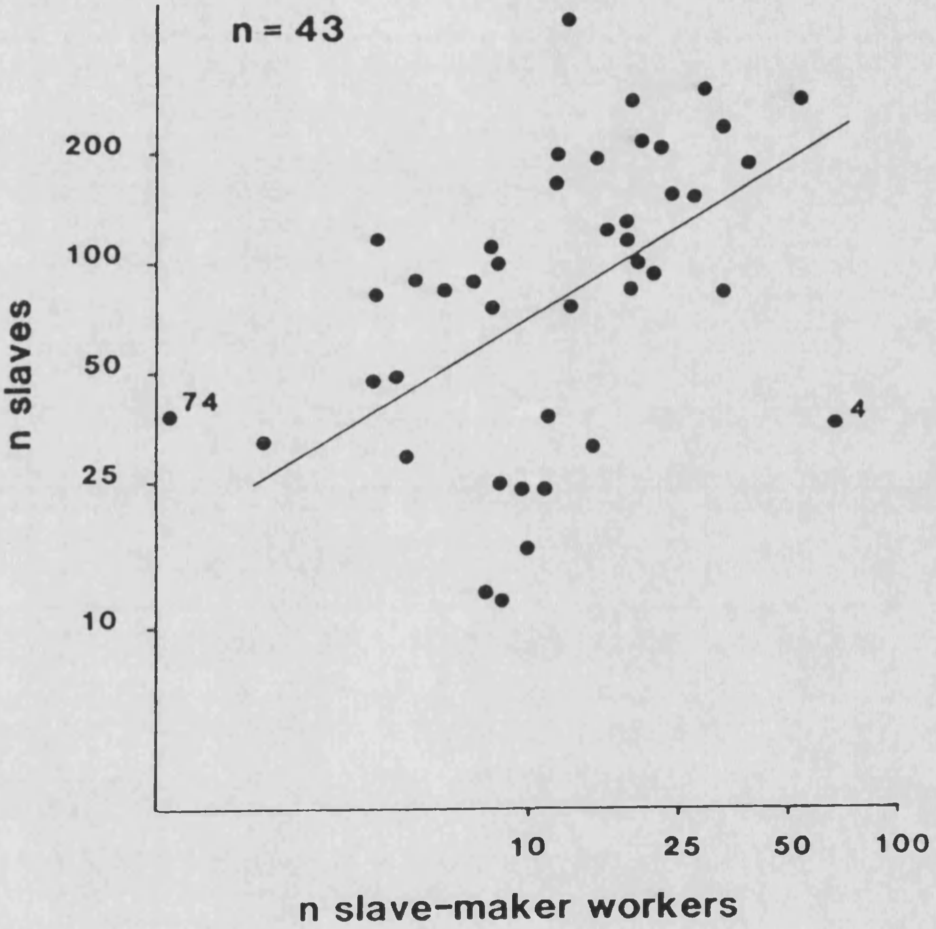


Figure 5.3 Relation between slave-maker worker number and size of slave force in H. sublaevis.

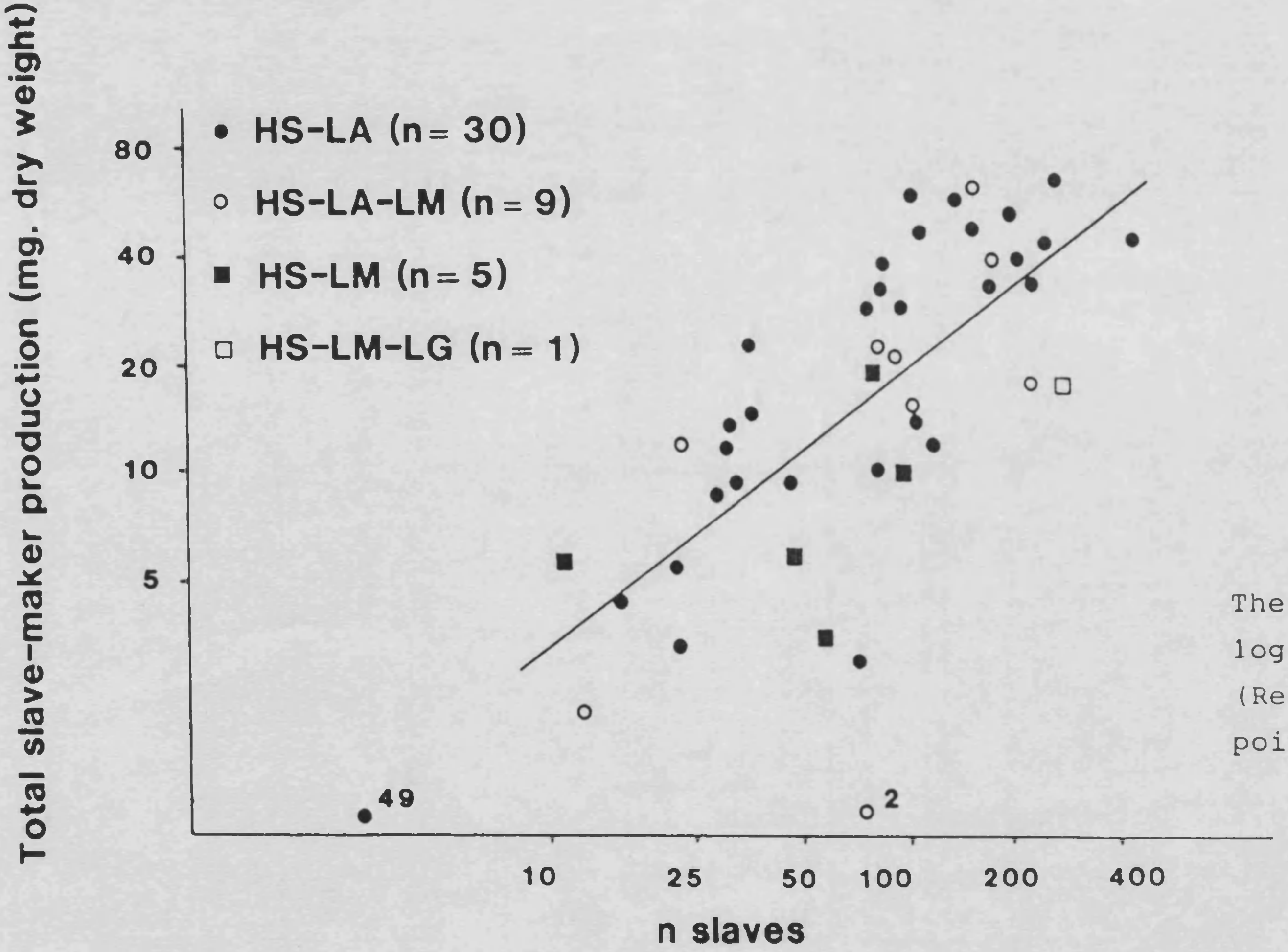


The equation of the regression line is:

$\log._{10}y = 0.6222\log._{10}x + 1.2411$

(Regression analysis omits outlying points from S 85 4 and 74).

Figure 5.4 Relation between slave number and total slave-maker biomass produced in H. sublaevis



The regression equation is:

$\log._{10}y = 0.7731\log._{10}x - 0.2465$

(Regression analysis omits outlying points from colonies S 85 2 and 49).

Figure 5.5 Production schedule of H. sublaevis colonies.

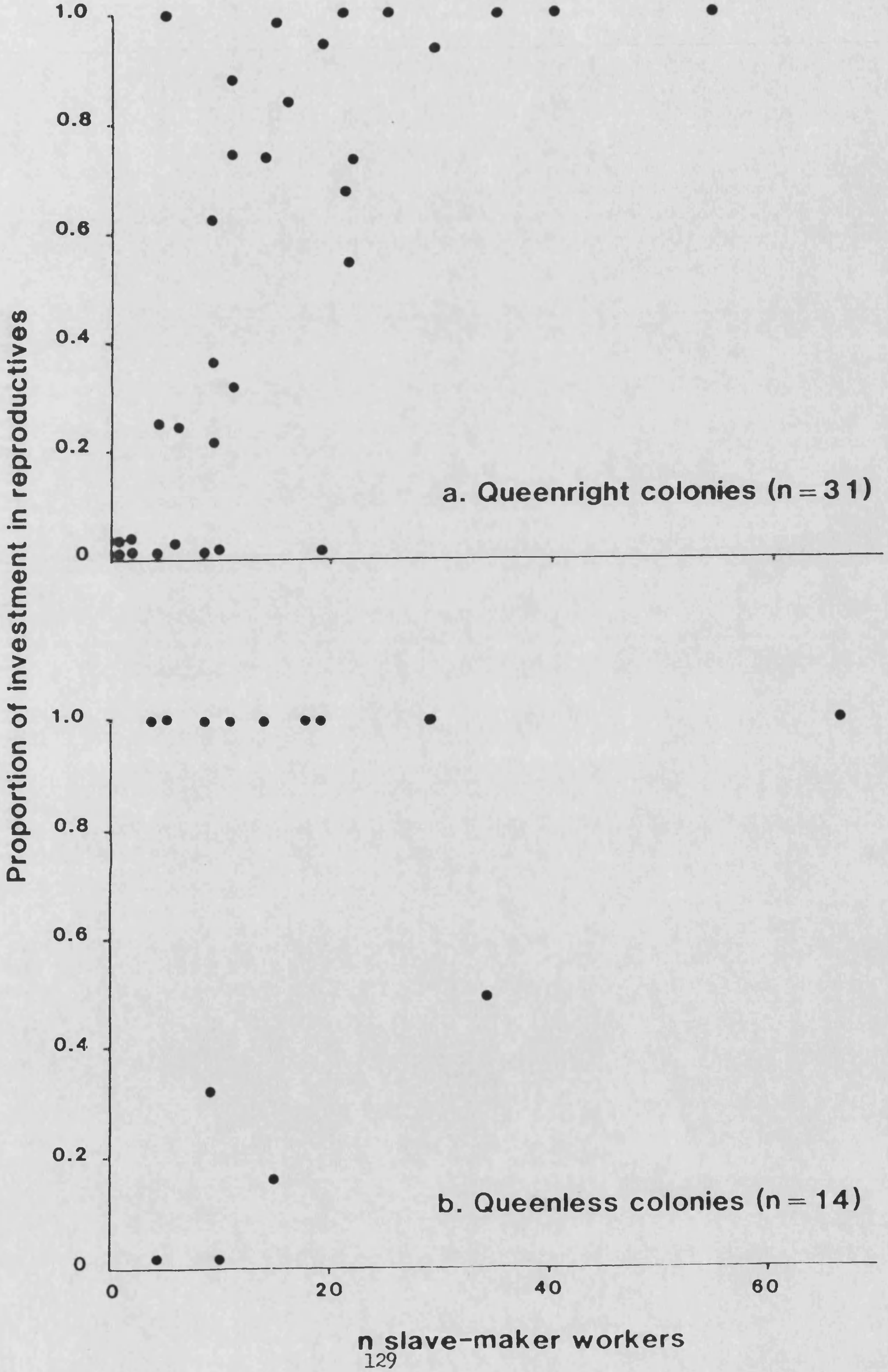
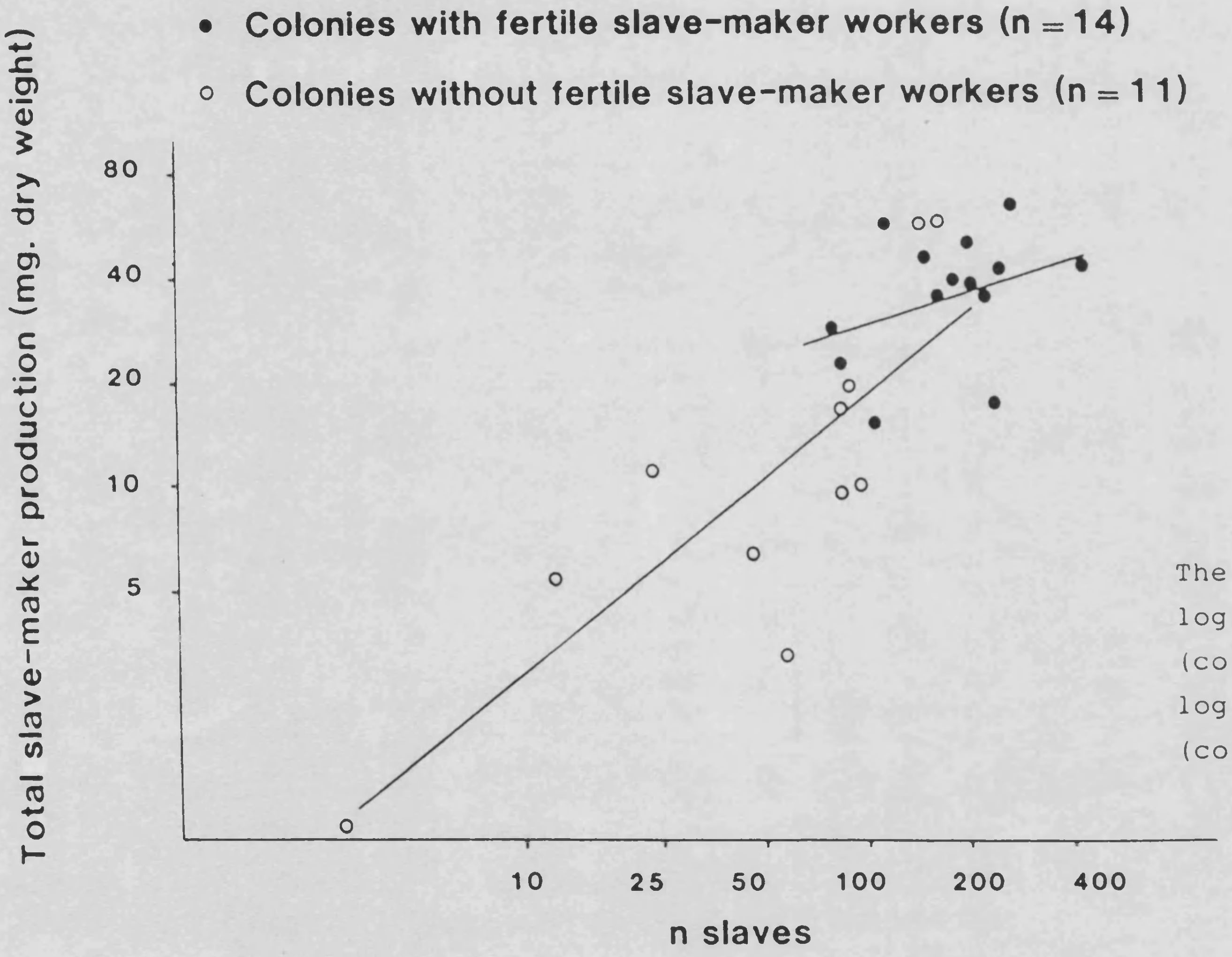


Figure 5.6 Productivity in queenright H. sublaevis colonies with and without fertile slave-maker workers.



The regression equations are:
$\log_{10}y = 0.3281\log_{10}x + 0.8085$
(colonies with fertile workers); and
$\log_{10}y = 0.8061\log_{10}x - 0.3381$
(colonies without fertile workers).

Chapter 6

Host-Parasite Relations between Harpagoxenus sublaevis and its Leptothorax Slaves

Introduction

Harpagoxenus sublaevis is a successful parasitic exploiter of its three slave species, Leptothorax acervorum, L.muscorum, and L.gredleri, with (as I will describe) some powerful adaptations for their manipulation. But since H.sublaevis lives in obligate, close association with its slaves, its biology is in turn partially influenced by theirs, as dissimilarities (also to be described) between H.sublaevis raised by the different hosts reveal. This chapter is about the reciprocal host-parasite relations between H.sublaevis and its slave species. The previous two chapters described work with H.sublaevis designed to test hypotheses concerned with kinship theory in social insects, and so involved issues such as worker reproduction and sex ratio determination. This chapter presents and analyzes data bearing on a different but equally intriguing set of problems, those posed by the widespread phenomenon of social parasitism in insects (see Ch.2), as exemplified by the H.sublaevis- Leptothorax association. However, some of the topics raised in chapters 4 and 5 re-appear, because it turns out that, for example, worker behaviour and colony sexual production may be among those aspects of slave-maker biology under subtle slave influence. In fact this chapter makes the general point that many important evolutionary consequences may arise accidentally in social parasites as the result of host-driven processes.

The first problem I deal with raised by the slave-maker / slave relationship concerns the lack in the Leptothorax hosts of H.sublaevis of specific defences for resisting enslavement. Dawkins (1982: Ch.4) suggested such a lack could occur if a social parasite were so rare that the low probability of infestation did not justify the cost to any one host lineage of developing defensive precautions (the "rare enemy effect"). One way to assess the importance of this hypothetical effect in slave species is, as in this study, to measure the frequency of parasitism in their populations. This information is also needed to determine whether any host is attacked preferentially by the slave-makers.

The next question I consider concerns the spatial distribution of H.sublaevis colonies. Although much work has been carried out on territoriality and space use in colonies of free-living ant species (Hölldobler 1979, Levings and Traniello 1981), little is known about such topics in slave-makers (but see Yasuno 1964). Yet slave-makers provide a particularly interesting test of the hypothesis that intraspecific competition influences colony spacing, because slave-maker colonies arguably risk becoming raid victims of their conspecific neighbours. In addition, knowledge of the pattern of exploitation of host colonies by slave-makers, and hence of the slave-makers' effect on host colony distribution, is essential for a full understanding of this host-parasite relationship. This chapter therefore reports the first field mapping survey of H.sublaevis colony distribution.

Another important question in the parasitology of H.sublaevis is whether the species "husbands" its slaves, that is defers immediate exploitation for future gain from them, as its North American relative H.americanus allegedly does. Alloway (1979) proposed that H.americanus released (rather than ate or enslaved) slave species queens and males derived from captured pupae, to guarantee a future supply of host colonies. In H.sublaevis slave sexuals cannot be either all eaten or all released, because slave-maker nests regularly contain Leptothorax queens which have lost their wings (dealates). Conceivably, H.sublaevis could alternatively husband its hosts by allowing slave queens to reproduce and hence provide further slave workers in the slave-makers' own nests. But this possibility was disproved by Buschinger and Winter (1978), who dissected dealate slave queens from German H.sublaevis colonies and found all were non-layers. This chapter supplements their study with a similar investigation of slave queen reproductive status in Swedish H.sublaevis nests, which suggests the slave-makers may deliberately inhibit reproduction by their slaves.

Turning to host influences on slave-makers, I next analyze the effects on H.sublaevis of the strong differences in body size between its three Leptothorax hosts. L.acervorum is a relatively large species (worker length 3.8-4.5 mm.), whereas L.muscorum is small (2.4-3.2 mm.), and L.gredleri intermediate (3.0-3.5 mm.) (Buschinger 1966c, Collingwood 1979:72). Slave size partly determines that of the slave-makers raised. For example, L.muscorum - reared H.sublaevis are considerably smaller than those reared by L.acervorum (Buschinger and Winter 1975). In this chapter I present a full statistical analysis of slave-maker queen and worker size in relation to host class. I furthermore argue that through their effect on body size, the influence of slaves reaches behavioural and social aspects of slave-maker biology, such as worker reproduction.

Finally, to examine host-induced differences further, I attempt to discover whether H.sublaevis raised by different slaves constitute genetically distinct host races. Recently, evidence has accumulated that where intimate, near host-specific associations occur, such as those between parasites and hosts or phytophagous insects and their food plants, populations of the parasitic or phytophagous organism may divide into separate subpopulations each specialized on one particular host (host race formation). Sympatric speciation may then follow (Bush 1975, White 1978). Since nearly all slave-makers have a selection of hosts (Table 19.1 in Wilson 1971), these social parasites would appear especially prone to such a process. This speculation is fuelled by the observation that the socially parasitic leptothoracine ant genus *Epimyrma* strongly resembles the expected end-product of speciation through host race formation (see Discussion). Therefore it seems worthwile to investigate the genetic population structure of a single slave-maker species with several hosts, such as *H.sublaevis*, for evidence of genetic differentiation of host classes. In this chapter I analyze the electrophoretic data presented in chapter 5 (Table 5.4), jointly collected with Dr. T.M. van der Have, for such evidence.

Methods

Score of the relative frequencies of Leptothorax and H.sublaevis colonies

All data in this chapter derived from the same population of *H.sublaevis* and *Leptothorax* in the Bröms-Kristianopel pinewoods, Sweden, which features in chapters 4 and 5. In these woods leptothoracine colonies occurred inside hollow dead twigs on the ground. When collecting ants in June-July 1983-1986 I kept a score - classified by species and woodland area - of all occupied twigs found. Since all species involved are monodomous (single colonies occupy single nest-sites), this score recorded the number of colonies of each species found. Further, because determining each colony's species composition involved breaking open part of the nest twig, previously encountered but uncollected colonies remained recognizable. Hence I avoided duplicate recordings. Therefore, assuming all colony types were found equally easily, the score provided a measure of the relative frequencies of the unparasitized and parasitized colony types. Although the score was not kept continually, the total number of colonies recorded in this way exceeded 900.

Mapping survey of H.sublaevis colony distribution

The high density of twigs both uninhabited and occupied, coupled with the relatively large distances between slave-maker colonies, made mapping large plots of woodland floor impracticable. Instead the following method of investigating the distribution of H.sublaevis colonies was adopted. In June and July 1985 and 1986 thirty-one H.sublaevis colonies were located in areas not previously disturbed by collecting. All twigs were examined in the region defined by a circle with a 2m. radius around each colony. Occupied twigs were marked with stakes and their positions recorded. If no colonies were found within 2m. of the focal slave-maker nest, searching continued outwards until the nearest Leptothorax or slave-maker neighbour was found. This way, a combined area of roughly 380 $m.^2$ was examined and mapped.

Ovarian dissections of dealate Leptothorax queens from H.sublaevis nests

H.sublaevis colonies collected in June-July 1985 and 1986 were inspected for the presence of dealate Leptothorax queens. Shortly after collection, all such queens (numbering 218) were removed from 22 colonies, frozen, and subsequently dissected. The ovaries were removed and examined for eggs, sperm, and corpora lutea, following the method described for H.sublaevis females in chapter 4.

Slave-maker size measurements

I measured the pronotal (thoracic) width of 1150 female H.sublaevis, constituting all or nearly all the female members of 37 colonies in the three main host classes of the 1985 H.sublaevis population sample (Table 5.1). Pronotal width is a standard measure of body size and is proportional to dry weight on a log.-log. plot (see notes to Table 5.7). I measured each ant under a Zeiss binocular miscroscope to the nearest 0.05 eyepiece graticule unit (i.e. to the nearest 0.015 mm., since 1.0 graticule unit = 0.294 mm.). Each ant was classified as a colony (maternal) queen, produced (virgin) queen, or worker, on the basis of the ovarian dissections of the 1985 H.sublaevis described in chapter 5. The resulting data on body size in relation to caste and host class were statistically interpreted by analysis of variance, as detailed in the Results. I also investigated the relation between slave-maker body size and ovariole number, again using data from the ovarian dissections in chapter 5.

Electrophoresis and genetic data analysis

The electrophoretic data analyzed in this chapter to determine the genetic status of the H.sublaevis host classes are those already presented in chapter 5 (Table 5.4), where they were used to provide information on queen mating number, intra-colony relatedness, etc.. All electrophoretic methods were therefore as given in chapter 5.

The genetic data were analyzed for host class differentiation as follows. Treating each host class as if it were a geographically separate population, I calculated Nei's (1972) genetic distance between all host class pairs. Genetic distance is a measure of the divergence time between isolated populations. In two randomly-mating populations X and Y, the probability of identity of two randomly chosen genes is $jx = \sum x_i^2$ in X, and $jy = \sum y_i^2$ in Y, where x_i and y_i are the frequencies of the ith alleles in X and Y respectively. The probability of identity of a gene from X and a gene from Y is $jxy = \sum x_i y_i$. Over all loci (two were sampled in the slave-maker case), the normalized identity of genes between X and Y is

$$I = Jxy/\sqrt{(JxJy)}$$

where Jx, Jy and Jxy are the arithmetic means, over all loci, of jx, jy and jxy respectively. The genetic distance between X and Y is

$$D = -\log._e I$$

Hence, when two populations are genetically identical (have the same alleles in identical frequencies), I = unity and D = zero (Nei 1972).

Results

Relative frequencies of Leptothorax and H.sublaevis colonies

Table 6.1 gives the score totals for all categories of *Leptothorax* and *H.sublaevis* colony in each year and woodland area. The commonest *Leptothorax* species was *L.acervorum* (64% of unparasitized colonies, calculated from pooled scores), followed by *L.muscorum* (34%) and *L.gredleri* (2%). *L.gredleri*, as well as being rare, was restricted to two areas. Within areas 1,2 and 5, where data for more than one year are available, the relative frequency of *L.acervorum* and *L.muscorum* colonies did not significantly alter from year to year (X^2 tests, all X^2 corr.<1.7, all d.f.=1, all p>0.05). But this frequency was significantly different from area to area (X^2 test pooling year scores within areas, X^2=46.8, d.f.=4, p<0.001). The varying relative abundance of *L.acervorum* and *L.muscorum* in different parts of the woods presumably reflected differences in microhabitat.

Most H.sublaevis colonies contained either L.acervorum slaves (70% of colonies, calculated from pooled scores), or L.muscorum (16%), or both these species (11%) (Table 6.1). L.gredleri, reflecting its scarcity, was present in only 3% of H.sublaevis colonies. The following analysis aims to determine the proportion of enslaved colonies in L.acervorum and L.muscorum. It also determines whether these two principal host species were parasitized at identical frequencies, or whether colony-founding H.sublaevis queens preferred one species to the other (or one was easier to enslave than the other). Colonies containing both L.acervorum and L.muscorum slaves were omitted from this analysis. This was because, given such colonies must have arisen when slave-maker workers captured slaves different in species to those initially acquired by the queen, determining the original choice of slave species was impossible.

In two areas with two-year scores, the frequency of L.acervorum parasitism by H.sublaevis did not change significantly from year to year (both X^2 corr.<0.01, d.f.=1, both $p>0.9$). In the third such area, area 5, this frequency did significantly alter, between 1985 and 1986 (X^2 corr.= 3.95, d.f.=1, $p<0.05$). But the 1986 sample of parasitized colonies was very small (2), weakening this conclusion: a single extra parasitized colony in this sample would have yielded a non-significant result. Hence, at the risk of type II error, I conclude the frequency of L.acervorum enslavement remained stable from year to year within areas, to justify pooling year scores within areas below. The parasitism frequency of L.muscorum remained approximately constant from year to year in all three areas with two years' data (all X^2 corr.<0.26, d.f.=1, all $p>0.5$). Turning to area comparisons, the infestation rate of L.acervorum was found to be the same between areas (X^2 test pooling years within areas as explained above, $X^2=5.36$, d.f.=4, $p>0.1$). This was also true for L.muscorum (X^2 test pooling years within areas, and areas 2 with 4, and 3 with 5 [to avoid excess low cell totals], $X^2=2.27$, d.f.=2, $p>0.1$). In other words, within each principal Leptothorax host, the frequency of parasitism by H.sublaevis was uniform throughout the woods.

This meant I could pool area scores to compare the parasitism rate of L.acervorum with that of L.muscorum (Table 6.2). I found L.acervorum was parasitized at a significantly higher frequency (7.6%) than L.muscorum (3.4%) (X^2 corr.=5.40, d.f.=1, $p<0.05$). This conclusion held even if L.acervorum colonies containing the workerless inquiline L.kutteri were included in the unenslaved L.acervorum sample (Table 6.1). (Later findings suggested such colonies were liable to H.sublaevis attack). However, the preference of H.sublaevis for L.acervorum over L.muscorum was not demonstrable within any single area (all X^2 corr.<2.08, d.f.=1, all $p>0.1$), presumably due to low sample sizes. Furthermore, the original host classes of mixed slave colonies remained uncertain. So the conclusion H.sublaevis parasitized L.acervorum more frequently than L.muscorum is tentative.

Spatial distribution of Leptothorax and H.sublaevis colonies

Data from the mapping survey appear in Table 6.3. Conventional nearest neighbour analysis (Clark and Evans 1954) of H.sublaevis colony distribution was not possible because the distance between most H.sublaevis colonies and their nearest conspecific neighbour could not be measured (see Methods). However, in 1985 (area 3) I did measure the distance between eleven H.sublaevis colonies and their nearest known slave-maker neighbours. The mean maximum nearest neighbour distance thereby estimated was 6.6 m. (range 1.2-15.1 m.). This was a maximum estimate because additional, undiscovered slave-maker colonies could have been present in the unsearched spaces between colonies of known position.

If H.sublaevis colonies were distributed randomly in a population of Leptothorax colonies, the proportion of slave-maker nests with a slave-maker nearest neighbour would match the frequency of parasitism. For example, if one in ten Leptothorax colonies were parasitized, one in ten H.sublaevis nests would have another as nearest neighbour, provided parasitized colonies occurred randomly. I therefore compared the frequency of H.sublaevis colonies with other H.sublaevis for nearest neighbours in the Bröms-Kristianopel population (3 out of 31: Table 6.3), with the frequency of parasitism of Leptothorax by H.sublaevis (23 out of 306 colonies parasitized: Table 6.1) in the principal mapping areas (area 3 in 1985, area 5 in 1986). These frequencies were not significantly different (Two tailed Fisher's exact test, p=0.87). To have detected significant aggregation of H.sublaevis colonies on the basis of these sample sizes would have required finding 6 of 31 H.sublaevis colonies with others for nearest neighbours (One tailed Fisher's exact p=0.04). But greater than expected separation of slave-maker colonies (overdispersion) could not have been detected in this sample, since the one tailed Fisher's exact probability if none of the 31 colonies had a slave-maker nearest neighbour=0.1. Hence the results suggested H.sublaevis colonies were not clumped in distribution, but may have been either randomly distributed or overdispersed.

On average, each H.sublaevis colony had nearly 4 Leptothorax colonies within 2m. of it (range 0-16) (Table 6.3). In 13 mapped patches it was possible to measure and compare the distance from the focal slave-maker colony to its Leptothorax nearest neighbour (mean ±S.D.=83±37cm.), and the distance from that Leptothorax colony to its nearest *Leptothorax* neighbour (mean±S.D.=62±35cm.) (Table 6.3). Analysis of these results showed *Leptothorax* colonies were not significantly closer to each other than to *H.sublaevis* colonies (Paired t test, $t=1.393$, d.f.=12, $p>0.1$). *H.sublaevis* colonies did not appear to be surrounded by a *Leptothorax*-free area.

Comparison of the relative frequencies of *L.acervorum* and *L.muscorum* colonies derived from the mapping data and from the collecting scores considered previously revealed a strong discrepancy. In area 3 (1985), scoring recorded 124 *L.acervorum* to 81 *L.muscorum* colonies (Table 6.1). But when mapping in the same time and place I found 37 *L.acervorum* colonies to 51 *L.muscorum* (Table 6.3), a significant difference (X^2 corr.=7.73, d.f.=1, $p<0.01$). Two explanations exist for why mapping, unlike scoring, suggested *L.muscorum* to be commoner than *L.acervorum*. First, mapping might have detected *L.muscorum* colonies in small twigs overlooked during general collecting, if the diminutive *L.muscorum* nested in smaller twigs than *L.acervorum*. Second, *H.sublaevis* colonies (which were the focus of all maps) conceivably occurred in patches with above-average relative frequencies of *L.muscorum* colonies. The slave-makers perhaps favoured local areas with high densities of both *Leptothorax*, which might have been disproportionately populated by the less obtrusive *L.muscorum*. Which of these two explanations is correct is unknown. However, even if the first is true, this does not affect earlier calculations on the frequency of *L.muscorum* enslavement. For if in collecting I overlooked *L.muscorum* colonies, I must also have overlooked *L.muscorum* colonies parasitized by *H.sublaevis*, since free-living and enslaved colonies almost certainly occupied similar twigs. Hence the estimate of percentage infestation of *L.muscorum*, and the comparison of *L.muscorum* and *L.acervorum* parasitism rates

(Table 6.2), remain valid. But biased scoring could have meant that, contrary to the figures at the head of this Results section, L.muscorum was commoner than L.acervorum at the study site.

Reproductive status of Leptothorax queens from H.sublaevis colonies

Of 47 H.sublaevis colonies collected in June-July 1985, 28 (60%) contained dealate Leptothorax queens, and 19 (40%) contained none. In colonies where they occurred, such queens constituted 4.5% of the adult slave population. Their mean number was 6 per colony (range 1-21). Table 6.4 gives the results of ovarian dissections of 218 slave queens collected in 1985 and 1986. No queen was inseminated. Further, although 15(6.9%) had ovaries containing yolky eggs, the total absence of corpora lutea indicated none had laid any eggs. I conclude with Buschinger and Winter (1978) that Leptothorax queens are never reproductive inside H.sublaevis colonies.

Fifty dealate Leptothorax queens from the slave-maker nests belonged to the workerless inquiline species L.kutteri (see Ch.2). This ant is a relatively common parasite of L.acervorum in the Bröms-Kristianopel woods (Table 6.1). However, the lack of reproductive L.kutteri queens in H.sublaevis colonies suggested the inquiline does not parasitize enslaved nests. On the contrary, H.sublaevis queens must have usurped - or their workers raided - L.acervorum colonies harbouring L.kutteri, to account for non-laying L.kutteri queens appearing in slave-maker nests.

Analysis of H.sublaevis body size in relation to caste and host class

The results of the size measurements of H.sublaevis queens and workers are displayed as histograms in Figure 6.1. Basic statistics for individual colonies are given in Table 6.5, and the data summarized in Table 6.6.

(a) Variation in worker and queen size between host classes

The initial analysis presented in Table 6.7 (pairwise comparison of means of pooled data) suggests that *H.sublaevis* workers of the three host classes all differed significantly in pronotal width. The order of decreasing size - *L.acervorum* - reared *H.sublaevis*, mixed slave-reared *H.sublaevis*, *L.muscorum* - reared *H.sublaevis* - matched that predicted from slave size. In contrast to workers, *H.sublaevis* queens produced by different slaves did not have significantly different pronotal widths (Table 6.7). Very few had pronotal widths less than 1.9 graticule units (0.56mm.) (Fig.6.1). However, since colonies with *L.muscorum* slaves produced few sexuals (Table 5.1), the sample of *L.muscorum* - reared queens was very small. Colony (maternal) queens in mixed slave nests were significantly smaller than those in nests with *L.acervorum* slaves, which were of almost identical size to queens heading nests containing *L.muscorum* (Tables 6.6, 6.7).

The data were further examined by analysis of variance. I carried out a two-level nested ANOVA of the data from both workers and produced queens. In each analysis the higher-level classification was defined by host class, the lower by colony. Since sample sizes within colonies were unequal, in both cases the between host classes: between colonies variance ratio (F) was calculated using a synthesized between colonies mean square (Satterthwaite's approximation: Sokal and Rohlf 1969: 280). The statistics used in each ANOVA were those in Table 6.5, and the results are shown in Table 6.8. The results indicated significant pronotal width variation between host classes in workers but not in queens, confirming the earlier analysis with pooled data. They also revealed significant variation between colonies of the same host class in both workers and queens. Hence not all variation in slave-maker body size is explained by slave type.

The validity of these ANOVAs was called into question because in both castes pronotal width variances in individual colonies (Table 6.5) proved significantly heterogeneous. This was demonstrated with Bartlett's test (Sokal and Rohlf 1969:370) (Workers: $X^2=115.4$, d.f.=35, $p<0.001$; Queens: $X^2=49.3$, d.f.=18, $p<0.001$). Log. transformation did not remove this problem. However, ANOVA of the worker data excluding colonies with fewer than ten workers, and utilizing colony sample sizes which had been equalized by randomly picking ten widths from each remaining colony (with the net effect of homogenizing variances: $X^2=36.9$, d.f.=27, $p>0.05$), gave the previous results. As before, there was significant size variation between host classes, and between colonies within host classes. Hence, in the absence of comparable non-parametric tests with nested designs, the overall conclusions of the original ANOVAs seem justified.

Single classification ANOVA of colony queen size (Table 6.8) suggested there was no significant variation between colony queens from different host classes, in minor disagreement with the results from the earlier pairwise comparison of means from pooled data (see above and Table 6.7). Uniformity or near-uniformity of colony queen size was expected given the sizes of queens produced by each host class were also similar.

(b) Variation in worker and queen size between colonies: influence of slave number

The mean pronotal width of *H.sublaevis* workers in colonies with *L.acervorum* slaves increased with slave number (Fig.6.2) (Regression analysis with log. transformed slave number [derived from Table 5.1]

: $F=23.3$, $v_1=1$, $v_2=21$, $p<0.001$). There was also a positive relation between the sizes of L.acervorum -reared queens and slave number, but it was not significant ($F=0.98$, $v_1=1$, $v_2=13$, $p>0.25$). This lack of significance could have resulted from the relatively low number of colonies sampled, which also precluded seeking similar relationships in the other host classes. However, the conclusion that colonies with larger slave workforces tended to produce larger slave-maker females almost certainly accounts for the significant between colony variation in queen and worker body size detected in the ANOVAs.

(c) Comparison of worker and queen size

Within each host class, H.sublaevis queens were significantly larger than workers (pairwise comparison of means from pooled data [Table 6.6], all $d>5.1$, all $p<0.001$) (see also Fig.6.1). Queens and workers were closest in size in the L.acervorum host class, as expected since L.acervorum - reared workers were the largest. In fact, in this host class, queens and workers from just those colonies producing queens (n colonies=15: Table 6.5) were not significantly different in size (queen mean pronotal width ±S.D.=2.034±0.097 graticule units, n=280; worker mean ±S.D.=1.991±0.117, n=297 [Table 6.5]; this difference is less than the 0.05 measurement error). This was because queen-producing colonies tended to be those with many slaves (see Fig.6.2 and Ch.5, e.g. Fig.5.5) which, as reported above, also produced larger than average workers. Thus, within the L.acervorum host class, colonies with high slave populations produced queens and similarly-sized, large slave-maker workers, whereas small, unproductive colonies produced no queens and small workers.

(d) Comparison of colony and produced queen size

In two host classes (H.sublaevis with L.acervorum, H.sublaevis with

L.muscorum), colony queens were on average larger than produced queens (Table 6.6), although this difference was only significant in the L.acervorum host class ($d=3.08$, $p<0.01$). In the mixed slave host class, colony queens were smaller overall than produced queens, but this difference was not significant ($d=1.29$, $p>0.1$). The conclusion colony queens tended to be larger than produced queens matched the expectation that large size (indicating superior fighting ability) should favour queens at colony foundation.

(e) Size and ovariole number

The ovariole number of H.sublaevis females ranged from 2 to 10, the modal number being 6 or 3 per ovary (Fig.6.3). However, the frequency distributions of ovariole number for the different castes and host classes paralleled the size-frequency distributions (compare Figs.6.1 and 6.3). Ovariole number was therefore positively related to body size, so that queens had most ovarioles on average and L.muscorum - reared workers least.

Genetic status of H.sublaevis host classes

The mean allele frequencies in each host class were calculated from the electrophoretic data in Table 5.4 and are displayed in Table 6.9. At each locus, the frequency of the commonest allele was higher in one host class than in the others. Thus Me 100 was particularly common in the mixed slave host class, and Mdh-2 100 in colonies with L.muscorum slaves. In fact the rarer Mdh-2 allele (97) was totally absent in L.muscorum-enslaving H.sublaevis : all slave-makers in this host class were homozygous for Mdh-2 100 (Tables 5.4, 6.9).

At first sight these unequal allele frequencies suggested there was genetic differentiation between host classes. But from further analysis I concluded they instead reflected chance variation due to the relatively low numbers of colonies analyzed in the mixed slave and L.muscorum host classes (7 and 9 respectively : Table 6.9). Thus, the proportion of colonies with Mdh-2 97 in the L.muscorum host class (0 out of 9) proved to be not significantly different from the proportion of colonies with this allele in the L.acervorum host class (8 out of 31: Table 5.4) (Two tailed Fisher's exact test, p=0.21). In addition, calculation of Nei's (1972) genetic distance between each of the three host classes - a measure comparing allele frequency differences at both loci simultaneously - indicated overwhelming genetic identity between them (Table 6.10). The conclusion that genetic differentiation between sympatric host classes was lacking in H.sublaevis was also easiest to reconcile with the finding that, for alleles at both loci, individual host classes and the whole population were in Hardy-Weinberg equilibrium. This was demonstrated in the previous chapter when calculating inbreeding coefficients (Table 5.5), since inbreeding coefficients of or around zero indicate observed heterozygosities equal or approximately equal to heterozygosities expected on the basis of Hardy-Weinberg equilibrium (see Ch.5, Methods).

Discussion

Frequency of enslavement and the lack of anti-slavery defences in Leptothorax

In the Bröms-Kristianopel population the percentage of Leptothorax colonies parasitized by H.sublaevis was 7.6% in L.acervorum, and 3.4% in L.muscorum. Figures of 12.9% (Sturtevant 1927) and 6.3% (Wesson 1939) were reported for the parasitism rate of L.curvispinosus by H.americanus. The percentage of Leptothorax nests locally affected by both slave-maker species must in fact have been higher, since every enslaved colony launches raids on one or more unparasitized neighbours.

In later discussion I consider reasons why L.acervorum was parasitized more frequently than L.muscorum. Here I wish to discuss, in the light of the above rates of parasitism, the absence of anti-slavery defences in Leptothorax. Despite the reproductive damage they incur under slavery, all the Leptothorax hosts of H.sublaevis apparently lack specific, adaptive defences against enslavement. That is, they have no defences to distinguish them from unenslaved species. When attacked by raiding H.sublaevis, Leptothorax workers sting and bite, but this defence is clearly imperfect and is identical to the workers' response to other Leptothorax in territorial disputes. In addition, Leptothorax workers have no resistance to a chemical produced by raiding H.sublaevis which subverts their nestmate recognition system and causes them to attack each other (see Ch.7). Further, after capture as brood, Leptothorax workers emerging in slave-maker nests perform work~~er~~ for their captors, yet conceivably they could instead rebel and kill all the slave-maker brood in their charge. All these reasons support the conclusion the Leptothorax slave species have failed to develop specific defences or retaliatory measures against slave-makers, and this requires explanation.

Dawkins (1982:72) explained the absence of slave mutinies as follows. Suppose a gene for mutiny arose and slave workers successfully rebelled. Being sterile they could not transmit the gene and thereby further its spread. So, in principle, mutinous behaviour cannot evolve in slave species. However, this argument is undermined by the fact workers of most slave species (e.g. Leptothorax and Formica spp.), like those of H.sublaevis and many other ants, are not totally sterile. They are instead capable of reproduction by parthenogenetic male production (see Ch.8, especially Table 8.1). This possibility also upsets Gladstone's (1981) contention that slaves do not rebel because they have no alternative to behaving slavishly. The reason slaves remain in servitude more probably stems from a developmental than from an evolutionary constraint, involving imprinting. Experiments show most ant workers cannot innately recognize their colony - specific odour, but learn it through imprinting as young adults (Jaisson 1985). This system is clearly exploited by slave-makers and other ant social parasites (Le Moli 1980, Jaisson 1985, Ch.2). It almost certainly constitutes a formidable obstacle to the evolution of the ability in slaves to appreciate their condition.

However, the constraint of imprinting is not in principle insurmountable, and explanation is still required for why slave species have no effective defences against the initial attacks of the slave-maker queen or workers. The fundamental reason for slaves' vulnerability to slave-makers appears to be the "rare enemy effect", also proposed by Dawkins (1982:Ch.4). He argued that in predator-prey or host-parasite systems the prey or host lineage will inescapably lose the evolutionary "arms race" of adaptation and counter-adaptation (Dawkins and Krebs 1979) if its opponent is rare. For counter-adaptations are not worth their cost to any single host or prey lineage if the probability of attack is very low. Unfortunately, no formal models exist of this intuitively convincing effect. But in the Leptothorax-H.sublaevis system, the percentage chance of any one Leptothorax lineage encountering slave-makers is probably far smaller than indicated by local frequencies of parasitism. This is because Leptothorax populations are more widespread than those of their parasites. Hence the rare enemy effect, combined with developmental barriers to slaves' recognizing their predicament, is the best explanation for the otherwise puzzling lack of anti-slavery adaptations in slave species. The arms race between H.sublaevis and its Leptothorax hosts appears to have a permanently asymmetric outcome in favour of the slave-makers.

Distribution of H.sublaevis colonies, raiding policy, and effects on Leptothorax colony distribution

H.sublaevis colonies were distributed randomly, or possibly were overdispersed, among the Leptothorax colony population. The evident absence of aggregation was interesting given the wingless queens of H.sublaevis must disperse for colony foundation from as many centres as there are colonies. By contrast, most ant queens mate aerially, so their distribution prior to colony foundation does not reflect the existing colony distribution, but is instead determined by where each mated queen chances to land. Therefore the observed distribution of H.sublaevis colonies suggests young slave-maker queens deliberately leave their home neighbourhood. If queens usurped the first Leptothorax colony they were likely to encounter, many more slave-maker colonies would have occurred near others.

The reasons encouraging foundress slave-maker queens to disperse all involve the avoidance of intra-specific competition. As described in chapter 3, evidence exists that H.sublaevis queens may attempt to usurp other queens in newly-founded colonies. In addition, incipient slave-maker colonies are probably vulnerable to raids from their queen's maternal colony, if founded too close. Buschinger and Alloway (1977) described the apparent aftermath of such an occurrence in H.americanus. In fact, in most ants established colonies are hostile to nearby conspecific foundation attempts (Wilson 1971:453).

In the slave-maker Polyergus breviceps, which raids over large distances, even mature colonies were attacked by other Polyergus (Topoff, LaMon, Goodloe and Goldstein 1984). But in H.sublaevis mature colonies probably rarely occur within mutual raiding distance, because colonies cannot raid far (see below) and the younger colony could not become established. Thus, the three H.sublaevis colonies with slave-maker nearest neighbours in Table 6.3 were either incipient or small (max. 5 workers). Moreover, the genetic data in chapter 5 indicating all H.sublaevis workers within single colonies were full siblings (possible exceptions - i.e. colonies S 85 96 and S 84 73 - numbered just 2 out of 49 colonies), provided positive evidence against intra-specific raids in H.sublaevis, at least as a regular occurrence. In sum, the distribution of H.sublaevis colonies almost certainly reflected strong intra-specific competition, but between queens or colonies and queens, rather than between mature colonies.

The effect of H.sublaevis on Leptothorax colony distribution remains unclear. No evidence was found for the existence of Leptothorax-free regions around H.sublaevis nests. For comparison, Yasuno (1964) found that colonies of the slave-maker Polyergus samurai cleared their surroundings of slave species nests, although the host colonies reoccupied the empty space each autumn after raids had ceased. In H.sublaevis it is also unknown how far colonies raid in nature. However, in July 1985 I encountered two apparent instances of raids in progress in the field. The distances between each slave-maker colony and its suspected victim were respectively 113 and 214 cm.. These figures fit expectation. It seems unlikely that H.sublaevis workers could orient effectively over more than about 3m., for reasons involving their small size, the roughness of the leaf-litter terrain, and the relative inefficiency of recruitment in pairs (tandem recruitment : see Ch.3).

Even supposing *H.sublaevis* workers raid no further than 3m., the mapping results indicated prospective *Leptothorax* targets were rarely inaccessible. On average, slave-maker colonies lay within 2m. of nearly 4 *Leptothorax* colonies. Further, unlike the large slave-makers such as *Polyergus*, *H.sublaevis* probably do not raid multiply each season. In 1984 I compared the number of *L.acervorum* worker pupae found in unparasitized *L.acervorum* nests (collected in July and censused in August) (mean ±S.D.=20.7±14.5 pupae per colony, n=51 colonies), with the number simultaneously occurring in *H.sublaevis* nests containing *L.acervorum* slaves (mean ±S.D.=26.8±23.9, n=20). These means were not significantly different (d test with log. transformed data, d= 0.36, $p>0.1$). Although this comparison was obviously imperfect, since it ignored brood development, the observed quantity of captured brood suggested *H.sublaevis* colonies raid only a low number of *Leptothorax* nests each summer.

Slave-maker colonies face a variant of the widespread central place foraging problem - how to optimize resource utilization from a fixed centre (Orians and Pearson 1979) - with respect to their slave supply. I conclude from the arguments just presented that most *H.sublaevis* colonies could live permanently in one nest-site, rather than be periodically obliged to emigrate to find fresh slaves. The apparently low rate at which they deplete their surroundings of slaves is probably more than adequately balanced by *Leptothorax* colonies repopulating the vacant territory.

Such repopulation probably occurs through the immigration of entire *Leptothorax* colonies or sizeable colony fragments. *Leptothorax* colonies exhibit an efficient and stereotyped nest relocation behaviour (Möglich 1978), and in the Bröms-Kristianopel woods the available Leptothorax habitat appeared saturated, implying vacant nest-sites were quickly exploited. Further, incipient Leptothorax colonies were notably rare, suggesting colony reproduction regularly occurred through splitting as in other polygynous species. Alternatively, raided colonies may often escape total destruction, and themselves reoccupy their previous nest-sites (Buschinger, Ehrhardt and Winter 1980:260). Hence H.sublaevis colonies are probably usually close to host colonies with substantial brood supplies. Nevertheless, the need to relocate is presumably occasionally unavoidable. Such a requirement could explain observations in other slave-maker species of raids which end with part or all of the slave-maker colony migrating to the victim's nest-site (e.g. H.americanus, Wesson 1939; L.duloticus, Alloway 1979; H.canadensis, Stuart and Alloway 1983), or records of slave-makers emigrating to wholly new sites (e.g. Polyergus lucidus, Kwait and Topoff 1983), a behaviour probably shared with free-living ant species when short of resources (Smallwood 1982). But, to summarize my conclusion concerning the effects of H.sublaevis on the colony distribution of its hosts, the slave-makers' impact seems to be generally short-term and local.

Slave sterility, the treatment of captured pupae, and "husbandry"

I found all dealate Leptothorax queens in H.sublaevis colonies to be unmated and non-reproductive. Buschinger and Winter (1978) reported identical results from 484 dissections of enslaved queens, except that some L.muscorum queens in their sample (though still non-laying) were inseminated, having undoubtedly returned to their captors' nests after mating close by. (L.muscorum is a species in which queens "call" and mate on the ground: see Ch.3). The sterility of enslaved Leptothorax queens is suggestive, because both L.acervorum and L.muscorum are facultatively polygynous species in whose colonies multiple, egg-laying queens regularly coexist (Buschinger 1968c). Further, in free-living L.acervorum from Swedish populations, such queens are sometimes unmated. In dissections, P.Douwes (unpublished observations) found two layers among 29 naturally occurring unmated queens. This frequency (2 out of 29), though low, is significantly higher than the frequency of laying, unmated queens in the Bröms-Kristianopel H.sublaevis colonies (0 out of 108: see Table 6.4) (One tailed Fisher's exact test, p=0.04). This finding suggests H.sublaevis deliberately suppresses reproduction by captured Leptothorax queens, presumably pheromonally. Slave reproduction would divert resources away from slave-maker brood, since the Leptothorax queens could probably not be prevented from producing sexuals.

Leptothorax slave workers, like queens, are almost certainly never reproductive in slave-maker colonies. I have no dissection evidence, but in nearly 140 hours of observations of H.sublaevis nests, the only egg I saw laid by a slave worker appeared non-viable and was immediately eaten by a slave-maker and another slave (see Ch.4, Discussion). In addition, Leptothorax males (conceivably worker progeny) rarely occurred in slave-maker nests. Only 10 out of 92 colonies (10.9%) censused in their entirety (1983-1986) contained such males (nine per colony on average). The sporadic occurrence of these males is best explained if they originated from captured brood (see below). The extent of male production by free-living workers of the Leptothorax hosts of H.sublaevis is unknown (see Ch.8). However, if workers are commonly reproductive in these species, their sterility when enslaved must (as in slave queens) be enforced by their slave-maker captors.

Since none reproduce, all adult *Leptothorax* in slave-maker colonies must derive from captured brood. When such brood ecloses, interesting differences arise in the treatment the emerging *Leptothorax* workers, queens and males receive from the slave-makers. Workers are evidently left to function as slaves as their behavioural pre-programming dictates. If necessary, any tendency they have to produce males is suppressed, as just discussed. Emerging *Leptothorax* queens have their wings gnawed off by the slave-makers (Buschinger, Ehrhardt and Winter 1980:257), accounting for their habitual dealate condition. These queens are also prevented from reproducing, and seemingly make useful slaves like the workers. Otherwise, their toleration by the slave-makers is curious. Some *Leptothorax* males eclosing in *H.sublaevis* nests must survive for the duration of their brief adult life-span (c.14 days) unmolested, since intact *Leptothorax* males occasionally occurred among slave-makers (see above). But observations suggest that others are destroyed by the slave-makers as soon as they eclose. Slave-makers can therefore apparently only discriminate between *Leptothorax* and their own morphologically extremely similar males when the males reach adulthood, suggesting species-specific odours are only expressed by adults among these ants (as mentioned in Ch.3). Since leptothoracine males perform no work, their destruction by the slave-makers is probably adaptive.

The treatment of slave sexuals by *H.sublaevis* contrasts strongly with that described by Alloway (1979) in *H.americanus*. This slave-maker allowed recently emerged alate slave sexuals to leave the nest unharmed. Alloway proposed *H.americanus* was thereby "husbanding" its slave resources, by ensuring the survival of the progenitors of its future stock of slave colonies. But there could only be selection for such behaviour in slave-makers if released queens generally founded colonies near the nest which released them, so benefitting that colony most. Yet, despite Alloway's (1979) opposite speculation, there is no evidence the queens behave this way. Further, the possibility seems intrinsically unlikely, since selection would presumably act strongly on the freed queens to found colonies in safety elsewhere. Hence the release of host sexuals by *H.americanus* is probably not adaptive in the way Alloway suggested. Other explanations for the habit are required. For similar reasons - the unlikelihood such practices would preferentially benefit their originators - occurrences of "husbandry" appear improbable throughout the slave-making ants.

Body size, host class, caste determination and productivity in H.sublaevis

Worker size in *H.sublaevis* was influenced by both slave type and colony size. Small slaves raised small slave-maker workers and within host classes, worker size was also reduced by low slave numbers. Similar results with regard to host class were reported by Buschinger and Winter (1975) in a comparison of 100 *L.acervorum*- and 50 *L.muscorum* - reared *H.sublaevis* workers. The findings suggest *H.sublaevis* worker size was strongly influenced by resource acquisition.

By contrast with the situation in workers, *H.sublaevis* queen size appeared independent of host class. Queens were similar in size whatever slaves raised them. In addition, queens were larger than workers of corresponding host classes, as also found by Buschinger and Winter (1975). These results imply only female larvae which pass a certain size threshold (corresponding to an adult pronotal width of c.1.9 graticule units, or 0.56mm.: Fig.6.1) can become queens. This requirement, if true, matches similar thresholds found elsewhere in social insects (Wheeler 1986). The finding does not contradict the discovery of a genetic influence on caste in German *H.sublaevis* (Buschinger 1978 a, Winter and Buschinger 1986) because, as discussed in chapter 3, all Swedish *H.sublaevis* females appear to be homozygous for one of the alleles (E) at the caste-biasing locus. So in Swedish *H.sublaevis* the genetic variation which is the partial basis of caste determination in German populations is absent. Hence caste in the Swedish ants must be environmentally determined, as here suggested.

H.sublaevis queens were larger than workers for reasons presumably involving selection on queens for successful non-independent colony foundation and for greater fertility than workers. The advantage of large size to colony foundresses was demonstrated within the queen caste by the finding maternal queens tended to be larger than virgin queens. The reason why large size would confer greater fertility on queens stems from the positive association found to exist between body size and ovariolar number. Thus queens tended to have more ovarioles than workers, and large workers more than small ones. This worker-queen difference again matches findings of Buschinger and Winter (1978).

Although *H.sublaevis* colonies with a mixed slave population, or with *L.muscorum* slaves exclusively, produced smaller workers, they did not appear to fall below colonies in the *L.acervorum* host class on the 1985 population production curve (Fig.5.4). In other words, as mentioned in chapter 5 (Results), these colonies apparently yielded a biomass of new slave-maker production in the same proportion to their slave population sizes as the L.acervorum-enslaving H.sublaevis. However, the sample of colonies with L.muscorum slaves for which production data exist was small. But despite not disadvantaging colonies in terms of total biomass produced, slave-type arguably did affect the kind of slave-maker produced. Colonies with L.muscorum slaves had a seemingly abnormally low output of H.sublaevis sexuals (see Table 5.1). At the end of this chapter, I return to this point when discussing whether L.muscorum is in fact an inferior slave species.

Host race formation in slave-making ants

The host classes of H.sublaevis were probably not genetically distinct, since the allele frequency differences between them could be explained by chance variation due to small sample sizes. However, by the same token, such genetic differentiation cannot be conclusively ruled out. A study of electrophoretic variation over numerous loci, in several populations, including more colonies per host class, would be required to settle the question definitively.

The reason such an investigation would be worth pursuing concerns the mode of speciation in taxa of slave-making ants. As explained in chapter 2 in the context of the origin of social parasites (here I am concerned with their radiation), sympatric speciation is held to be rarer than speciation in the allopatric mode, because of the difficulty of seeing how co-existing populations could attain reproductive isolation (White 1978). But, as mentioned in the Introduction, evidence exists that organisms (e.g. parasites) living closely with other organisms may be exceptionally prone to sympatric speciation through host race formation (Bush 1975, Diehl and Bush 1984, White 1978). In this process competition for hosts causes the parasite population to subdivide into host races, which by selection against non-specialists then achieve genetic isolation. As also mentioned previously, slave-making ants, with their several hosts, seem likely candidates for speciation through host race formation. In fact, preliminary evidence for host specificity among slave-makers - a precondition of the process - has recently been found by Goodloe and Sanwald (1985) in choice experiments with foundress queens of the slave-maker *Polyergus lucidus*. Further, again as stated earlier, members of the socially parasitic ant genus *Epimyrma* closely resemble the expected end-product of speciation by this route.

The genus *Epimyrma* is known in unusual and fascinating detail (see Buschinger and Winter 1982,1983,1985, Winter and Buschinger 1983, Jessen 1986). It is monophyletic, and consists entirely of slave-making, "degenerate" slave-making (see below), and workerless inquiline representatives. Several species are sympatric in Southern Europe and most - apart from the slave-makers - have only one host each. The host species are not particularly closely related to each other, suggesting that successive allopatric speciation of an ancestral host-parasite pair has not occurred. Moreover, the life-history strategies of the different *Epimyrma* appear to be linked in an evolutionary sequence. All the inquilines are of the kind which (like slave-makers) kill host queens (see Ch.2). The "degenerate" slave-makers are slave-makers with reduced numbers of workers which, though capable of raiding, rarely raid in the field. Hence they appear intermediate between the obligate slave-maker and the inquiline members of the genus.

I therefore speculate that speciation in *Epimyrma* could have occurred by subpopulations of an ancestral parasitic form specializing upon different hosts, and eventually forming new species. At the same time, this radiation was accompanied by a divergence in life-history strategies. Such divergence could have stemmed from the nature of the new host species. For example, a relatively sparsely distributed host might enforce a "big bang" life history policy on its parasites, involving exclusively sexual production in the inquiline manner. Conceivably, the process of speciation and life history divergence in Epimyrma occurred allopatrically, but a more parsimonious explanation of the present day overlapping distributions of some Epimyrma is that it came about sympatrically through host race formation. Further work on host specificity, geographical occurrence, and genetic variation in Epimyrma, could be used to test these ideas.

General discussion

I now conclude this chapter with a general discussion of the influence of H.sublaevis and its Leptothorax hosts on one another. Since the H.sublaevis slave-makers in the Bröms-Kristianopel woods most probably constituted a single population with two principal host species, as a preliminary I first consider possible reasons guiding the slave-makers' choice of host, and the consequences of such decisions.

As earlier stated, the data suggested L.acervorum was parasitized at a higher frequency than L.muscorum. Since the larger L.acervorum is unlikely to be easier to enslave than L.muscorum, this finding implies L.acervorum was the slave-makers' preferred host. The larger size of L.acervorum workers was in fact almost certainly the reason for this preference. As the results showed, L.acervorum slaves produced large slave-maker workers, which presumably made better, more combative raiders. In addition, as I discuss later, L.acervorum-enslaved colonies appeared to achieve better sexual output than colonies with L.muscorum slaves. In other words, H.sublaevis probably preferred to enslave L.acervorum because it was the superior host. But why then was L.muscorum parasitized at all?

There could be several reasons. First, as already indicated, L.muscorum colonies were perhaps easier for slave-maker queens to usurp. This advantage could have partially offset the apparently reduced sexual productivity of mature H.sublaevis colonies with L.muscorum slaves (see below). But if L.muscorum nests were more vulnerable to usurpation, their frequency of enslavement should have exceeded that of L.acervorum, unless colonies founded in L.muscorum nests later changed host classes. Yet, as I explain below, evidence such a transition regularly occurred is not strong. Therefore ease of takeover alone does not seem to explain the choice of L.muscorum as hosts. A second possible reason involves intra-specific competition for L.acervorum nests, which could have driven some slave-maker queens to turn to L.muscorum. But the low overall frequencies of parasitism of both species indicate such competition was not sufficiently strong, except perhaps locally. Thirdly, searching costs could have constituted another reason for the enslavement of L.muscorum. Since slave-maker queens were presumably at risk the longer they spent seeking colonies to usurp, they might have been selected to enter L.muscorum nests if L.acervorum proved difficult to find. Selection on H.sublaevis queens to parasitize any potential host colony rather than none seems the best explanation for their attacking a seemingly inferior host.

Conceivably, slave-maker queens attacked L.muscorum colonies because they were easier to overpower, and colonies thus initiated then later acquired the more prized L.acervorum slaves. Mixed slave colonies therefore represented a transition between young *L.muscorum*-enslaving colonies, and mature colonies with *L.acervorum* slaves. Data on the colony age structure of the host classes partly supported this idea. Thus, among the 1985 slave-makers (see Table 5.1), 2 out of 7 *L.muscorum*-enslaving colonies were incipient, compared with 1 out of 30 colonies in the *L.acervorum* host class. Further, none of 5 non-incipient *L.muscorum*-enslaving colonies had lost their queen, in contrast to 11 out of 29 such colonies with *L.acervorum* slaves. However, neither of these frequency differences was significant (One tailed Fisher's exact tests, p=0.17 and 0.12 respectively). Hence there is no conclusive evidence against the idea some *L.muscorum*-enslaving colonies could have persisted in the *L.muscorum* host class for life.

This being so, it is interesting to examine the consequences of enslaving *L.muscorum* for *H.sublaevis*. These were evidently small worker size and, less clearly, reduced sexual output. Table 5.1 shows that the four 1985 *L.muscorum*-enslaving colonies for which production data exist together produced just 6 slave-maker queens and 8 males. However, the small number of colonies in the sample makes it difficult to tell whether these colonies simply yielded the sexual production expected in colonies of their size. For the same reason, it is hard to say whether the lack of larger, productive colonies in the host class was a chance omission in collecting, or a genuine feature. But, arguably, the small *L.muscorum* slaves were hard pushed to rear sexual slave-maker brood, for example to raise female larvae beyond the size threshold for queen development (see earlier discussion). If so, *L.muscorum* was a greatly inferior host.

Interestingly, the small slave-maker worker size characteristic of the L.muscorum host class appeared to have adverse consequences for worker fertility. Only one L.muscorum-raised slave-maker worker in the 1985 collection was fertile (Table 5.1), although the small size of the host class sample again prevented drawing definite conclusions from this. This proposed consequence of worker size did not contradict the finding in chapter 5 (Table 5.3) that within the L.acervorum and mixed slave host classes sterile workers were no smaller than fertile ones, since all workers in these host classes were on average larger than those reared by L.muscorum. In addition, small body size in L.muscorum - reared workers certainly placed a lower limit on their potential fecundity, because compared to their L.acervorum-reared counterparts these workers had fewer ovarioles, some as few as two (Fig.6.3). Hence, overall, enslaving L.muscorum did appear to have a negative effect on the reproductive capabilities of H.sublaevis workers. Given this, their behaviour must also have been affected, since dominance activity in H.sublaevis workers is tightly correlated with their degree of ovarian development (see Ch.4).

I now summarize the reciprocal influences of H.sublaevis and its Leptothorax slaves on each other. At the level of individual behaviour, H.sublaevis is a powerful manipulator of its hosts. Admittedly, the slave-makers do not need to force their hosts to act as slaves, but rather exploit the pre-existing inclination of the Leptothorax ants to work for the colony in which they eclose. All the slave-makers must do is substitute their colony for the Leptothorax colony while their future slaves are still brood. Direct manipulation occurs when on raids the slave-makers disrupt their opponents' nestmate recognition system with chemical weaponry (see next chapter) and when, following capture, they prevent the

Leptothorax from laying eggs. Conceivably, the transfer of fluid substances from slave-maker abdominal tip to slave ("abdominal trophallaxis" : see Ch.4, Discussion) observed by Stuart (1981) in *H.americanus* is implicated in ovarian inhibition of slaves in this species. Alloway (1982) proposed an interesting additional aspect of its slaves' behaviour which *H.americanus* may influence. He found evidence that the North American *Leptothorax* hosts of *H.americanus* rejected fewer *Leptothorax* pupae from other colonies when enslaved than when free-living. Similar observations were made in the hosts of *H.canadensis* (Stuart and Alloway 1983). But the mechanism by which slave-makers could enhance pupa - acceptance by slaves remains unclear. Turning to colony level, all slave-makers, including *H.sublaevis*, clearly have an extremely adverse effect on their hosts. Both usurpation by *H.sublaevis* queens and attack by workers must destroy a *Leptothorax* colony's reproductive chances, unless sufficient members manage to escape. But as far as the *Leptothorax* population is concerned, *H.sublaevis* has little effect. As previously discussed, the slave-makers appear too rare.

Conversely, *H.sublaevis* is evidently influenced by the nature of its hosts. These influences are all accidental in the sense that they are not brought about by counter-adaptations to enslavement in *Leptothorax*, but rather stem from the necessary intimacy of the host-parasite relationship. Thus, when *L.muscorum* is chosen as host, the *L.muscorum* workers rear undersize *H.sublaevis* workers with apparently diminished reproductive potential and hence, conceivably, reduced dominance behaviour. *L.muscorum*-enslaving colonies also appear to suffer a depressed sexual output, meaning lower fitness for all members. *H.americanus* again provides an illuminating extra example in this context. Alloway and Del Rio Pesado (1983) inferred that the habit in *H.americanus* colonies of occupying multiple nest sites (polydomy) was imposed on the slave-makers by their hosts, which in a free-living state are routinely polydomous. *H.americanus* would arguably function more efficiently as a slave-maker if colonies tended to centralization, because this would allow more rapid recruitment of a raiding force. Since *H.americanus* is monogynous, polydomy also allows the establishment of worker-only groups free from queen inhibition, which in turn could facilitate the habit of worker reproduction and dominance behaviour also found in *H.americanus* (Franks and Scovell 1983; Ch.4, Ch.8). In other words, a relatively minor detail of the hosts' biology may have important consequences for that of its parasite. Returning to *H.sublaevis*, conclusive evidence was lacking that the slave-maker population underwent genetic subdivision into host races. Yet elsewhere among slave-makers, in the genus *Epimyrma*, such a process could have occurred, even resulting in the diversification of life history strategies and speciation.

In conclusion, the evidence indicates that *H.sublaevis*, by its comparative rarity, is the outright winner in a permanently asymmetrical evolutionary arms race against its *Leptothorax* hosts. But for the same reason, the slave-maker has no lasting effects on *Leptothorax* on a gross scale. On the contrary, the intimacy of the host-parasite relationship ironically renders *H.sublaevis* subject to various host influences, which may profoundly affect its future evolution.

Summary of Chapter 6

1. In the Bröms-Kristianopel population, 70% of H.sublaevis colonies contained L.acervorum slaves, 16% L.muscorum, 11% both these species, and 3% L.gredleri. The proportion of Leptothorax colonies parasitized was significantly higher in L.acervorum (7.6%) than in L.muscorum (3.4%). L.acervorum therefore appeared to be the preferred slave species, probably because its workers produced larger slave-maker workers and more sexuals.

2. H.sublaevis colonies were not aggregated but were distributed randomly, or possibly were overdispersed, among the population of Leptothorax colonies. This distribution arguably arose because young wingless slave-maker queens dispersed prior to colony foundation to avoid intra-specific competition. The mean maximum nearest neighbour distance between H.sublaevis nests was 6.6m.. Leptothorax colonies were not significantly closer to each other than to the slave-makers, suggesting H.sublaevis did not have long-term effects on their distribution. Correspondingly, it is unlikely slave-maker colonies were routinely obliged to emigrate to find fresh slave supplies.

3. Dealate Leptothorax queens occurred in 60% of H.sublaevis colonies. However, dissections showed none were inseminated or egg-laying. In fact, H.sublaevis appeared to deliberately suppress reproduction by enslaved queens. Leptothorax workers were also never reproductive in slave-maker nests. H.sublaevis is, in principle, unlikely to practice "husbandry" of its slave resources.

4. L.acervorum-reared H.sublaevis workers were larger than those from mixed slave colonies, which in turn were larger than workers raised by L.muscorum. This order matched that predicted from slave size. H.sublaevis worker size was also positively correlated with slave

number. The level of resource acquisition therefore strongly affects worker size in H.sublaevis. Queen size, by contrast, appeared uniform and hence independent of host class. In addition, queens were larger than workers from corresponding host classes. These findings suggested that a necessary condition for queen determination was that female larvae exceeded a certain size threshold. This could explain why L.muscorum-enslaving colonies appeared to have a low sexual output.

5. Colony queens tended to be larger than virgin queens, in agreement with the expectation that large size should have favoured queens at colony foundation. In both queens and workers, size was positively correlated with ovariolar number.

6. Allele frequency differences between host classes in H.sublaevis probably resulted from chance variation due to small sample sizes. Also, estimates of Nei's (1972) genetic distance between all host class pairs were very close to zero. Therefore no conclusive evidence was found for genetic differentiation of the slave-maker population into host races.

7. Colonies founded in L.muscorum nests suffered reduced sexual productivity compared to L.acervorum-parasitizing colonies. L.muscorum-reared slave-maker workers appeared to have less reproductive potential, as a result of their small size. L.muscorum was therefore arguably an inferior host species to L.acervorum, only parasitized when slave-maker queens failed to find L.acervorum.

8. H.sublaevis is in some ways a powerful manipulator of its hosts' behaviour. The Leptothorax slave species have probably failed to evolve specific anti-slavery devices because of the slave-makers'

comparative rarity. *H.sublaevis* therefore appears to be outright victor in a permanently asymmetrical arms race against its hosts. But, in common with other slave-makers, it is evidently subjected to various host influences, which could have far-reaching evolutionary consequences.

Table 6.1 Numbers of Leptothoracine colonies found in the Bröms-Kristianopel woods, Sweden, 1983-1986

		Host *Leptothorax*			*H.sublaevis*					*L.kutteri*
Area	Year	LA	LM	LG	HS+ LA	HS+ LM	HS+ LA+LM	HS+ LG	HS+ LM+LG	LA+ LK
1	1983	129	23	0	11	0	0	0	0	5
	1985	18	7	0	2	0	0	0	0	0
2	1984	130	57	0	10	4	0	0	0	8
	1985	49	24	0	3	1	0	0	0	2
3(A+B)	1985	124	81	10	11	2	2	0	1	3
4	1985	47	45	0	1	1	3	0	0	1
5	1985	13	22	1	5	0	0	0	0	0
	1986	37	28	3	2	2	2	1	0	5
	Tots.	547	287	14	45	10	7	1	1	24
							Grand total			936

Notes: a. Specific names are abbreviated as in Table 5.1, e.g. LA = *Leptothorax acervorum*. LK = *Leptothorax kutteri*, a workerless inquiline parasite of LA occurring in the Bröms-Kristianopel woods (see Ch.2).

b. Area names are as described in chapter 5 (Table 5.4, notes).

Table 6.2 Comparison of frequencies of parasitism of *Leptothorax acervorum* and *L.muscorum* by *H.sublaevis* (pooled scores)

	Total n colonies	n colonies (% of total)	
		Not parasitized	Parasitized
L.acervorum	592	547(92.4%)	45(7.6%)
L.muscorum	297	287(96.6%)	10(3.4%)

$X^2_{corr.}$ [with original scores] = 5.402, d.f.=1, $p<0.05$.

Table 6.3 Summary of Harpagoxenus sublaevis mapping data

Colony no.	Host class	Area	Nearest leptothoracine neighbour (NN)	Distance to NN (cm.)	Distance to NN'S nearest neighbour (cm)	No.LA colonies within 2m.	No.LM colonies within 2m.	No.LG colonies within 2m.
S 85 1	HS+LA	6	LA	213	-	0	0	0
2	HS+LA+LM	3	LA	86	126	1	0	0
4	HS+LA	3	LA	198	-	2	0	0
7	HS+LA	3	LM	41	37	1	2	0
9	HS+LA	3	LM	31	60	4	8	0
10	HS+LA	3	LM	53	50			
11	HS+LA	3	LA	59	17	4	11	1
12	HS+LM	3	LM	137	30	1	4	0
i13	HS+LM	3	LM	90	79	0	6	0
14	HS+LM+LG	3	LM	178	-	1	1	0
15	HS+LA	3	HS:S 85 16	117	-	2	3	0
16	HS+LM	3	LM	89	-			
17	HS+LA	3	LA	107	66	3	0	1
18	HS+LA	3	LM	124	38	1	3	1
22	HS+LA	3	LM	46	123	9	3	0
23	HS+LA	3	LM	95	26	0	4	0
24	HS+LA+LM	3	LA	139	-	1	0	3
26	HS+LA	3	LM	80	-	5	4	0
27	HS+LA	3	LM	107	-	1	2	0
29	HS+LA	3	LA	139	-	1	0	1
S 86 1	HS+LA+LM	5	HS:S 86 2	129	-	0	5	0
i2	HS+LM	5	HS:S 86 1	129	-			
5	HS+LM	5	LG	180	-	0	0	1
6	HS+LA	5	LM	140	69	1	4	0
12	HS+LA+LM	5	LA	183	-	1	0	0
13	HS+LA+LM	5	LM	335	-	0	0	0
17	HS+LM	5	LA	64	-	1	1	0
18	HS+LA	5	LM	67	83	1	1	0
19	HS+LA	5	LM	122	-	0	1	0
24	HS+LG	5	LG	230	-	0	0	0
25	HS+LA+LM	4	LM	145	-	0	2	0

Notes: a. Where the 2m. radius circles surrounding two HS colonies overlapped, e.g. as in S 85 9 and 10, the no. Leptothorax colonies given in the right hand columns are those in the resulting figure of eight-shaped area.

b. i = incipient colony

c. Other species found near HS colonies but not represented in this table included: Leptothorax (Myrafant) tuberum, Myrmica spp., Formica fusca, F.polyctena

Table 6.4 Reproductive status of dealate *Leptothorax* queens in *H.sublaevis* colonies

Leptothorax species	No. queens removed	No.colonies of origin	No.queens dissected	No. mated	No. with yolky eggs	No. with corpora lutea
L.acervorum	110	11	108	0	8	0
L.muscorum	45	11	44	0	1	0
L.gredleri	21	2	20	0	6	0
L.kutteri	50	5	46	0	0	0
		Tots.:	218	0	15	0

Notes: a. The queens were removed for dissection from 15 and 7 *H.sublaevis* colonies collected in June-July 1985 and 1986 respectively.

b. All queens with yolky eggs had a maximum one egg per queen.

Table 6.5   Mean female pronotal width in 36 *H. sublaevis* colonies

Workers						Queens					
Colony no.	Σx	Σx^2	n	$\bar{x}$	S.D.	Colony no.	Σx	Σx^2	n	$\bar{x}$	S.D
HS+LA n = 23											
S 85 1*+	101.55	211.088	49	2.072	0.114	S85 1	131.25	269.978	64	2.051	0.114
+ 4	115.5	223.06	60	1.925	0.111	4	36.75	71.148	19	1.934	0.060
+ * 15	77.05	152.613	39	1.976	0.101	15	7.65	14.633	4	1.913	0.025
+ 26	30.8	52.865	18	1.711	0.098	29	13.75	27.053	7	1.964	0.085
+ 29	21.6	42.52	11	1.964	0.103	41	56.9	115.79	28	2.032	0.077
+ 41	13.8	27.225	7	1.971	0.057	83	80.55	162.473	40	2.014	0.082
+ 42	45.35	82.313	25	1.814	0.045	11	35.65	74.788	17	2.097	0.041
* 49	3.55	6.313	2	1.775	0.106	17	21.4	45.81	10	2.14	0.039
+ * 83	23.3	45.35	12	1.942	0.100	18	100.1	204.755	49	2.043	0.074
+ 84	20.55	38.548	11	1.868	0.125	22	13.85	27.453	7	1.979	0.091
+ * 11	39.75	83.218	19	2.092	0.056	96	3.8	7.24	2	1.9	0.141
+ 17	24.85	51.503	12	2.071	0.062	37	4	8	2	2	0
+ * 18	38.7	79.015	19	2.037	0.103	71	28	56.055	14	2	0.065
* 22	7.95	15.833	4	1.988	0.103	82	4	8.005	2	2	0.071
96	3.9	7.625	2	1.95	0.141	97	32	68.375	15	2.133	0.088
* 10	89.25	169.818	47	1.899	0.086						
* 37	12.4	22.04	7	1.771	0.111						
* 40	21.45	38.413	12	1.788	0.080						
* 48	5.45	9.913	3	1.817	0.076						
71	81.25	161.218	41	1.982	0.071						
* 74	37.25	69.463	20	1.863	0.067						
82	3.9	7.61	2	1.95	0.071						
* 97	25.8	51.33	13	1.985	0.103						
Tots.	844.95	1648.894	435			Tots.	569.65	1161.556	280		
HS+LA+LM n = 7											
S 85 2+	23.6	46.575	12	1.967	0.121	S85 2	4.3	9.25	2	2.15	0.071
+ * 24	72.7	132.405	40	1.818	0.084	24	3.8	7.22	2	1.9	0
+ 47	20	36.545	11	1.818	0.135	54	11.9	23.67	6	1.983	0.117
* 54	38.5	67.695	22	1.75	0.123	60	15.8	31.23	8	1.975	0.060
+ * 60	54.3	98.895	30	1.81	0.145	62	77.75	163.568	37	2.101	0.072
+ * 62	85.1	165.165	44	1.934	0.116	68	3.75	7.033	2	1.875	0.035
+ * 68	48.35	93.928	25	1.934	0.132						
Tots.	342.55	641.208	184			Tots.	117.3	241.971	57		
HS+LM n = 6											
S 85 12*	99.5	172.055	58	1.716	0.155	S85 53	9.9	19.645	5	1.98	0.104
* 13	12.05	20.843	7	1.721	0.129						
* 16	31.6	59.145	17	1.859	0.159						
* 38	32.2	51.935	20	1.61	0.070						
* 53	53.15	97.893	29	1.833	0.131						
+ * 61	55.95	95.273	33	1.695	0.113						
Tots.	284.45	497.144	164			Tots.	9.9	19.645	5		

+ = colony with fertile workers (see Table 5.1).

* = colony in which colony (maternal) queen size also measured (+ S 85 72, HS+LM).

Table 6.6 Mean pronotal width of H.sublaevis females from different host classes (pooled colony data)

Host class	Workers				Queens				Colony queens			
	n_i	n_c	$\bar{x}$	S.D.	n_i	n_c	$\bar{x}$	S.D.	n_i	n_c	$\bar{x}$	S.D.
HS+LA	435	23	1.942	0.133	280	15	2.034	0.097	13	13	2.150	0.134
			0.571	0.039			0.598	0.029			0.632	0.039
HS+LA+LM	184	7	1.862	0.138	57	6	2.058	0.102	5	5	1.990	0.114
			0.547	0.041			0.605	0.030			0.585	0.034
HS+LM	164	6	1.735	0.152	5	1	1.980	0.104	7	7	2.136	0.141
			0.510	0.045			0.582	0.031			0.628	0.041

Notes: a. n_i = no. individuals measured, n_c = no. colonies of origin.

b. In each host class the table shows the mean ($\bar{x}$) and standard deviation (S.D.) pronotal width in graticule units (upper row) and millimetres (lower row). 1 graticule unit = 0.294 mm. Measurements were made to the nearest 0.05 graticule unit (0.015 mm.).

Table 6.7 Results of comparing pronotal widths of H.sublaevis from different host classes, using pooled data (d or t-test)

Workers (d)

	HS+LA	HS+LA+LM	HS+LM
HS+LA	___	6.663***	15.355***
HS+LA+LM		___	8.124***
HS+LM			___

Queens (d)

	HS+LA	HS+LA+LM	HS+LM
HS+LA	___	1.633	1.152
HS+LA+LM		___	1.610
HS+LM			___

Colony queens (t (d.f.))

	HS+LA	HS+LA+LM	HS+LM
HS+LA	___	2.354(16)*	0.224(18)
HS+LA+LM		___	1.905(10)
HS+LM			___

Notes: *** = $p<0.001$

* = $p<0.05$

No asterisk(s) = no significant difference

Table 6.8 Results of comparing pronotal widths of H.sublaevis from different host classes (ANOVA)

Workers	Source of variation	d.f.	SS	MS	F
	Between host classes	2	5.23	2.615	9.164^{***a}
	Between colonies within host classes	33	5.911	0.179	14.917***
	Within colonies	747	9.008	0.012	
	Total	782	20.149		

Queens [HS+LM omitted]	Source of variation	d.f.	SS	MS	F
	Between host classes	1	0.026	0.0260	0.272^{a}
	Between colonies within host classes	19	1.019	0.0536	7.768***
	Within colonies	316	2.184	0.0069	
	Total	336	3.229		

Colony queens	Source of variation	d.f.	SS	MS	F
	Between host classes	2	0.0970	0.0485	2.771
	Within host classes	22	0.3856	0.0175	
	Total	24	0.4826		

Notes: a. These two F values calculated by dividing between host classes MS by a synthesized between colonies MS (see text).

b. SS = Sum of squares, MS = mean squares.

*** = $p<0.001$, no asterisk = not significant.

Table 6.9 Allele frequencies in a population of Harpagoxenus sublaevis

Host class	n_c	Me locus	n_c	Mdh-2 locus
HS+LA	31	f(96) = 0.267	31	f(97) = 0.080
		f(100) = 0.733		f(100) = 0.920
		f(104) = 0		
HS+LA+LM	7	f(96) = 0.076	6	f(97) = 0.031
		f(100) = 0.883		f(100) = 0.969
		f(104) = 0.041		
HS+LM	9	f(96) = 0.265	9	f(97) = 0
		f(100) = 0.735		f(100) = 1.000
		f(104) = 0		
All colonies	47	f(96) = 0.238	46	f(97) = 0.058
		f(100) = 0.756		f(100) = 0.942
		f(104) = 0.006		

Notes: a. Original data are shown in Table 5.4.

b. n_c = number of colonies in sample (S 84 73 and S 85 96 were omitted because they possibly contained contaminants: see Ch.5).

c. The allele frequencies (f) shown are mean per colony frequencies.

Table 6.10 Genetic distances between Harpagoxenus sublaevis host classes

		Host classes		
		HS+HA	HS+HA+HM	HS+HM
	HS+HA	___	0.0171	0.0030
Host classes	HS+HA+HM	0.9830	___	0.0179
	HS+HM	0.9970	0.9823	___

Notes: a. The values above the diagonal are estimates of genetic distance, D, and the values below the diagonal estimates of normalized genetic identity, I (Nei 1972). I = 1.0 indicates total genetic identity; $D = -\log_e I$.

b. The allele frequencies from which these estimates were calculated are shown in Table 6.9.

Figure 6.1 Pronotal width distribution in H. sublaevis

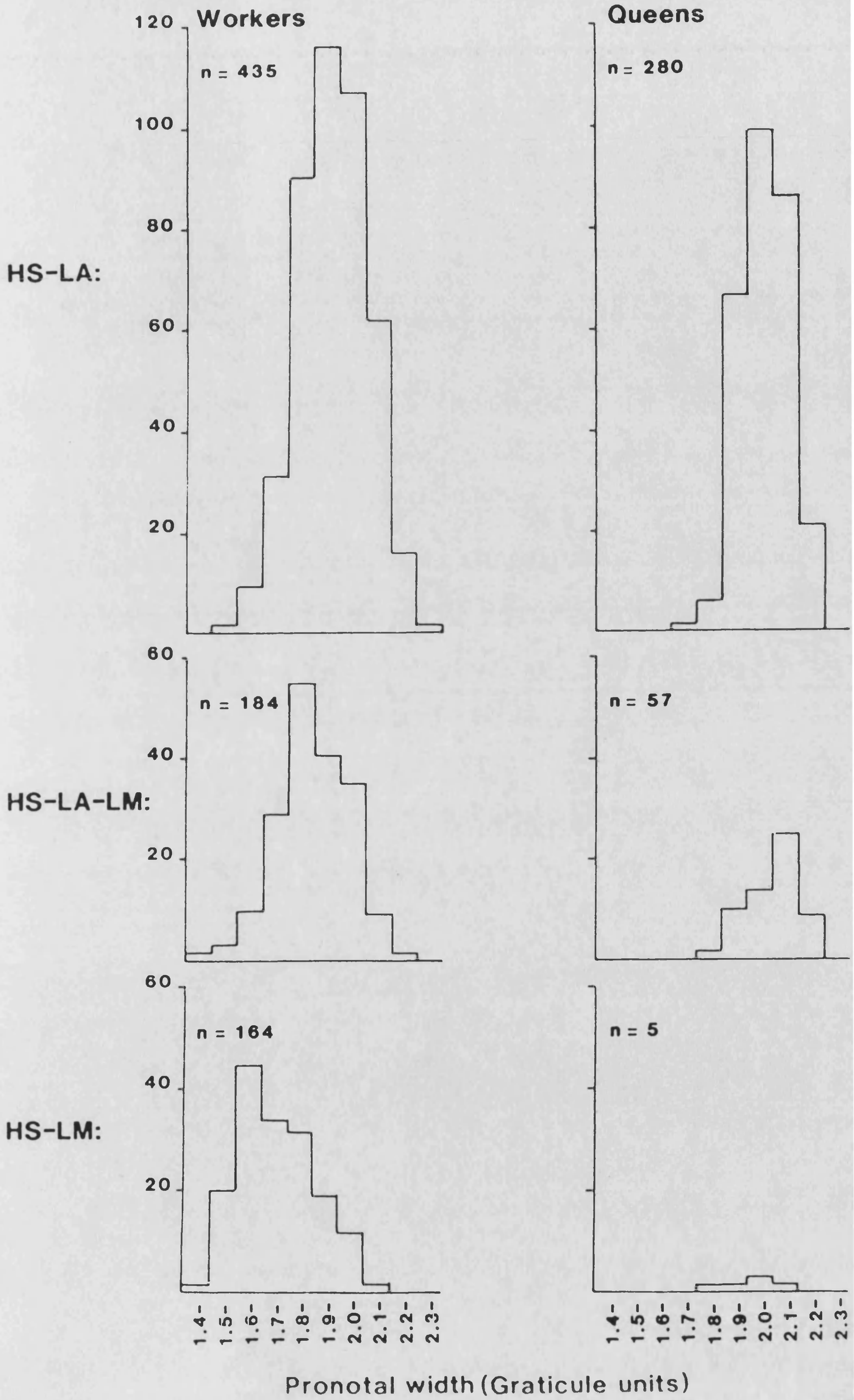
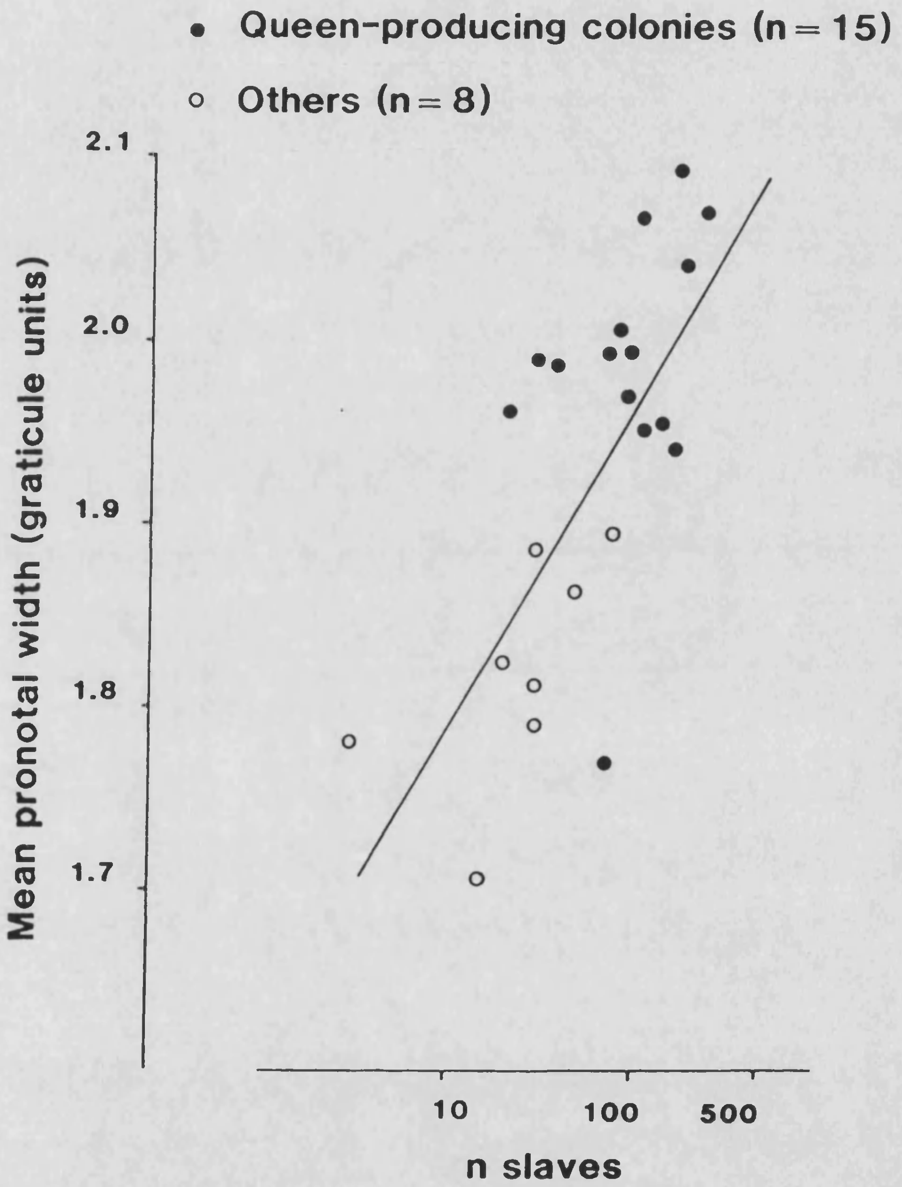
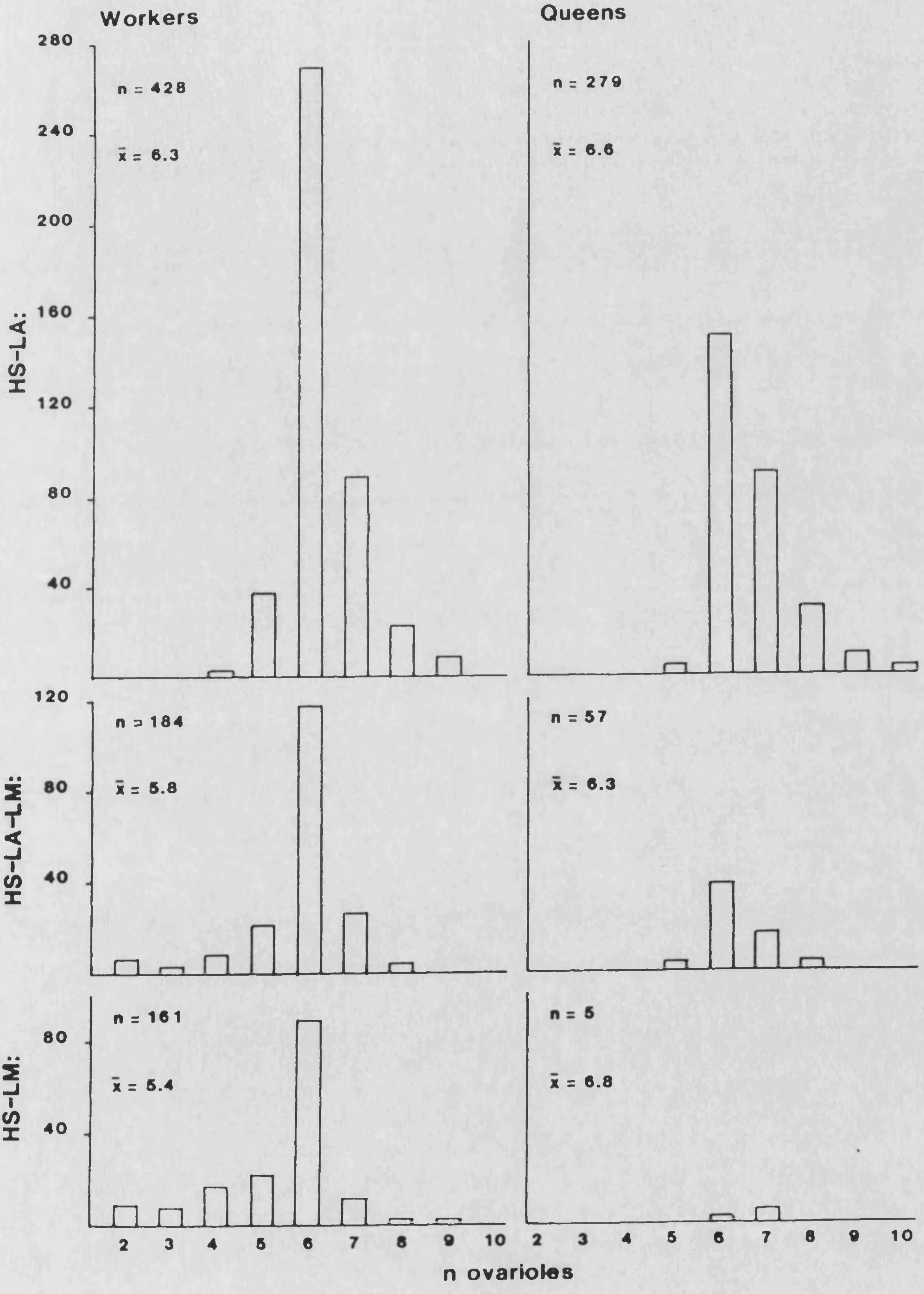


Figure 6.2 Relation between L. acervorum-reared H. sublaevis worker pronotal width and slave number



The regression equation is: $y = 0.174\log_{10}x + 1.615$

Figure 6.3 H. sublaevis ovariole number.



Chapter 7

"Propaganda Substances" in *Harpagoxenus sublaevis*

Introduction

In 1971, Regnier and Wilson described a remarkable chemical strategy employed by North American *Formica* slave-maker workers on slave raids. During attacks on nests of their slave species (also *Formica*), these slave-makers released large quantities of decyl, dodecyl, and tetradecyl acetates, which caused the slave species workers to panic and scatter, so leaving their brood undefended. The slave-maker chemicals therefore acted like exaggerated versions of their hosts' alarm pheromone, undecane (Wilson 1975c). The slave-makers produced these chemicals in their Dufour's gland, which was found to be hypertrophied compared to that of the slaves. This gland, with the poison gland, forms part of the sting apparatus throughout the Aculeate Hymenoptera (Wigglesworth 1972:607). Among ants, its secretions vary greatly in chemical composition, and known functions include alarm, defence, and recruitment (Hermann and Blum 1981, Morgan 1984, Bradshaw and Howse 1984). Regnier and Wilson termed the *Formica* slave-maker Dufour's secretions "propaganda substances".

In 1974, Buschinger suggested that *Harpagoxenus sublaevis* also possessed a chemical weapon. The most obvious adaptations of *H.sublaevis* females for fighting are their large and secateur-like mandibles, with which they dismember their opponents (see Ch.3). But, in colony foundation experiments, Buschinger (1974b) noted that *H.sublaevis* queens introduced to *Leptothorax* nests fought both by biting and by smearing the *Leptothorax* workers with secretions from their stings. Remarkably, the affected *Leptothorax* ants were then attacked by their own nestmates. Similar phenomena were observed when *H.sublaevis* workers attacked *Leptothorax* ants during slave raids (Winter 1979, Buschinger, Ehrhardt, and Winter 1980:255). I have also commonly observed such occurrences. Typically, *H.sublaevis* queens or workers immobilize hostile *Leptothorax* ants by biting off their appendages. But if gripped by several attackers, they daub them with a secretion from the extruded sting. New *Leptothorax* arrivals then attack their contaminated nestmates. This way, slave-makers both escape their attackers, and disable and confuse them. Clearly, *H.sublaevis* possesses its own "propaganda substance".

Since it is released by the sting, this substance originates from either the poison or the Dufour's gland. But the functions of poison gland secretions are already known in *H.sublaevis*. In queens they act as mate attractants, in workers as recruitment pheromones (Buschinger 1972, Buschinger and Winter 1977) (see Ch.3). Further, the Dufour's gland in *H.sublaevis* is far larger than in the related host *Leptothorax* species, while the poison gland reservoir (poison vesicle) is similar in size (Buschinger 1968a, Winter 1979). Also, in dissections of queens following colony foundation attempts, Buschinger (1974b) found the Dufour's glands were empty. Therefore strong indirect evidence existed that *H.sublaevis*, like the *Formica* slave-makers, produced its "propaganda substance" in the Dufour's gland.

This chapter describes experiments to test this hypothesis. I recorded the response of *Leptothorax* workers to nestmates treated with extracts of the Dufour's and poison glands of *H.sublaevis* and, as controls, *Leptothorax* ants. At the same time, Anthony Allies and Nigel Franks tested the response of *Leptothorax* workers to the

Dufour's secretions of L.kutteri. This species is a workerless inquiline parasite of L.acervorum (see chapters 2 and 6), which infiltrates L.acervorum colonies and then co-exists with its hosts. In introduction experiments, L.kutteri queens attacked by L.acervorum workers also secreted a substance from the sting which caused contaminated workers to be attacked by their nestmates. Moreover, the Dufour's gland in L.kutteri is again hypertrophied (Buschinger 1974a). Hence it appeared L.kutteri also produced a "propaganda substance" from its Dufour's gland when attempting to enter host colonies. The results of our experiments have been published in Allies, Bourke and Franks (1986).

Methods

All ants used in the experiments came from the Bröms-Kristianopel or other southern Swedish populations. We tested the response of L.acervorum workers to nestmates treated with glandular extracts from L.kutteri queens, H.sublaevis females, and L.acervorum queens. The H.sublaevis females were not classified as workers and queens, because of practical difficulties arising from the similar external morphology of the two castes (see Ch.3). But as already mentioned, the "propaganda substances" of both castes have identical behavioural effects. All Leptothorax workers tested were from unparasitized L.acervorum colonies. In every trial, a set of four replicate portions was made from each colony by arbitrarily selecting four groups of three workers and housing each group in its own small arena ($4cm^2$.). We then added to three of these replicate portions a nestmate treated with either (1) an extract of Dufour's glands, or (2) an extract of poison vesicles, or (3) solvent alone.

Glandular extracts were prepared by first dissecting ants in distilled water. Glands were then removed and crushed in the non-toxic solvent, liquid paraffin (mineral oil). The Dufour's gland volumes of L.kutteri and H.sublaevis are, respectively, about 40 and 60 times that of queen L.acervorum. Hence L.kutteri Dufour's extracts contained 6 glands/10 μl of solvent (giving a concentration of 1.5×10^{-2} μl gland contents/μl solvent), and H.sublaevis Dufour's extracts contained 4 glands/10 μl of solvent (concentration 1.6×10^{-2} μl gland contents/μl solvent). In the queen L.acervorum trials, we crushed each, small Dufour's gland singly in a paraffin droplet (concentration 1.3×10^{-3} μl gland contents/μl solvent). Poison extracts contained the same number of poison vesicles as corresponding Dufour's extracts contained Dufour's glands. Concentrations of poison extracts were therefore lower than those of Dufour's extracts in proportion to the size difference between the poison vesicle and Dufour's gland in each species.

All ants introduced to the worker groups were first marked with paint dots on their heads. The glandular extracts (or liquid paraffin alone) were then applied to the thorax with a pinhead. The average volume (±S.D.) applied was 0.50±0.15 μl, determined by weight. Therefore each ant treated with Dufour's extracts received the equivalent of either 0.29 of an average L.kutteri gland, or 0.21 of a H.sublaevis gland, or a whole queen L.acervorum gland (since here the entire paraffin droplet containing each gland was applied). Sixty minutes after the addition of each treated ant to the arena of nestmates, we recorded whether it was being bitten (attacked) or not (not attacked). The time interval guaranteed any attack was not a temporary, alarm response to a new object in the arena. Finally, a worker - also marked with a paint dot - from a different unparasitized L.acervorum colony was added to the fourth replicate portion of each trial colony. Only results from trials in which this alien was attacked were considered valid, to exclude trials in which the unfamiliarity of the test arena might have lead to a lack of hostility to any introduced worker.

Results

L.acervorum workers treated with Dufour's extracts of L.kutteri and H.sublaevis were significantly more likely to be attacked by their nestmates than workers treated with either poison extracts or liquid paraffin alone (Table 7.1). In contrast, Dufour's extracts of L.acervorum queens did not induce attack by nestmates at a significantly greater frequency than control solvent (Table 7.1). These results suggested the hostility-inducing substances of L.kutteri and H.sublaevis originate in the Dufour's gland, and that the active agents in these secretions are either absent or present in only very small amounts in the Dufour's glands of L.acervorum queens.

Discussion

The behavioural effects of the Dufour's secretions of H.sublaevis queens and workers are evidently different to those of the Formica slave-makers studied by Regnier and Wilson (1971). The H.sublaevis substances do not simply disperse hostile slave species ants, but by somehow disrupting their nestmate recognition system cause them to attack each other. This is clearly a potent strategy for weakening the slave species' defences, especially since - as the results showed - the slave-maker secretions have persistent effects. Such subversive chemical weapons are unknown outside H.sublaevis, L.kutteri, and possibly some related, leptothoracine social parasites (see below). The Dufour's secretions of *H.sublaevis* may also serve another purpose during slave raids. Buschinger, Ehrhardt and Winter (1980:257) observed the slave-makers smearing captured brood with an abdominal secretion, apparently to deter would-be *Leptothorax* rescuers. However, from my observations, such behaviour does not invariably occur on slave raids.

Interestingly, several other slave-making species may also employ chemical weapons. Alloway (1979) recorded that *Leptothorax* ants attacked by *H.americanus* workers fought among themselves. He suggested the slave-makers induced such behaviour with a contaminating secretion. If so, the use of disruptive chemical weaponry represents another remarkable parallelism between *H.americanus* and *H.sublaevis*, since these two species are not true congeners (see chapters 3 and 4). The existence of chemical weapons in *H.canadensis* (which is a genuine sibling species of *H.sublaevis*:Ch.3) is uncertain. *H.canadensis* females reportedly never use their stings in fights with their host species, but are instead exclusively mandible fighters (Stuart and Alloway 1983, Stuart 1984). Further, the Dufour's gland in *H.canadensis* is only half its length in *H.sublaevis* (Buschinger and Alloway 1978). Yet this gland is still larger than the host's, and Stuart and Alloway (1983:67) did note fighting among host species nestmates following attack by *H.canadensis*. Hence chemical "propaganda" remains a possibility in this species. *Polyergus* slave-makers probably possess a panic-inducing pheromone like that of the North American *Formica* raiders, to judge from the behaviour of victims of *Polyergus* attack. But, by the same token, the European slave-maker *F.sanguinea* lacks such substances (Buschinger, Ehrhardt and Winter 1980:256).

In sum, there is strong evidence that the use of chemical weapons, though not universal, is widespread among slave-making ants. But the North American *Formica* and *H.sublaevis* are the only slave-makers in which the behavioural effects and glandular origin of such substances have been elucidated.

Our findings showed the Dufour's secretions of *L.kutteri* queens had identical behavioural effects to those of *H.sublaevis*. *L.kutteri* is the sole inquiline known to possess such disruptive chemicals. Yet the means by which *L.kutteri* succeeds in infiltrating *L.acervorum* colonies remains a mystery. This is because all laboratory introductions performed to date have ended fatally for the *L.kutteri* queens (Allies, Bourke and Franks 1986). We could only suggest the inquilines deploy their "propaganda substance" as a defence against the initial attacks of *L.acervorum* workers, and then later somehow acquire the hosts' colony odour, thereby subsequently allowing peaceful co-existence. Remarkably, the use of Dufour's secretions to facilitate penetration of host colonies may also occur in the inquiline *Doronomyrmex pacis*. This very close relative of *L.kutteri* also parasitizes *L.acervorum* and has a hypertropied Dufour's gland (Buschinger 1974a). But yet another related inquiline parasite of *L.acervorum*, *L.goesswaldi*, lacks an enlarged Dufour's gland (Buschinger 1974a). The behaviour of *D.pacis* and *L.goesswaldi* queens when introduced to *L.acervorum* colonies is apparently unknown.

The strong similarity between the chemical weapons of *H.sublaevis* and *L.kutteri* in both behavioural effect and glandular origin is striking. This similarity could stem from convergent evolution, especially since the parasites share *L.acervorum* as host. Alternatively, the close phylogenetic relatedness of these two parasites, and of both to *L.acervorum* (Buschinger 1981; Ch.2), suggests common ancestry is the explanation. Recently, E.D.Morgan and his group at the University of Keele have chemically analyzed the Dufour's secretions of H.sublaevis, L.kutteri, and L.acervorum. Their results could help solve this problem.

Using gas chromatography and mass spectrometry, these researchers found the Dufour's gland of H.sublaevis workers contained about 20 components, all hydrocarbons. Most were linear alkanes and alkenes from C_{15} to C_{23}, but two were non-aliphatic hydrocarbons, identified as (E)-β-farnesene and a homologue of this compound (Ollet, Morgan, Attygalle and Billen 1987). The Dufour's secretions of L.acervorum queens and workers were similar to each other and to those of H.sublaevis, except that they lacked longer chain hydrocarbons (most were C_{15} to C_{19}), as well as (E)-β-farnesene and its homologue (Ali, Morgan, Attygalle and Billen 1987). These findings suggested the farnesenes were the substances responsible for the disruptive effects of H.sublaevis Dufour's secretions on L.acervorum. But in preliminary analyses, no farnesenes were found in L.kutteri. If these compounds are the active constituents of the slave-maker "propaganda substances", and L.kutteri on further analysis is found to possess them, common ancestry would be the best explanation for similar chemical strategies in these parasites. This would raise interesting questions as to whether the common ancestor of H.sublaevis and L.kutteri was itself a social parasite and, if so, what kind. But if H.sublaevis and L.kutteri utilize different compounds with similar effects, convergence is the favoured explanation for the parasites' shared means of chemical attack. In addition, further work on the chemistry of the Dufour's secretions of these ants is of great interest in another context. Identification of any chemical which disrupts nestmate recognition could provide special insights into the chemical basis of colony-specific odour in ants, a subject about which almost nothing is known (Hölldobler and Michener 1980).

Summary of Chapter 7

1. *Harpagoxenus sublaevis* queens and workers utilize "propaganda substances" in fights with hostile ants of the host species, *Leptothorax acervorum*. These substances are used by the slave-makers as chemical weapons during non-independent colony foundation and on slave raids. They have the unusual behavioural effect of causing *L.acervorum* nestmates to attack each other, and therefore appear to override nestmate recognition in host species colonies.

2. Laboratory experiments demonstrate the slave-makers produce these substances in the Dufour's gland.

3. Queens of the closely related workerless inquiline *L.kutteri* produce Dufour's secretions with identical behavioural effects, in their fights against hostile *L.acervorum* ants.

4. Published observations indicate the use of chemical weaponry may be widespread in slave-making ants.

5. Preliminary chemical analysis of Dufour's secretions in *H.sublaevis*, *L.kutteri* and *L.acervorum* suggests farnesene compounds could be major constituents of the parasites' "propaganda substances".

Table 7.1 Response of Leptothorax workers to nestmates treated with gland extracts

	(A) L. kutteri gland extracts			(B) H. sublaevis gland extracts			(C) L. acervorum gland extracts		
	Attacked	Not attacked	Total trials	Attacked	Not attacked	Total trials	Attacked	Not attacked	Total trials
Dufour's extract	19	11	30	15	15	30	4	11	15
Poison extract	3	27	30	6	24	30	2	13	15
Liquid paraffin control	4	26	30	4	26	30	3	12	15
		$X^2 = 26.2$, $p < 0.001$			$X^2 = 11.4$, $p < 0.01$			$X^2 = 0.8$, $p > 0.05$	

Chapter 8

Worker Reproduction in the Higher Eusocial Hymenoptera

Introduction

Eusocial animal societies are characterized by co-operative care of young, overlap of generations within the group or colony, and reproductive division of labour - meaning (in eusocial Hymenoptera) the differentiation of females into queen and worker castes (Wilson 1971). The higher eusocial Hymenoptera are those social bees, wasps and ants in which queen-worker dimorphism is strongest. Essentially, queens and workers in these groups differ in their reproductive systems. The workers are morphologically incapable of mating, either because of spermatheca loss (as in Harpagoxenus sublaevis), or vaginal constriction. However, if such workers retain ovaries, they are capable of reproduction despite their inability to mate because, in all Hymenoptera, males arise parthenogenetically from unfertilized eggs. This chapter is a review of the occurrence and significance of worker reproduction, defined as parthenogenetic male production by workers, throughout the higher eusocial Hymenoptera.

Earlier, I concluded worker reproduction in H.sublaevis not only strongly influences colony social structure, but also figured prominently in the species' social history (see chapter 4). Further, I indicated H.sublaevis was not alone either in having reproductive workers, or in these other respects. This chapter supports these claims. It demonstrates that workers of many advanced social species have reproductive ability even though, as their morphology indicates, they are also adapted for helper behaviour. In addition, I present evidence that through its historical role, worker reproduction greatly influenced many characteristic traits of advanced Hymenopteran colonies, including queen regulation of worker fertility (queen control), the temporal division of labour, and physical caste polymorphism. I also argue that worker reproduction, in colonies of those species where it still occurs, remains a powerful influence on social cohesion, colony efficiency, and sex investment ratios. I therefore assert the importance of worker reproduction in our understanding of Hymenopteran societies (see also Lin and Michener 1972, Evans 1977, West-Eberhard 1981, 1982).

I begin by considering the theoretical basis of worker reproduction. One reason why worker reproduction has interest to evolutionists in general is that the phenomenon itself - the almost unique existence in the advanced social Hymenoptera of a morphological worker caste furnishing both helper and reproductive individuals - is seemingly paradoxical and so challenging to evolutionary theorists. To illustrate this challenge, in some species there exists an extraordinary reproductive division of labour, in which queens produce exclusively female offspring, and the worker caste all the males. In the first section, therefore, I describe how two leading theories of eusocial evolution, the mutualistic and kinship theories, each explain the existence of a reproductive, worker caste. I further discuss recent models suggesting worker male production may actually have facilitated the origin of eusociality. Next, I outline why queen-worker conflict over worker reproduction is expected. I also describe models predicting the optimum proportion of worker-produced males, and how sex investment ratios may consequently alter. I present evidence suggesting worker-produced males are a general influence on sex investment in monogynous ants, contrary to the conclusions of Nonacs (1986a). Finally, I review links between worker reproduction and social variables such as gyny levels (queen number), in preparation for testing the relevant hypotheses later in the review.

The second section is a summary from the literature of records of worker reproduction in the higher eusocial Hymenoptera, with systematic consideration of such important details as whether workers lay eggs in queenright or queenless conditions, and the proportion of worker-produced males. This section therefore supplements previous compilations of cases of worker reproduction (e.g. in Wilson 1971, Lin and Michener 1972, Hamilton 1972, Oster and Wilson 1978, Brian 1979, 1980, 1983, Fletcher and Ross 1985).

In the third part of the review I use the data assembled on worker reproduction in ants to test the hypotheses referred to above, linking worker reproduction with gyny levels. The results suggest worker reproduction may be promoted by monogyny because of the high probability of orphanage in monogynous colonies. I discuss how to reconcile this conclusion with the observation that monogyny is also apparently conducive to worker sterility, and argue this can be done assuming queen-worker conflict over worker reproduction has been a dynamic, extended process.

The final section describes features of advanced eusocial colonies which are conceivably direct consequences of worker reproduction. These further exemplify worker reproduction's importance. West-Eberhard (1981) pointed out that every advanced insect society can only have attained its high level of organization after undergoing a lengthy evolutionary history. She also proposed a major theme of this history in each species was intra-colony reproductive competition, particularly queen-worker conflict over worker reproduction. As will be evident, this is the viewpoint I adopt in the third part of the review, as just described. In the final section I return to this theme, and describe how West-Eberhard's hypothesis provides a satisfying theory for the evolution of queen control. It suggests queen control arose in direct response to the threat to queen fitness posed by reproductive workers. This idea gains extra support if, as indicated above, worker reproduction was implicated in eusociality's early stages. Queen control is hence one major consequence of worker reproduction. Others include more overt manifestations of intra-colony reproductive competition, such as worker dominance orders, also discussed in this section. In addition, I report how selection for worker reproduction has influenced the development of a near-universal system of temporal division of labour in social Hymenoptera. Finally, I describe Oster and Wilson's (1978) proposal that worker reproduction has also constrained the proliferation of specialist, physical worker castes. These lines of evidence in turn provide reciprocal support for West-Eberhard's hypothesis. For all these reasons, my conclusion is that worker reproduction deserves recognition as an integral feature of Hymenopteran eusociality.

Theoretical Basis of Worker Reproduction

A Worker reproduction and the evolution of eusociality.

To explain the existence of sterile workers was the evolutionary puzzle that prompted all the various theories of Hymenopteran eusociality (reviewed by Starr 1979, Brian 1983, Brockmann 1984, Andersson 1984, Jaisson 1985). But what needed explaining in many cases was a worker caste which managed to combine worker morphology and behaviour with some degree of reproductive activity. A common strength of two main theories of eusocial evolution is that each offered an explanation of this situation. First, the mutualistic theory maintained the earliest workers were individuals which joined semisocial (single generation) groups to gain the advantages of group living, and found it paid to defer reproduction and work because all workers had some probability of later reproduction. Their work was therefore an investment in future offspring, although these were not guaranteed to all. The forerunners of the worker caste were, in other words, "hopeful reproductives" (Lin and Michener 1972, West-Eberhard 1978, Ross 1985, Fletcher and Ross 1985).

Second, as mentioned in chapter 4, Hamilton's (1964, 1972) kinship theory also provided an explanation for reproduction in the worker caste. In the Hymenoptera the haplodiploid sex determination system by which females usually develop from fertilized eggs and are diploid, whereas males develop from unfertilized eggs (arrhenotoky) and are haploid, leads to unusual asymmetries in genetic relatedness (r) between colony members. Assuming a subsocial (matrifilial) route to eusociality, Hamilton argued that on the basis of their greater relatedness to their sisters ($r=0.75$) compared to their daughters ($r=0.5$) Hymenopteran workers should rear sisters; but, by the same token, they should produce sons ($r=0.5$) instead of rearing brothers ($r=0.25$). Trivers and Hare (1976) pointed out that Hamilton's theory in fact implied workers had two options, either to rear sisters and produce sons, or to forfeit personal reproduction and rear a female-biased brood of sisters and brothers. Only workers following the second option would be completely sterile. Workers following the first option would combine worker behaviour with reproduction.

Recently, Aoki and Moody (1981) concluded from allele frequency models of eusocial evolution (by the subsocial route) that if the first workers had taken the fertile rather than the sterile course, worker behaviour would have arisen more easily. This was because conditions for the fixation of a worker behaviour allele were less restrictive if workers substituted sons for brothers than if they raised a bias of sisters (single locus model). Also, in a more realistic two locus model (where one locus controlled worker behaviour, the other worker ability to raise a bias of sisters or substitute sons for brothers), the worker allele was favoured without assuming tight linkage of the loci if workers laid, but was only favoured given such linkage when workers did not lay. Hence, together, the models suggested the laying worker route to eusociality was more likely than the nonlaying one. Significantly, each using different modelling techniques, several other authors have also recently concluded that male-producing workers would have facilitated the evolution of eusociality (by the subsocial route) (Iwasa 1981, Bartz 1982, Pamilo 1984), for example by removing the requirement for female-biased sex ratios to create average degrees of relatedness favourable to worker evolution (Bartz 1982). These findings imply that total worker sterility arose relatively late in eusocial evolution. They also suggest queen control of worker reproduction - as indicated by Aoki and Moody (1981) - is a secondary feature. I return to these ideas at various points throughout this review.

B Worker reproduction in present day colonies

Even if it did facilitate the origin of eusociality, the persistence of worker reproduction in present day colonies requires explanation. This is because the interests of queens and workers conflict over the parentage of males (Trivers and Hare 1976, Oster, Eshel and Cohen 1977, Bulmer 1981). A queen should prefer the colony to invest in her sons and daughters ($r=0.5$) rather than in her less closely related grandsons (r=0.25), and should therefore oppose worker male production in her presence. This argument underlies the explanation of the evolution of queen control later in the review. But if the queen dies and there are no more of her offspring to be reared, queen and worker interests concur. Both parties favour worker male production, in the case of the departed queen because posthumous grandson production is better than no reproduction at all (Alexander 1974:365, Owen and Plowright 1982). Such reasoning is supported by the strong, observed association between worker reproduction and colony orphanage (see following section).

Cases of workers continuing to lay with the queen present cannot be so simply explained. These cases have often been ascribed to accidental failures in queen control. However, this interpretation ignores selection on workers to pursue queenright worker reproduction and hence actively to circumvent queen control. Such selection could arise for the reasons deriving from the mutualistic and kinship theories of eusociality already discussed, or because of low worker-brood relatedness as later explained. In any event, such selection may be very strong, as indicated by game theoretic models (Charnov 1978a), and by calculations that a worker *Apis mellifera scutellata* honey bee could increase its inclusive fitness twenty-five-fold by producing just a single son (West-Eberhard 1981). Lin and Michener (1972) and Alexander (1974) suggested the interesting possibility that males themselves could also be influencing workers to reproduce. Hymenopteran arrhenotoky means a male is unrelated to his mate's sons, and so can only contribute to the next male generation through reproductive worker daughters. However, no evidence exists that males somehow manipulate their worker offspring into reproducing (see Starr 1984 for further discussion of the possible role of male interests in queen-worker conflicts). Hence the best explanation for queenright worker reproduction is that in some circumstances, despite queen opposition, it is favoured by selection on workers.

C The proportion of worker-produced males and sex investment ratio

If workers are to be reproductive at all, what proportion of males should they produce? Oster, Eshel and Cohen (1977) and Oster and Wilson (1978) developed optimization models from kinship theory which predicted workers should produce all, or none, of a colony's males. Reproductive workers do produce all males in some species, but data in the following section indicate that more often they produce only a proportion. Oster and Wilson (1978) proposed three hypotheses to account for these instances of mixed male parentage: (a) laying workers are subsidized by energetic surpluses in the colony; (b) queen control fails; (c) competitive group selection permits a stable equilibrium of laying and nonlaying workers. In chapter 5, I found some evidence for the first hypothesis in *H.sublaevis*. Owen and Plowright (1982) tested the first two hypotheses with data from the bumble bee *Bombus melanopygus*, and rejected them in this case. However, Oster and Wilson themselves acknowledged the tentative nature of their proposals, and pointed out the striking variation among social Hymenoptera in the proportion of worker-produced males (documented in the next section). This variation still awaits explanation.

When workers produce males in colonies with a queen the expected equilibrium sex investment ratios of the queen and nonlaying workers alter: relative male investment should increase (Trivers and Hare 1976, Oster, Eshel and Cohen 1977, Charnov 1978b, Benford 1978). The precise sex ratio depends on the proportion of worker-produced males, the number of laying workers, and which party controls investment.

For example, if all males arise from one or a few laying workers, the expected sex ratio in most models of the above authors is 1:1 males:females (queen or nonlaying worker control) or 4:3 males:females (laying worker control). Alternatively, workers may only produce males in orphaned conditions. As discussed in chapter 5, Taylor (1981) presented a model showing that in this case (assuming queen control) queens should compensate for the extra males by producing a more female-biased brood than previously, but not sufficiently to restore equal investment (resulting in a male-biased overall population sex ratio). Such sex ratio compensation has been reported in bumble bees (Owen, Rodd and Plowright 1980) and free-living ants (Forsyth 1981). It also appeared to occur in the slave-maker Epimyrma ravouxi (Winter and Buschinger 1983), but in H.sublaevis I concluded the proportion of worker-produced males was not large enough to provoke this effect (Ch.5).

At present, too few data exist with which to assess critically the overall importance of worker male production for sex investment ratios on the basis of the above models, although its influence has long been suspected (Trivers and Hare 1976, Alexander and Sherman 1977). However, Nonacs' recent (1986a) conclusion that it is relatively unimportant in ants seems premature. Nonacs reanalysed published sex ratios from 33 monogynous ant species and calculated the mean per species proportion of investment in males to be 0.282 (Nonacs 1986a:Table 3). This figure closely approximated the 0.25 proportionate investment in males expected assuming a non-reproductive workforce (Trivers and Hare 1976). But, as Table 8.1 of the present review later shows, workers in 10 of the 33 species listed by Nonacs reportedly possess reproductive capability. Accordingly, I calculated from Nonacs' data the mean proportion of investment in males in these 10 species: it was 0.343. The mean male investment in the remaining 23 species was 0.256. Hence, first, relative investment in males is greater where worker male production is suspected, as predicted above (whether workers reproduce in queenright or queenless conditions). Second, sex investment in the species not suspected of having reproductive workers is closer to the 0.25 expectation than the previous estimate. Admittedly, the difference between the two new means is only at the borders of significance (One tailed Mann-Whitney U-test, $t=1.724$, $0.1>p>0.05$). Nevertheless, the results of this analysis at least suggest more data, including explicit measures of levels of worker male production, are required before dismissing worker reproduction as a significant influence on sex investment in monogynous ants.

D Social correlates of worker reproduction

Several authors have supposed workers are more likely to reproduce in some social regimes than in others. For example, Hamilton (1972) argued for an association between worker reproduction and monogyny, and worker sterility and polygyny. Trivers and Hare (1976), rejecting the hypothetical connection between polygyny and inbreeding on which this argument was based, reversed Hamilton's predictions. They reasoned that in a monogynous colony a worker which killed the queen in a physical contest over egg-laying would greatly reduce its own inclusive fitness by destroying the one source of new workers and queens. But if the queen killed the worker her fitness loss would be minimal. However, in the presence of several queens to which it was closely related, the prospectively reproductive worker would not be handicapped in this way. Therefore, Trivers and Hare argued, workers might reproduce more readily in polygynous than in monogynous colonies. Similarly, worker reproduction would be more likely in annual colonies, since a worker killing the queen of a perennial colony would have more to lose. However, evidence to be discussed later suggests workers of some monogynous (albeit annual) bees and wasps do kill their queen to reproduce, in apparent contradiction to Trivers and Hare's hypothesis.

A more important factor governing the amount of worker reproduction is almost certainly intra-colony relatedness. The converse of Hamilton's (1964) original 3/4 relatedness hypothesis for worker sterility is that a worker should not rear brood less closely related to it (on average) than any offspring it might bear. This again implies that polygyny (which reduces worker-brood relatedness) promotes worker reproduction, and monogyny (raises worker-brood relatedness) worker sterility, although as already described kinship theory permits worker reproduction in monogynous colonies if workers substitute sons for brothers. Similar considerations suggest worker reproduction is promoted by multiple mating of queens, since multiple mating also reduces relatedness assuming simultaneous usage of sperm from different males (as confirmed by Ross, 1986, in vespine queens). However, these effects of polygyny and multiple mating could be mitigated if workers recognize and selectively rear the brood most closely related to them, a possibility now receiving intensive attention (e.g. Visscher 1986; see review of Gadagkar 1985). In the third section of this review I test some of the relations predicted above between gyny levels and worker reproduction with data from ants, and tentatively conclude that although most species with reproductive workers are monogynous, their workers reproduce less in queenright and more in queenless conditions than reproductive workers of polygynous species, in keeping with the arguments from relatedness.

The Occurrence of Worker Reproduction

This section is a survey of records of worker reproduction in the higher eusocial Hymenoptera. These, as explained in the Introduction, constitute those groups where workers are morphologically distinct from queens (bumble bees, stingless bees, honey bees, vespine wasps, higher ants). Groups where workers are defined predominantly by their behaviour (halictine bees, polistine wasps, polybiine wasps, some primitive ants) are not included: aspects of reproduction by workers in these groups are reviewed by Fletcher and Ross (1985). The morphological-behavioural distinction is not in fact clear-cut. But I make it because I wish only to consider reproduction by workers unequivocally adapted for a helper role, as evidenced most convincingly by differences in morphology between workers and queens. Such differences include small worker body size, lack of wings (in worker ants), and as previously explained worker inability to mate and reproduce sexually. By concentrating on morphological workers, I therefore exclude workers which could found their own colonies. I also avoid confusion of worker reproduction with male production by uninseminated queens. Caste differences in social insect reproductive systems are reviewed by Brian (1979). As earlier described, since workers incapable of mating are being considered, all offspring are parthenogenetically-produced males. Thelytoky - the parthenogenetic production of females (Wilson 1971:325, Crozier 1975) - does occur in social insect workers, e.g. in the ants Cataglyphis cursor (Cagniant 1979, 1982) and Pristomyrmex pungens (Itow et al. 1984), and the honey bee race Apis mellifera capensis (Ruttner 1977, Moritz and Hillesheim 1985). However, it is rare and so will not be further considered.

Particular attention is paid to: (a) whether worker reproduction occurs in queenright or queenless conditions; (b) the proportion of all males which workers produce; (c) the frequency of layers in the worker population; and (d) whether fertile workers show dominance behaviour.

A Bumble Bees (Bombinae)

Worker reproduction is widespread in bumble bees, and there is great variation in the proportion of males workers produce. Zucchi (cited in Kerr 1969:158) found workers of the perennial and polygynous *Bombus atratus* produced nearly all (up to 98%) of the males, apparently in the presence of queens. In a *B.terrestris* colony studied by van Honk, Röseler, Velthuis and Hoogeveen (1981), 82% of males were worker-derived; laying began with the queen present, and eventually involved 25 of the colony's 99 workers. *B.melanopygus* workers produced 19% of the males in queenright colonies, and accounted for 39% of males overall, since laying continued after the queen's death (Owen and Plowright 1982). In fact worker laying when the queen is dead occurs "in almost all bumble bee species" (Owen and Plowright 1982:92). In orphaned colonies of *B.terricola* reproductive workers were thought to account for the male-bias of the population sex ratio (Owen, Rodd and Plowright 1980). On the other hand, in several bumble bee species in Canada, the percentage of males coming from workers was low, reaching zero in *B.polaris* (Richards 1977).

Reproduction in worker bumble bees frequently involves aggressive dominance behaviour among workers and between workers and the queen (Free 1955, Katayama 1971, 1974, Röseler and Röseler 1977, van Honk, Röseler, Velthuis and Hoogeveen 1981, van Honk and Hogeweg 1981, van Honk 1982, Hogeweg and Hesper 1983, van Doorn and Heringa 1986;

reviews of Michener 1974, Morse 1982, Plowright and Laverty 1984). A prominent feature of this aggression is reciprocal oophagy (egg cannibalism) by workers and queens (e.g. *B.lapidarius*, Free, Weinberg and Whiten 1969; *B.ruderatus*, Pomeroy 1979).

B Stingless Bees (Meliponini)

Egg-laying by workers in stingless bees is very common and has been reviewed by Sakagami (1982). When the queen is present workers of many species lay non-viable trophic (nutritional) eggs, which the queen eats during the complicated cell-provisioning and queen oviposition process characteristic of these bees (see Wilson 1971:93). Workers frequently only lay reproductive eggs in queenless conditions (Sakagami 1982). However, queenright worker reproduction occurs in some species. For example, in *Trigona postica* colonies Beig (1972) reported that the queen laid one egg per cell, and that such cells nearly always yielded females. But in 27% of cells, fertile workers (numbering an estimated 23 per colony) laid an extra egg. These two-egg cells always yielded males, because the worker-produced male larva killed the female larva or egg produced by the queen (Beig 1972, Beig, Bueno, da Cunha and de Moreas 1982). Therefore in queenright colonies of this species workers produce nearly all males, at the expense of 27% of the queen's almost exclusively female offspring. Beig also found most males came from workers in three more stingless bee species (cited in Kerr 1969:169). In another species, *Melipona subnitida*, Contel and Kerr (1976) showed by electrophoretic analysis that queenright laying workers produced an average 39% of males.

In *Melipona favosa* worker laying appeared to be associated with aggression among workers, at least in orphaned colonies (Sommeijer and Velthuis 1977, Sommeijer 1984).

C Honey bees (Apini)

Even the worker honey bee, the epitome of the self-sacrificing worker social insect, has the capacity for personal reproduction. When worker honey bees are kept in queenless groups, some develop their ovaries and start to lay eggs, and aggression breaks out in the group (Sakagami 1954, 1958, Jay 1968, 1970, 1972, 1975, Jay and Jay 1976, Bai and Reddy 1975, Velthuis 1970, 1977, Hesse 1979, Korst and Velthuis 1982; reviewed by Michener 1974, Velthuis 1985, Seeley 1985). The aggression is frequently directed at the workers with ovarian development (Sakagami 1954, Velthuis 1970, 1977). Honey bee workers within a hive constitute patrilines, because the single honey bee queen mates multiply (review of Seeley 1985). Evers and Seeley (1986) recently found that in queenless groups half sisters were preferentially attacked over full sisters: this is the first evidence to suggest the existence of kin-defined factions among groups of reproductive workers (see final section). Intriguingly, one laying worker in a queenless colony may start to behave and to attract a retinue like a queen (Sakagami 1958). Further, this "false queen" appears to inhibit ovarian development in the other workers (Sakagami 1958). Velthuis, Verheijen and Gottenbos (1965) found extracts from ordinary laying workers could also restrict ovarian development in other bees (see also Jay and Nelson 1973). In fact, in Apis mellifera capensis, both "false queens" and laying workers produce 9-ODA (9-oxo-trans-2-decenoic acid), a major component of honey bee "queen substance", i.e. the pheromonal mix with which the hive queen suppresses worker ovarian growth (Ruttner, Koeniger and Veith 1976, Velthuis 1985).

In natural colonies it might seem worker bees never experience the queenless conditions necessary for laying, since a new queen is always reared in preparation for swarming and the old queen's departure. However, orphaning could occur if the old queen died in winter when there was no brood to supply her replacement, or in summer a young queen failed to return from her nuptial flight having previously killed her royal sisters (J.B.Free, pers. comm.). Interestingly, Page and Metcalf (1984:696) suggested the frequency of orphaned nests in A.mellifera populations is "relatively high". They also reported the production by one orphaned hive of over 6000 male bees. The proportion of worker-produced males in natural honey bee populations may therefore be significant. Furthermore, Free and Williams (1974) showed laying A.mellifera workers preferred to oviposit in drone cells rather than in the smaller worker cells (which would yield undersize males), in contrast to queens laying only unfertilized eggs who displayed no such preference. This discrimination by laying workers provides additional evidence for the importance of worker male production in orphaned nests (Page and Metcalf 1984). In the giant honey bee, A.dorsata, male production by naturally occurring queenless workers has also been reported (Velthuis, Clement, Morse and Laigo 1971).

Laying by worker honey bees may not be confined to queenless nests. Taber (1980) reported that even in queenright hives about one in every hundred workers had ovaries in egg-laying condition. Although in honey bees (as in some ants) ovary-developed workers are not always layers (Ribbands 1953, Sakagami 1958, Velthuis 1977), these bees were described as a "possible source of a few males" by Fletcher and Ross (1985:328). Since a hive contains 20,000 to 80,000 workers (Wilson 1971), one percent represents many potential layers. Even if unable to produce eggs in normal circumstances, such workers might take advantage of periods in the colony cycle (e.g. swarming) when queen control is temporarily weak or absent to start laying. On the other hand, they might still be prevented by the inhibitory effect queen-produced brood also evidently has on worker ovarian development (Jay 1970, 1972, Jay and Jay 1976, Kropáčová and Haslbachová 1971, Seeley 1985). But despite this, Kropáčová and Haslbachová (1969, 1970) found worker ovarian development was greatest shortly after swarming, and Velthuis (1985:348) wrote "once swarming preparations are being made, workers often have well developed oocytes and may occasionally lay an egg". Also, Fletcher (in Fletcher and Ross 1985) found that in emergency queen rearing, laying *A.m.adansonii* workers could arise rapidly enough to produce drone pupae before the new queen's appearance. However, in general, the extent of worker male production in natural queenright colonies seems a little-explored area of honey bee biology.

D Vespine Wasps (Vespinae)

Previous reviews of vespine worker reproduction include those of Richards (1971), Spradbery (1973), Jeanne (1980), and Akre (1982). Ishay (1964) found workers of the oriental hornet *Vespa orientalis* laid eggs both in orphaned colonies (up to 40-50% of all colonies) and in queenright colonies at the season's end. He suggested workers produced an "important percentage" of late season males. The death of the queen was accompanied by: (a) fighting between workers, sometimes fatal; (b) partition of the nest into egg-laying territories; (c) worker killing of queen-produced larvae. Often the death of the queen herself was caused by the workers: she was literally licked to death by them (Ishay 1964). In both *V.orientalis* and the European *V.crabro* small groups of workers can also reportedly found new nests in which they rear males (Hamilton 1972, pers. comm., Ishay 1976, pers. comm., Kugler, Motro and Ishay 1979).

Workers of Dolichovespula maculata habitually lay in the presence of the queen. She attacks them and eats their eggs, and workers also eat each other's eggs (Greene 1979). A similar situation was found in D.arenaria (Greene, Akre and Landolt 1976).

In the genus Vespula workers removed from the queen's influence also laid eggs, fought, and ate rivals' eggs (Landolt, Akre, and Greene 1977). Queenright laying has been reported too, in V.vulgaris, V.germanica (Montagner 1966, Spradbery 1971) and V.consobrina (Akre 1982). Montagner estimated that in half his colonies workers produced 75 to 100% of the males. Worker laying was associated with occasionally fatal aggression among workers and between workers and the queen (Montagner 1966). Furthermore, Archer (1981) found field evidence suggesting laying V.vulgaris workers destroyed the queen's male brood while sparing their own. However, after finding only 4% of workers in natural V.vulgaris populations had developed ovaries, Spradbery (1971:513) concluded such workers "would be unlikely to make a significant contribution to male production." Similar conclusions have recently been reached in other Vespula studies. Akre, Garnett, MacDonald, Greene and Landolt (1976), in prolonged observations of V.pensylvanica and V.atropilosa, never witnessed worker oviposition. Ross (1985), investigating several North American species, discovered only 1.6% of workers had ovarian development in queenright colonies, a figure that rose to 30-45% in colonies lacking a queen. He considered the proportion of worker-produced males "insignificant" (Ross 1985:420). In addition, he questioned the reproductive success of worker-produced males, which would have emerged late in the season when mates are few (Ross 1985, Fletcher and Ross 1985). Montagner's findings may have resulted from artificial weakening of queen control, since his experimental techniques involved temporarily removing and radio-labelling the queen (Hamilton 1972, Ross 1985). In the first electrophoretic investigation of vespine male parentage, Ross (1986) also showed that in field queenright V.maculifrons and V.squamosa colonies, males almost certainly originated exclusively from the queen. But workers did produce males when queenless. Hence, overall, vespine workers appear to be reproductive mostly in queenless conditions.

E Ants (Formicidae)

As pointed out by Cole (1986), ants present a variety of worker reproductive systems. They include species in which workers possess both ovaries and a spermatheca, species in which they have ovaries only, and species in which all reproductive apparatus has been lost. In addition, as in other groups considered, ants show great differences between species in worker ability to reproduce with queens present.

Workers with ovaries and a spermatheca occur in the primitive ponerine ants (Brian 1979). However, their potential to mate and reproduce sexually excludes them from this review. Table 8.1 lists species where workers have ovaries but no spermatheca in which either (a) male production by workers occurs, or (b) worker laying of reproductive eggs has been reported. Table 8.1 therefore groups together examples of worker reproduction of widely varying quality, depending on the method of determining worker reproduction (specified in the table). The interpretation of egg-laying and ovarian development in ants is complicated by the following pair of widespread phenomena: (a) As in stingless bees, in many ants workers produce non-viable trophic eggs as food for other colony members (Wilson 1971:281). Cases of trophic egg-laying alone are not included in Table 8.1; (b) In some species workers' ovaries undergo a cycle of growth and resorption correlated with the temporal division of labour, without eggs ever being laid (e.g. Formica spp., Dumpert 1981:118) (see final section). Therefore ovarian dissections alone provide the weakest evidence for worker reproduction. I reserve discussion of the Table 8.1 data until the following section.

Workers entirely lacking reproductive organs, or with vestigial ovaries, occur in species of the large ant genera Solenopsis, Monomorium, Pheidole, Tetramorium, and Eciton (Wilson 1971, Oster and Wilson 1978, Fletcher and Ross 1985). Here worker reproduction is obviously impossible.

Worker Reproduction and Gyny Levels: Tests of the Hypotheses

This section uses the data from ants in Table 8.1 to test the hypotheses earlier described predicting whether worker reproduction should be associated with mono- or polygyny.

Of the 40 ant species with reproductive workers in Table 8.1 whose gyny is known, 29 are monogynous and 11 are polygynous. Buschinger (1974c) found in a survey of European ants that the overall ratio of mono- to polygynous species is 50:50. Hence, if this ratio is universal, a significantly greater number of species with reproductive workers are monogynous than expected by chance (X^2 test, $X^2=8.1$, d.f.=1, $p<0.01$), suggesting an association between worker reproduction and monogyny. This conclusion is tentative because (a) some authorities (e.g. Hölldobler and Wilson 1977) state most ant species are monogynous; (b) the analysis ignores the influence of multiple mating by queens, since present data are too scant (Table 8.1, notes). Another, more general problem of an analysis of this sort is its assumption that each species constitutes an independent test of the null hypothesis (i.e., here, that worker reproduction is equally likely to be associated with mono- or polygyny). But closely related species are less likely to be independent in this way than distantly related ones, because of their common descent. Hence more refined comparative method would replace individual species with separately evolved lineages as the unit of test (Ridley 1983). However, ant phylogeny is too poorly known for such a procedure to be followed in this case. But the use of individual species was arguably justified anyhow, since gyny levels are not uniform within ant taxa, e.g. species of the same genus often differ in gyny (Table 8.1).

Despite the above reservations, the finding worker reproduction is associated with monogyny could be informative when coupled with further analysis of Table 8.1. For data in the table also indicate that in monogynous species worker reproduction occurs mostly in queenless conditions, whereas in polygynous species it occurs in queenless and queenright conditions equally often. Of 29 monogynous species, workers reportedly reproduce in the absence of the queen in 21 and in her presence in 8, but of 11 polygynous species workers reproduce with queens absent in 6 and queens present in 5 (Table 8.1). However, the apparent association between monogyny and queenless worker reproduction is not statistically significant (X^2 test for association, X^2 corr.=0.49, d.f.=1, $p>0.1$), although this could be the fault of small sample sizes. In addition, the data in Table 8.1 concerning whether workers reproduce in the presence or absence of queens may simply reflect the greater ease with which worker reproduction is detectable in worker-only groups, rather than the real-life situation. Nevertheless, the suggestion that workers in monogynous ant species, if reproductive, reproduce mostly in queenless conditions, is interesting because it could help explain the tentative association between worker reproduction and monogyny detected above. This is because the greater probability of worker-only groups arising from queen mortality in mono- than in polygynous species could mean there has been greater selection on workers in monogynous species to retain reproductive capability, through greater opportunity to exercise it unhindered by queens. In H.sublaevis and three other monogynous species in Table 8.1, Myrmica sulcinodis, Epimyrma ravouxi, and Apterostigma dentigerum, queenless colonies produced substantial proportions of all males (see also Ch.5).

Monogyny, therefore, seems to favour the retention of worker reproductive activity because of the concomitant high probability of orphanage. I now discuss this idea and its implications more fully. It is important first to note that this suggestion does not undermine the earlier argument that polygyny promotes worker reproduction because of low worker-brood relatedness (ignoring the possibility of kin recognition). The crucial distinction is again between queenright and queenless worker reproduction. Formerly, workers may have been universally selected to reproduce in queenright conditions, in polygynous societies because of low relatedness, and in monogynous ones if selection - as many indications suggest - favoured the worker strategy of producing sons and rearing sisters earlier described. In every case, such worker reproduction would have met with queen opposition, because of queens' greater relatedness to their own brood. Hence workers might have been forced to reproduce mostly in queenless conditions where (again as previously mentioned), since their interests coincide, queens could even have favoured workers reproducing. But queenless conditions presumably arise comparatively rarely in polygynous species, both because the probability of queenlessness occurring through mortality must be lower when there are many queens per colony, and because polygynous colonies frequently adopt new queens. By contrast, monogynous species almost never replace queens, and further - as evidenced by H.sublaevis (Ch.5) - a relatively high proportion of their colonies become queenless through orphaning. Therefore, workers have far greater opportunity to reproduce free from queen interference in mono- than in polygynous species. Hence monogyny, more than polygyny, could prolong the existence of a reproductively active worker caste.

However, ironically for this very reason, monogyny might also ultimately lead to total worker sterility. To see why, it is necessary to consider the queen's position. From the arguments already advanced, workers in monogynous species would be selected to grow ovaries as larvae, for use when orphaned as adults. But because workers might only be prevented from also reproducing in the queen's lifetime by queen control, the queen would therefore be faced with a workforce constantly equipped and prepared to reproduce. If the cost to the queen of continual inhibition of worker laying outweighed the benefits to her of posthumous grandson production, she might be selected - as suggested by Fletcher and Ross (1985) - to prevent somehow ovaries developing in worker larvae, for example by secreting a growth regulating pheromone. Thus she might render workers totally sterile. Hence the suggestion is that monogyny first promotes retention of reproductive activity in workers because of the orphanage effect, then as a result provokes extreme measures by queens to sterilize workers. This reasoning could help explain the observation that species with sterile workers, the greatest queen-worker dimorphism, and the most powerful queen control, also tend to be monogynous (e.g. West-Eberhard 1982, Hölldobler and Bartz 1985).

But it is also important to state, in a further twist, that workers under a single queen may acquiesce to the imposition of sterility. This is because sterile workers have recourse to the Hamiltonian strategy of raising a female-biased brood of sisters and brothers, which under monogyny (and single queen mating) is equivalent in terms of worker inclusive fitness to raising an evenly-balanced brood of sisters and sons. Therefore in a monogynous society sterility need not be more disadvantageous to workers than reproduction. (This was of course the basis of Hamilton's theory: hence, in keeping with kinship theory, worker sterility still ultimately stems from high worker-brood relatedness in the above reasoning. On the same basis, workers in polygynous societies should not acquiesce to sterility, unless there is kin recognition). But in monogynous species the workers' new strategy of biassing sex ratio would again bring them into conflict with the queen, who favours equal investment in her sexual offspring (Trivers and Hare 1976). Intriguingly in this context, in monogynous free-living ants with non-reproductive workers, sex ratios are generally female-biased and hence apparently worker-controlled (Trivers and Hare 1976, Nonacs 1986a, Ch.5, and earlier discussion). I therefore suggest that in such species queens have won the queen-worker conflict over male parentage, but lost in their subsequent disagreement with workers over sex ratio. (Slave-making ants seem exceptions to this: as argued in chapter 4, H.sublaevis workers may be particularly reproductively active because they are unable to manipulate brood composition to their advantage). Of course, since evolution has no foresight, this conclusion does not imply queens should have refrained from initially suppressing worker reproduction.

Summing up, it seems that only by taking this dynamic and historical view of queen-worker differences (see also following section), is it possible to explain both the finding that most ant species with reproductive workers are monogynous, and the observation that monogyny also appears to be associated with total worker sterility.

As has been described, workers of the other groups considered - bumble bees, stingless bees, honey bees, vespine wasps - are frequently reproductive. Since nearly all species in these groups are monogynous, and their workers also reproduce mostly in queenless conditions, these groups further substantiate the idea that monogyny favours the retention of worker reproductive ability through the agency of orphanage.

Finally, returning to ants, workers in three species in Table 8.1 are suspected of producing all or nearly all the males. Two species are monogynous (number of queen matings unknown) and the third, *Myrmica rubra*, is polygynous with multiply-mated queens, leading to extremely low intra-colony relatedness (as confirmed electrophoretically by Pearson 1983). *M.rubra* workers reproduce in queenright conditions. Therefore, together with the relatively high proportion of polygynous species with queenright laying workers (Table 8.1 and above), the scale of worker reproduction in *M.rubra* supports the hypothesis that queenright worker reproduction is favoured by low worker-brood relatedness.

Consequences of Worker Reproduction

This section argues that the ability of workers to reproduce has had in the past, and continues to have in the present, important consequences for other members of the colony and for colony organization. One such consequence, the effect of worker-produced males on sex ratios, has already been discussed.

A The evolution of queen control

As already indicated, queen suppression of worker reproduction (queen control), both pheromonal and behavioural, is near-universal in social Hymenoptera (reviewed by Brian 1979, 1980, Fletcher and Ross 1985, Hölldobler and Bartz 1985). One explanation for this is Alexander's (1974) theory that parental manipulation of worker reproductive behaviour was itself primarily responsible for the original evolution of worker sterility. However, since worker reproduction was itself apparently implicated in eusociality's early stages (see first section), and as is now clear has been a persistent feature of eusocial colonies, a better explanation of queen control is that it evolved secondarily as a response to worker reproduction, over a long period. As explained earlier, faced with a reproductive worker caste, queens would have been selected (because of queens' greater relatedness to their own offspring) to compel the workforce to rear queen-produced brood exclusively. Therefore queen control as it now exists seems largely a consequence of worker reproduction.

Again in the previous section, I indicated queens may frequently have succeeded in suppressing queenright worker reproduction. However, this was almost certainly not a smooth process. Evidence exists that at each escalation of queen control, workers were counter-selected to evade reproductive inhibition and cling to their egg-laying ability. In other words, in numerous lineages there appears to have been an intra-specific evolutionary "arms race" (Dawkins and Krebs 1979) between the queen and worker castes over worker reproduction. The idea behind this suggestion was first proposed by West-Eberhard (1981). Supporting evidence includes the "multiplicity of queen control substances and the fact that each is only partially effective" in honey bees (West-Eberhard 1981:14), and the complex queen egg-laying ritual in stingless bees, which appears to be a relic of former dominance struggles with workers over egg-laying and oophagy (Hamilton 1972, West-Eberhard 1981, Sommeijer, Houtekamer and Bos 1984).

I also argued earlier that in some species total worker sterility appears to have arisen after a history of queen-worker conflict over worker laying, through a mixture of exceptionally potent queen control and eventual worker acquiescence. If this is correct, in these species queens seem the outright winners in the above-mentioned arms race, though their victory acquires a pyrrhic flavour owing to the workers' opportunistic manipulation of sex ratio. But in species where workers regularly egg-lay in queenright conditions, the arms race over worker reproduction must have had a fundamentally different outcome, or be at an earlier stage. Thus, the way in future to explain the enormous variation between species in the prevalence of worker reproduction, and the proportion of worker-produced males, would appear to be by studying enough species in sufficient depth for a comprehensive comparative analysis, invoking social structures, relatedness levels, and degrees of queen-worker conflict. Ecological factors, which through lack of data I have ignored, may also prove important.

B Conflicts between colony members

In every group considered in this review, worker reproduction was associated with aggression among worker nestmates. Such behaviour undoubtedly stems from reproductive competition. Worker layers are evidently selected to attack their fellows to inhibit rival reproductive activity, so increasing their own genetic representation among worker-produced males. This can lead to worker dominance hierarchies in which rank correlates with ovarian development, notably in bumble bees (and only recently detected in ants: Cole 1981, Franks and Scovell 1983, this study). In addition, as novel confirmation of the importance of kinship in insect societies, there is evidence that when not full sisters, reproductive workers form rival, kin-defined factions (Hölldobler 1984:360, Evers and Seeley 1986). In honey bees, workers have the queen-like ability to inhibit other layers pheromonally as well as physically (Velthuis 1985).

Worker reproduction also underlies further kinds of violent behaviour found in Hymenopteran colonies, such as oophagy and brood destruction. A remarkable example was earlier described in the stingless bee Trigona postica, in which male worker-produced larvae killed female larvae produced by the queen (Beig 1972). In Vespa orientalis (Ishay 1964), and possibly Vespula vulgaris (Archer 1981), laying workers ejected queen-produced brood. In V.vulgaris it seems male brood was selectively destroyed. Kinship theory predicts such selectivity would be a consequence of worker laying. However, in bumble bees, brood destruction is not linked with worker laying (Pomeroy 1979, Plowright and Laverty 1984). Alternative explanations for this behaviour other than kin-selected conflict include ecological factors such as protein shortage (Wilson 1971).

Finally, evidence exists that selection on workers to reproduce free from queen control may even provoke matricide. Workers of the hornet Vespa orientalis killed their queen prior to reproduction (Ishay 1964), and in colonies of the bumble bees Bombus ignitus (Katayama 1971) and B.terrestris (van Honk, Röseler, Velthuis and Hoogeveen 1981), the queen died after expulsion by laying workers. This behaviour is especially striking because these are monogynous species. It is therefore distinct from the phenomenon of worker culling of supernumerary queens found in some ants, which ends in monogyny, i.e. with one surviving queen (Forsyth 1980). Further, these examples apparently contradict Trivers and Hare's (1976) argument described earlier, that workers in monogynous species should avoid mortal conflict with the queen over reproduction. However, this conclusion requires qualification. Montagner (1966) found that *Vespula* wasp workers also killed their single queen if, following radioactive treatment, she laid only male eggs. This suggests that in the hornet and bumble bees matricide occurred because the queen had exhausted her sperm store and hence was producing solely haploid, male offspring. This would not refute Trivers and Hare's hypothesis, which predicted workers should not attack queens who might in future yield new queens. But, by the same token, the sperm depletion argument emphasizes the strength of queen-worker conflict over male parentage, if it implies workers only kill their queen in order to reproduce when she can yield nothing but male offspring.

C Colony efficiency and temporal division of labour

Cole (1986) estimated that in *Leptothorax allardycei* ant colonies, worker dominance activity reduced the time spent on brood care by 15%. Worker reproduction may also damage colony efficiency indirectly. In the present study I showed ovary-developed *H.sublaevis* workers were reluctant to scout and slave-raid, almost certainly to protect their reproductive futures (see Ch.4). They therefore constrained the temporal division of labour, i.e. the pattern of task allocation through time within the worker caste. I now propose to relate how former widespread worker reproduction could have strongly influenced the evolution of temporal division of labour among social Hymenoptera in general.

The argument begins with the suggestion that in its lifetime an individual reproductive worker could both engage in reproduction and help raise kin. This is implicit in the idea workers were selected to produce sons and rear sisters. To maximize its fitness, the worker should pursue these activities in a particular order. It should remain in the nest and lay eggs when young, and switch to altruistic or colony-beneficial tasks when old. This is because colony-beneficial tasks include risky activities outside the nest such as foraging. It has been proposed that a mixed worker reproductive strategy of this sort was the basis for a system of temporal division of labour almost universally found in social insects (West-Eberhard 1979, 1981, Wilson 1985). This is the "centrifugal" system (Wilson 1985), in which young workers perform brood care (nursing) and other tasks inside the nest, whereas external tasks such as foraging are performed by old workers. This system is clearly efficient in its own right from the colony's viewpoint, because it maximizes the numbers of nurses and foragers at each stage. But evidence exists that former selection on young workers to reproduce helped shape this system.

This is the finding that in various species which now almost certainly lack queenright worker reproduction, the behavioural changes workers undergo with age are paralleled by changes in their ovarian development. Young workers inside the nest are ovary-developed, older foraging workers have degenerated ovaries (Wilson 1985). Among ants, the ovarian development of the young workers cannot be explained by trophic egg-laying in every case. For remarkably, in _Formica_ species, the young nest workers apparently never lay but resorb all eggs they form (Wilson 1971:163, Dumpert

1981:118). Hence it is possible that as in some species queen control suppressed reproduction by young workers pursuing the mixed strategy, the difference in ovarian development between the worker age-classes (or the associated hormonal system) was sequestered to act as a mechanism for dividing the labour. Otherwise, the correlation between worker behaviour and ovarian development remains unexplained. Therefore what is now an adaptation promoting colony efficiency very probably originated in previous intra-colony reproductive competition, again in agreement with West-Eberhard (1981).

D Caste proliferation

I now come to my final example of the evolutionary consequences of worker reproduction. This involves physical worker castes. Such castes frequently exhibit bizarre body forms clearly adapted for specialist, colony-beneficial purposes, such as defence. Oster and Wilson (1978) therefore suggested that physical castes would only tend to arise and proliferate in conditions of worker sterility, since selection on reproductive workers to maximize the personal component of their fitness would counter the evolution of worker adaptations detrimental to workers' personal welfare. Oster and Wilson confirmed their hypothesis by demonstrating a highly significant statistical association between monomorphism (single worker caste) and the presence of worker ovaries in ants, and polymorphism (two or more castes) and the lack of ovaries. They also pointed out that polymorphism is in fact uncommon in social Hymenoptera: it is "virtually absent" in bees and wasps, and only 17% of ant genera have polymorphic species; a mere 2% have species with three or more castes. Although it is likely other factors, e.g. ergonomic (Oster and Wilson 1978) or developmental (Wheeler 1986), were also influential, these findings suggest worker reproduction has been a major constraint on physical caste proliferation, especially in bees and wasps.

Conclusion

The essence of eusociality is reproductive division of labour among the members of the society (Wilson 1971). An extraordinary feature of Hymenopteran eusociality is that this division of labour can involve the existence of a morphological worker caste furnishing both helper and (parthenogenetically) reproductive individuals. I propose that greater emphasis on the reproductive side of the Hymenopteran worker condition could enrich our understanding of queen-worker conflict, and of the emergence of a totally sterile worker caste. Further, whatever the reasons behind the enormous diversity in the occurrence and scale of worker reproduction, I conclude this behaviour has had and continues to have far-reaching effects on many characteristic features of Hymenopteran societies.

Summary of Chapter 8

1. Worker reproduction, defined as parthenogenetic male production by workers, is common among bumble bees, stingless bees, honey bees, vespine wasps, and higher ants, i.e. those advanced social Hymenoptera possessing a morphological worker caste incapable of mating.

2. The mutualistic theory ("hopeful reproductive" hypothesis) of Hymenopteran eusociality (semisocial or single generation route) states the first workers were reproductive because the possibility of future reproduction was the condition for their originally working. In Hamilton's kinship theory (subsocial or matrifilial route), workers produce males on account of their greater relatedness to sons than to brothers.

3. Several recent theoretical (subsocial) models indicate worker male production could have helped reduce the threshold for the evolution of worker behaviour. These imply worker sterility arose late in eusocial evolution, and that queen inhibition of worker fertility (queen control) is secondary.

4. Queen control arises because queens are more closely related to their own than to the workers' sexual offspring. Successful queen control explains why workers frequently reproduce only in queenless conditions. Conversely, queenright worker reproduction must reflect strong selection on workers to evade queen control.

5. Optimization models predict reproductive workers should produce all or none of a colony's males. But, though some produce 100%, reproductive workers more usually produce an extremely variable proportion of all males.

6. When either queenright or queenless workers reproduce, expected equilibrium sex ratios become more male-biased. Analysis of Nonacs' (1986a) compiled sex ratio data indicates this prediction is met in monogynous ants.

7. Kinship theory suggests polygyny and multiple mating both promote queenright worker reproduction because each leads to a reduction in worker-brood relatedness.

8. Analysis of data assembled on 40 ant species with reproductive workers reveals most are monogynous. Reproductive workers in monogynous species reproduce mostly in queenless conditions, in contrast to those in polygynous species which reproduce in queenright and queenless conditions equally often, in agreement with kinship theory.

9. I hypothesize monogyny favours the continuation of a reproductively active worker caste because the high probability of queenless conditions arising through orphanage in monogynous species allows workers more opportunity to reproduce free from queen control. This effect could explain why in ants worker reproduction appears associated with monogyny. Total worker sterility, also associated with monogyny, may therefore have arisen from queens' exerting exceptionally powerful control to combat worker reproduction, coupled with worker acquiescence to sterility because of high worker-brood relatedness.

0. The ability of workers to reproduce has important consequences for Hymenopteran colony organization and function. These include: queen control, viewed as the response of the queen caste in the "arms race" with workers over male parentage; dominance behaviour, oophagy, brood

destruction, and possibly even matricide in colonies with reproductive workers; the system of temporal division of labour based on worker age and ovarian development; and the rarity of physical castes among workers. For all these reasons, worker reproduction is crucial to our understanding of Hymenopteran societies.

Table 8.1 Reproduction (male production) in worker ants

Species	Gyny[a]	Method[b]	Remarks[c,d]	References[e,f]
Myrmeciinae				
Myrmecia and *Promyrmecia* spp.	M	OM	QA	Haskins and Haskins, 1950
Myrmecia gulosa	M	OM	QA	Freeland, 1958
Ponerinae				
Odontomachus haematodes	P	OL,OM	QP	Colombel,1971,1972
Neoponera obscuricornis	M(P)	D,OL	QP	Fresneau, 1984
Dorylinae				
Anomma spp.	M	OM	QA; but worker produced male larvae reportedly fail to reach adutlthood	Raignier, 1972
Myrmicinae				
Myrmica rubra	P	D,OL, OM,R,V	QP; all males possibly from workers	Weir,1959; Brian, 1953,1969; Brian and Rigby,1978; Smeeton,1981, 1982a,b,c
M.sulcinodis	M(P)	C	QA; orphaned colonies produced 45% of males	Elmes, 1974
Aphaenogaster rudis	M	E	QA	Crozier, 1974
A.subterranea		OM	QA	Bruniquel, 1972
A.senilis	M	OM	QA	Ledoux and Dargagnon, 1973
Stenamma fulvum		OM	QA	Fielde, 1905
Leptothorax tuberum unifasciatus	M	OL	QA	Bier, 1954
L.nylanderi	M	D,G,OL	QP; all males from workers?	Plateaux,1970,1981
L.ambiguus	P	D	QA	Alloway et al.,1982
L.curvispinosus	P	D	QA	Alloway et al.,1982
L.longispinosus	P	D,U	QP	Alloway et al.,1982; Herbers, 1984
L.allardycei	M	D,OM,V	QP; workers form dominance hierarchy and lay 22% of eggs	Cole, 1981, 1986
Temnothorax recedens	M	OL,U	QP	Dejean and Passera, 1974
Harpagoxenus americanus	M	C,D	QP; workers form dominance hierarchy	Buschinger and Alloway, 1977; Franks and Scovell, 1983

Species	Gyny^{a}	Method^{b}	Remarks^{c,d}	References^{e,f}
H.sublaevis	M	C,D,E, OL,OM	QA, possibly QP; workers form dominance hierarchy; orphaned colonies produced up to 22% of males	Buschinger and Winter, 1978; present study
H.canadensis	M	D	QP	Buschinger and Alloway, 1978
Epimyrma ravouxi	M	C	QA; orphaned colonies produced up to 27% of males	Buschinger and Winter,1983; Winter and Buschinger,1983
Messor capitatus	M	OL	QA	Delage, 1968
Novomessor cockerelli	M	OM	QA; worker aggression	Hölldobler and Bartz, 1985, p.248
Apterostigma dentigerum	M	C,D	QA, orphaned colonies produced 43% of males	Forsyth, 1981
Crematogaster impressa	M(P)	C	QA	Delage-Darchen,1974
Zacryptocerus varians	M	OM	QA	Wilson,1976
Dolichoderinae				
Dolichoderus quadripunctatus	M	OL,OM	QA; all males from workers?	Torossian, 1968
Formicinae				
Plagiolepis pygmaea	P	OL,U	QA	Passera, 1966
Lasius niger	M	E,OM	QP	Lubbock,1885; van der Have, Boomsma, and Menken (in prep.); *L.niger* workers may also be thelytokous (Crawley 1911).
Formica polyctena	P	OL,OM	QP	Ehrhardt, 1962; but see Schmidt, 1982
F.sanguinea	P	E	QP	Pamilo and Varvio-Aho, 1979; Pamilo, 1982b
F.exsecta	P,M	E,OM	QA	Pamilo and Rosengren, 1983
F.fusca	P,M	OM	QA	Lubbock, 1885
F.cinerea	P,M	OM	QA	Lubbock, 1885
F.pergandei		OL,OM	QA	Hung, 1973
F.canadensis		D,OM	QA	Hung, 1973
F.argentata		OM	QA	Fielde, 1905
Polyergus rufescens	M	OM	QA	Lubbock, 1885
P.breviceps	M	OM	QA	Hung, 1973
Campanotus herculeanus	M	OM	QA	Fielde, 1905
C.aethiops	M	D,OL,OM	QA	Dartigues and Passera, 1979

Species	Gyny[a]	Method[b]	Remarks[c,d]	References[e,f]
Oecophylla longinoda	M	OL,OM	QA	Hölldobler and Wilson 1983; these authors could not confirm Ledoux's (1950) report of thelytoky in O.longinoda workers.
O.smaragdina	M	OL,OM	QA	Hölldobler and Wilson 1983

Notes:

a. Gyny levels (where known): M = monogynous; M(P) = almost all colonies monogynous; P = polygynous; P,M = gyny levels reportedly differ between populations. Information on gyny comes from references in far right column and Buschinger, 1968c, 1974c; Collingwood, 1979. For comparisons in the text, M and M(P) species were classified together, P with P,M species. Buschinger (1974c) discusses fully the classification of gyny levels.

b. Method specified in references of determining worker male production or worker laying of reproductive eggs: C = census of production from queenless colonies; D = ovarian dissection; E = electrophoretic allozyme analysis; G = analysis of visible genetic markers; OL = worker egg laying observed;OM = worker male production observed (e.g. in captive worker-only groups); R = worker egg laying indicated by radio-labelling; V = worker egg laying indicated with vital dyes; U = reference asserts worker male production but evidence unspecified.

c. QA = worker reproduction reported in absence of queen(s); QP = worker reproduction reported with queen(s) present.

d. The number of queen matings is known for 9 species in the table: Aphaenogaster rudis, Harpagoxenus sublaevis, H.canadensis (1 mating); Lasius niger, Formica polyctena, F.sanguinea, F.pergandei (>1); F.exsecta (1-2); Myrmica rubra (5-6). Sources: Cole, 1983; Starr, 1984; Page, 1986; and contained references.

e. In over 60 species of leptothoracine (Myrmicinae) kept by A. Buschinger (pers. comm.) queenless workers produced males.

f. Bier (1953) reported egg-laying by queenless workers in several additional formicine and myrmicine species.

Chapter 9

Conclusion

In this final chapter, I first summarize some general implications of the findings described in this thesis. I then describe one way of synthesizing these findings, by detailing an attempt by Nigel Franks, Bryan Ireland (School of Mathematics, University of Bath) and myself to construct a life history model for Harpagoxenus sublaevis. This undertaking is not yet complete, so I cannot report its outcome. Nevertheless, I think it worthwhile to describe the aims and characteristics of the model, both for their own interest and to highlight so far unacknowledged connections between different aspects of the life of H.sublaevis. I also take the opportunity to review previous life history models for social insect colonies, and to discuss what is meant by "colony fitness", the quantity maximized in these models.

A major result from this study concerns the discovery in H.sublaevis colonies of linear, worker dominance orders. As shown in chapter 4, these arise because of reproductive competition among potentially male egg-laying workers. The three ant species in which dominance systems are now known - Leptothorax allardycei (Cole 1981), H.americanus (Franks and Scovell 1983), and H.sublaevis - are all leptothoracines, and therefore characterized by small colony size. Workers in species with large colonies are unlikely to form linear dominance systems, because a ranking order based on individual discrimination only seems possible when colony size is small (Wilson 1971:302). Since leptothoracines lack any obvious additional characteristics, apart from small colonies and ovary-bearing workers, conducive to the formation of worker dominance orders, it seems likely such orders will be found in species of other ant taxa which share these features.

As demonstrated in chapters 4 and 5 for H.sublaevis, and in chapter 8 for many other advanced social Hymenoptera, worker reproduction is an extremely pervasive influence on the organization of colonies. Numerous colony features are best explained by supposing that prolonged queen-worker conflict over male parentage has occurred, even in the most highly evolved bees, wasps and ants. Hence this study exemplifies the importance of intragroup reproductive competition in social evolution, in agreement with the arguments of West-Eberhard (1981). It also suggests that in monogynous social insects, colony orphanage has been an important factor in the maintenance of worker reproductive ability (see Ch.8), rather than the evolutionarily insignificant period of colony decline which it is sometimes labelled. In addition, this thesis points out that queen-worker conflict in Hymenopteran colonies can evidently result in a kind of reproductive division of labour which is at present not sufficiently appreciated, where queens produce all females, and workers all males (e.g. Myrmica rubra ants and other examples in Ch.8). Therefore the current definition of eusocial reproductive division of labour, involving "more or less sterile individuals working on behalf of individuals engaged in reproduction "(Wilson 1971:464) requires refining. More importantly, the richness of Hamilton's kinship theory, which helps explain both a totally sterile workforce, and a morphological worker caste which yields all males, needs to be recognized and elaborated.

Another principal conclusion of this thesis involves sex ratio. As chapter 5 showed, the genetic relatedness hypothesis of Trivers and Hare (1976), given the assumption slave-maker workers lack control of brood composition, correctly predicted the sex investment ratio observed in the Swedish H.sublaevis population. Relative sex investment in the slave-maker Epimyrma ravouxi also matched the expectation from the genetic relatedness hypothesis assuming a relatively high level of worker male production, but in German H.sublaevis local mate competition could not be entirely ruled out (see Ch.5). Therefore, though on balance supporting the genetic relatedness hypothesis of sex ratio in slave-making ants, this study also emphasizes that detailed investigations of single populations are required for sex ratio analyses, since sex ratio determination may vary between populations within individual species (see also Boomsma, van der Lee and van der Have 1982). In any event, slave-making ants provide a good example of the wide applicability of Fisher's theory of sex ratio, from which both Hamilton's (1967) theory of local mate competition and Trivers and Hare's (1976) genetic relatedness hypothesis ultimately derive.

I now turn to the life history strategy of H.sublaevis colonies, and the attempt to model it. The purpose of life history models is to determine how a biological system should allocate resources between growth (i.e. production of workers in the case of social insects) and reproduction (sexual production). According to the philosophy of optimization modelling, it is assumed natural selection has resulted in a life history policy that maximizes the fitness (generally classical fitness, i.e. lifetime rate of output of progeny) of the biological system under scrutiny. (What is meant by the fitness of an insect colony is discussed below). Therefore the ultimate aim of life history models proceeding by the optimization method is relatively modest. Since they assume - and hence do not test - the idea that natural selection in an optimizing agent, the purpose and value of these models lies in their being "a method for organizing empirical evidence, making educated guesses as to how evolution might have proceeded, and suggesting avenues for further empirical research" (Oster and Wilson 1978:295). Hence the attempt to build a colony life history model for H.sublaevis.

The earliest life history models for social insects (see Wilson 1971:Ch.21) concerned monogynous, annual colonies such as those of many wasps and bees, in which only overwintering mated queens link one generation and the next. In such species, Oster and Wilson (1978:Ch.2) found by optimization modelling that the policy which maximized colony fitness (see below) involved the colony producing first all workers, then all reproductives, with no mixing of resource allocation between the two groups (see Ch.5). Data from numerous annual social insects fitted this prediction (Oster and Wilson 1978:56).

However, as implied in chapter 1 when I questioned the validity of the concept of colony-level selection, and mentioned in chapter 5 in the context of Cole's (1986) model for the spread of a worker reproduction allele, Oster and Wilson's "colony fitness" is a problematic quantity. This is so whatever our definition of "fitness" (see Dawkins 1982:Ch.10). For example, colony fitness corresponds neither to classical fitness, since a colony is not an organism producing offspring, nor to genotypic fitness, since a colony is not a single genetic entity. Oster and Wilson's annual social insect model assumed worker sterility. Since all reproductives were therefore considered to arise exclusively from the queen, it would appear that by colony fitness these authors meant classical fitness of the queen. However, Oster and Wilson also classified queen and male reproductive progeny together, and hence their model incorporated no information, nor made any prediction, concerning relative resource investment between the sexes. Therefore, in the model queen fitness was not maximized in the sense that the queen necessarily achieved her optimal sex ratio. Hence colony fitness cannot be exactly equated with queen fitness. But clearly, when workers are sterile and there is only one queen per colony, the genetic interest (or inclusive fitness) of workers and queen coincides to the extent that both parties gain if the colony maximizes its lifetime rate of queen offspring output, the quantity Oster and Wilson termed colony fitness. However, it seems to me this quantity should only be so designated, and employed in models, in the realization that it is an incomplete description of the quantity natural selection will maximize. It is incomplete because it implies natural selection is indifferent to the proportion of queens and males produced. In other words colony fitness is an imperfect term currently necessary because present life history models are not sophisticated enough to incorporate the fact workers and queen may disagree over sex investment. The relevance of this problem to the H.sublaevis case becomes apparent below.

In addition to those for annual species, informative life history models have also been constructed for perennial species whose colonies reproduce by fission. In these species the question of how resources should be allocated between workers and reproductives is replaced by another, which asks at what size the colony should divide. This is because in fissioning species workers alive at the time of splitting count towards reproductive resource allocation. Franks (1985) concluded that in Eciton burchelli army ants, to minimize generation time and thereby maximize colony fitness (in the sense above, since *Eciton* workers lack functional ovaries), a colony should divide equally in two at such a size that the combined growth rate of the daughter colonies equalled its own growth rate. This prediction was met in colonies undergoing fission. Similarly, in honey bees, which also reproduce by colony fission, preliminary efforts have been made to predict the distribution of resources at swarming (Seeley 1985:Ch.5).

Most ant colonies, including those of *H.sublaevis*, are perennial and reproduce not by fission but by the release of queens and males. This kind of life history is the most complex to model, and only one previous attempt to build such a model has been made. This was by Brian, Clarke and Jones (1981) for *Myrmica* ants. *Myrmica* was chosen because, as the result of the work of Brian and his colleagues, it is one of the best known ant genera. Brian, Clarke and Jones incorporated data on sex ratio in their model by letting all males arise from workers (as in *M.rubra*) and consequently assuming the sex ratio reached the equilibrium value (under queen or nonlaying worker control) of 1:1 (Oster and Wilson 1978). The major outcome of their model was the finding that the scale of sexual emission should oscillate, so that productive seasons alternate with unproductive ones. Such periodicity appears to occur in some *Myrmica* populations.

Brian , Clarke and Jones were forced to assume monogyny in their *Myrmica* model, despite the fact most *Myrmica* are polygynous, to avoid unmanageable complexity. For similar reasons they ignored the likely possibility that colonies reproduced by budding, i.e. by the departure of queen and worker groups. Therefore *Myrmica* was a difficult subject to choose for a modelling exercise. By contrast, *H.sublaevis* is in some ways more tractable, because its colony structure (monogynous) and mode of reproduction (exclusively by sexual emission) are relatively straightforward. Of course, H.sublaevis does possess a complicating factor not found in free-living ant species, namely a workforce which only rears brood indirectly, by capturing slaves.

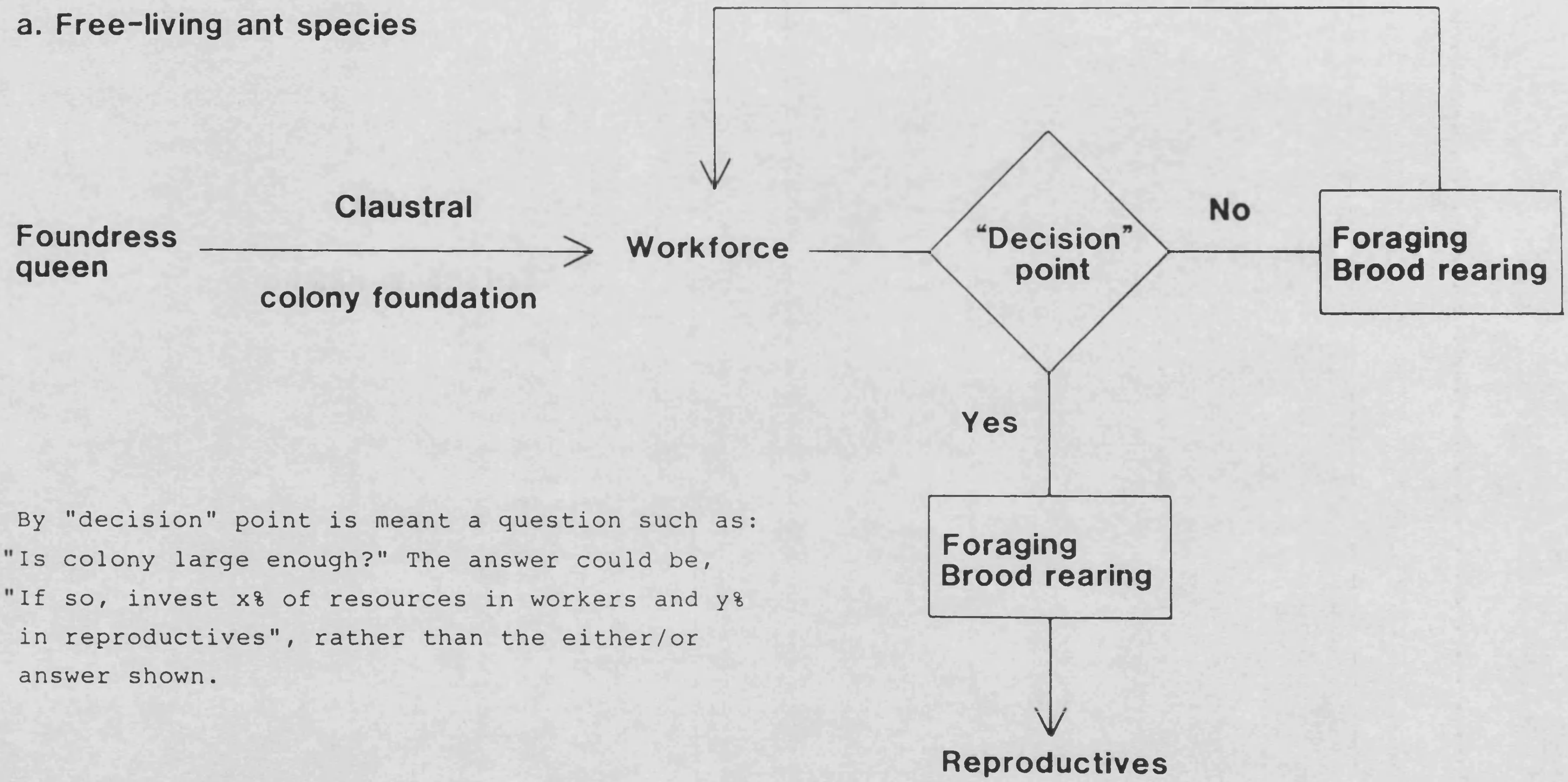
However, this is not a serious problem, because the slave-maker and free-living ant systems are basically similar. As illustrated in Fig.9.1a,b, the slave-maker workforce is simply inserted into the brood care system found in free-living ants. In fact, the slave-maker system may even turn out to be simpler to model. This is because, to model a free-living ant system, it is necessary to formulate and incorporate a mathematical description of the efficiency with which workers forage and thereby provide the colony with resources (Oster and Wilson's "return function"). It is not permissible to measure foraging efficiency in wild colonies and utilize this estimate in the model, because this procedure would introduce an unacceptable level of circularity. In the slave-maker model, we require both a formula for slave-raiding efficiency, and one for the efficiency with which slaves forage. But we can estimate slave foraging efficiency from the productivity of slave species workers in free-living host colonies, since these colonies constitute a natural control group. Hence we can obtain an independent estimate of resource gathering potential in slave workers, for use in the slave-maker model without circularity. This leaves slave-raiding efficiency still to be modelled. Yet we know that each slave-maker colony has on average 4 host colonies within raiding distance (see Ch.6), each containing a measurable number of host worker pupae. By contrast, nothing is known about the distribution of food around the colony, and it is far less easy to see how this information could be gathered and made the basis of a foraging model. It is for this reason - the greater ease of modelling raiding compared to foraging - that the slave-maker system holds special attractions.

The above argument also illustrates how some of the information presented earlier in this thesis will be used in constructing the H.sublaevis model. However, since I cannot report the model's findings, I am not going to specify each parameter in turn. But clearly, apart from raid efficiency and slave productivity, the model will involve information concerning such diverse quantities as queen and worker larval and adult longevity (known from Buschinger's data - see Ch.3), queen and worker survivorship (to be estimated), the relative costs of queens, workers and males (deducible from their dry weights - see Ch.5), and sex investment ratio (1:1, implying queen control). In addition, data on the observed production schedule of H.sublaevis colonies (Fig.5.5), will be used for testing the model's findings. Hence data concerning the ecology, population biology, behaviour and genetics of H.sublaevis will all be drawn together by this life history model.

As is now clear, the H.sublaevis model is possible because we possess a reasonable amount of information about this species. Simultaneously, it is desirable because without such a model it is impossible to organize this information. More fundamentally, it is only with the aid of such a model that it will be feasible to relate behavioural parameters such as raiding efficiency considered in this thesis, to reproductive fitness.

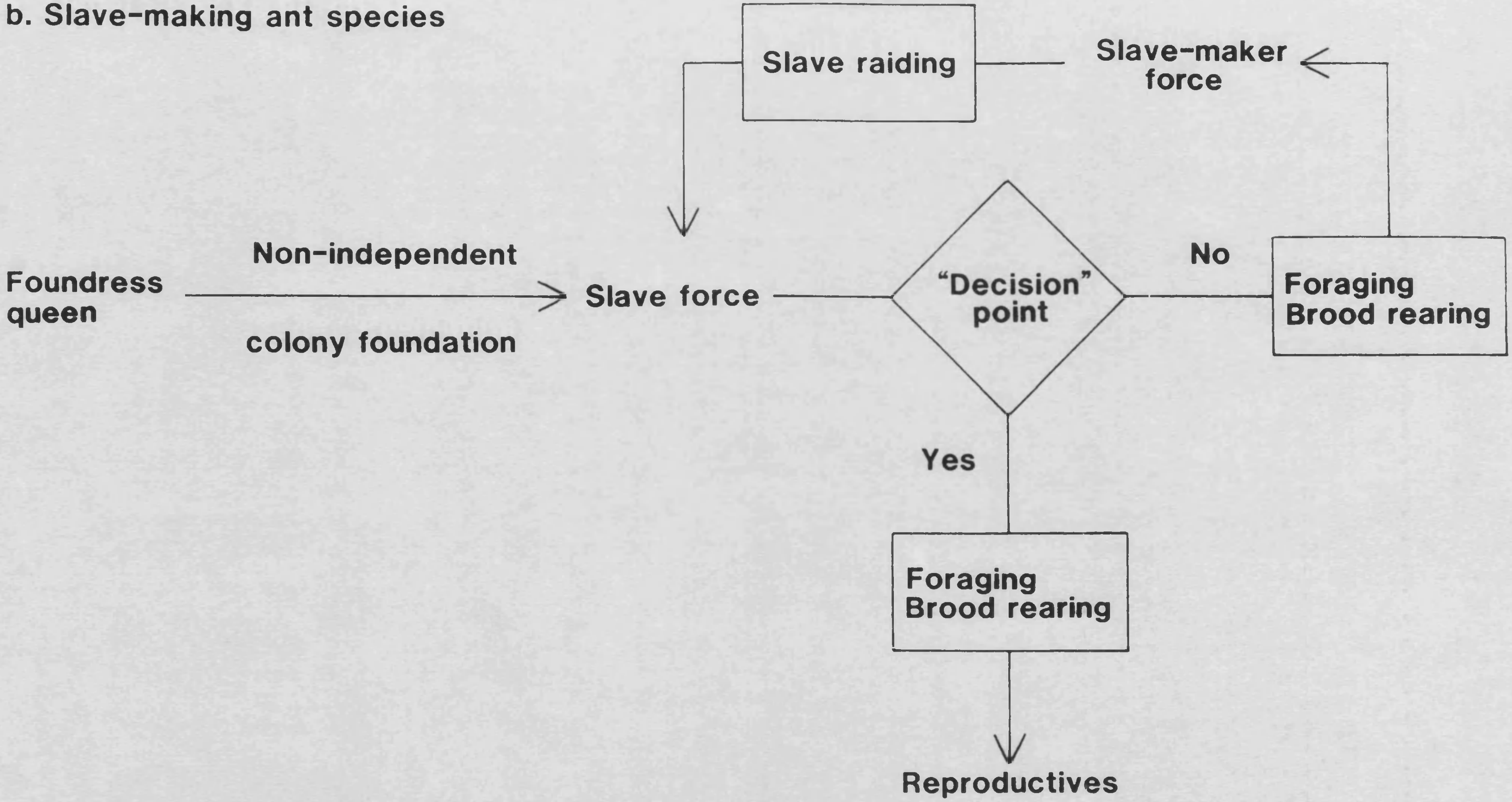
Finally, the H.sublaevis life history model could also help in the further analysis of queen-worker conflict in this species. In the model, the slave-maker colony will be allowed to live for 3 years after the queen's death in the orphaned state, when worker reproduction may occur. This is because we now know that orphaned colonies constitute a substantial proportion of all colonies, that their workers compete for male egg-laying opportunities, and that such workers give rise to most worker-produced males. The model could throw light on this state of affairs because it might predict, for example, that the queen should produce additional workers (at the expense of reproductives) in her declining years, to ensure grandson production beyond her natural life-span. This belated worker production would be contrary to the expectation based on assumptions of worker sterility. Clearly, to view workers simply as vehicles for queen reproduction constitutes a queen-centred view of worker reproduction. However, this refinement of the model could mark a first attempt to escape from the notion of colony fitness in similar life history models (undesirable for the reasons discussed earlier), to a more realistic notion of queen and worker inclusive fitness. Further, since orphanage is almost certainly an important period in the life history of many monogynous social insects (see above and Ch.8), yet one which tends to be ignored, the outcome of the model could yield generally applicable insights into the nature of queen-worker conflict. It could therefore help further our understanding of the gene-selected conflict which is at the heart of social organization.

Figure 9.1 Life histories of colonies of free-living and slave-making ants.



By "decision" point is meant a question such as: "Is colony large enough?" The answer could be, "If so, invest x% of resources in workers and y% in reproductives", rather than the either/or answer shown.

b. Slave-making ant species



References

Akre RD (1982) Social wasps. In: Hermann HR (ed.), Social Insects, Vol. IV, Academic Press, New York, London, pp. 1-105.

Akre RD, Garnett WB, MacDonald JF, Greene A, Landolt P (1976) Behavior and colony development of Vespula pensylvanica and V.atropilosa (Hymenoptera: Vespidae). J. Kans. Entomol. Soc. 49: 63-84.

Alexander RD (1974) The evolution of social behavior. Ann. Rev. Ecol. Syst. 5: 325-383.

Alexander RD, Sherman PW (1977) Local mate competition and parental investment in social insects. Science 196: 494-500.

Ali MF, Morgan ED, Attygalle AB, Billen JPJ (1987) Comparison of Dufour gland secretions of two species of Leptothorax ants (Hymenoptera: Formicidae) (In preparation).

Allies AB, Bourke AFG, Franks NR (1986) Propaganda substances in the cuckoo ant Leptothorax kutteri and the slave-maker Harpagoxenus sublaevis. J. Chem. Ecol. 12: 1285-1293.

Alloway TM (1979) Raiding behaviour of two species of slave-making ants, Harpagoxenus americanus (Emery) and Leptothorax duloticus Wesson (Hymenoptera: Formicidae). Anim. Behav. 27: 202-210.

Alloway TM (1980) The origins of slavery in Leptothoracine ants (Hymenoptera: Formicidae). Am. Nat. 115: 247-261.

Alloway TM (1982) How the slave-making ant Harpagoxenus americanus (Emery) affects the pupa-acceptance behavior of its slaves. In: Breed MD, Michener CD, Evans HE (eds.), The Biology of Social Insects, Westview Press, Boulder, Colorado, pp. 261-265.

Alloway TM, Buschinger A, Talbot M, Stuart R, Thomas C (1982) Polygyny and polydomy in three North American species of the ant genus Leptothorax Mayr (Hymenoptera: Formicidae).

Psyche 89: 249-274.

Alloway TM, Del Rio Pesado MG (1983) Behavior of the slave-making ant, *Harpagoxenus americanus* (Emery), and its host species under "seminatural" laboratory conditions (Hymenoptera: Formicidae). *Psyche* 90: 425-436.

Andersson M (1984) The evolution of eusociality. *Ann. Rev. Ecol. Syst.* 15: 165-189.

Aoki K, Moody M (1981) One and two-locus models of the origin of worker behavior in Hymenoptera. *J. Theor. Biol.* 89: 449-474.

Archer ME (1981) Successful and unsuccessful development of colonies of *Vespula vulgaris* (Linn.) (Hymenoptera: Vespidae). *Ecol. Entomol.* 6: 1-10.

Bai ARK, Reddy CC (1975) Ovary development and egg laying in *Apis cerana indica* workers. *J. Apic. Res.* 14: 149-152.

Bartz SH (1982) On the evolution of male workers in the Hymenoptera. *Behav. Ecol. Sociobiol.* 11: 223-228.

Beig D (1972) The production of males in queenright colonies of *Trigona (Scaptotrigona) postica*. *J. Apic. Res.* 11: 33-39.

Beig D, Bueno OC, da Cunha RA, de Moreas HJ (1982) Differences in quantity of food in worker and male brood cells of *Scaptotrigona postica* (Latr. 1807) (Hymenoptera: Apidae). *Insectes Soc.* 29: 189-194.

Benford FA (1978) Fisher's theory of the sex ratio applied to the social Hymenoptera. *J. Theor. Biol.* 72: 701-727.

Bernstein RA (1978) Slavery in the subfamily Dolichoderinae (F. Formicidae) and its ecological consequences. *Experientia* 34: 1281-1282.

Bhatkar A, Whitcomb WH (1970) Artificial diet for rearing various species of ants. *Fla. Entomol.* 53: 229-232.

Bier K (1953) Vergleichende Untersuchungen zur Fertilität der Ameisenarbeiterinnen. *Zool. Anz.* 150: 282-288.

Bier K (1954) Über den Einfluss der Königen auf die Arbeiterinnen Fertilität in Ameisenstaat. *Insectes Soc.* 1: 7-19.

Boomsma JJ, Isaaks JA (1985) Energy investment and respiration in queens and males of *Lasius niger* (Hymenoptera: Formicidae). *Behav. Ecol. Sociobiol.* 18: 19-27.

Boomsma JJ, van der Lee GA, van der Have TM (1982) On the production ecology of *Lasius niger* (Hymenoptera: Formicidae) in successive coastal dune valleys. *J. Anim. Ecol.* 51: 975-991.

Bradshaw JWS, Howse PE (1984) Sociochemicals of ants. In: Bell WJ, Cardé RT (eds.), *Chemical Ecology of Insects*, Chapman and Hall, London, New York, pp. 429-473.

Brian MV (1953) Oviposition by workers of the ant *Myrmica*. *Physiol. Comp. Oecol.* 3: 25-36.

Brian MV (1969) Male production in the ant *Myrmica rubra* L. *Insectes Soc.* 16: 249-268.

Brian MV (1979) Caste differentiation and division of labor. In: Hermann HR (ed.), *Social Insects*, Vol. I, Academic Press, New York, London, pp. 121-222.

Brian MV (1980) Social control over sex and caste in bees, wasps and ants. *Biol. Rev.* 55: 379-415.

Brian MV (1983) *Social Insects*, Chapman and Hall, London.

Brian MV, Brian AD (1955) On the two forms macrogyna and microgyna of the ant *Myrmica rubra* L. *Evolution* 9: 280-290. (Note: The species dealt with in this paper is in fact *Myrmica ruginodis* Nylander, the subject of former nomenclatural confusion with *M. rubra* - see Wilson 1971: 148 and Elmes 1973: 133).

Brian MV, Clarke RT, Jones RM (1981) A numerical model of an ant society. *J. Anim. Ecol.* 50: 387-405.

Brian MV, Rigby C (1978) The trophic eggs of *Myrmica rubra* L. *Insectes Soc.* 25: 89-110.

Brockmann HJ (1984) The evolution of social behaviour in insects. In: Krebs JR, Davies NB (eds.), *Behavioural Ecology*, 2cd ed., Blackwell, London, pp. 340-361.

Bruniquel S (1972) La ponte de la fourmi *Aphaenogaster subterranea* (Latr.): oeufs reproducteurs - oeufs alimentaires. *C. R. Acad. Sci. Paris Ser. D.* 275: 397-399.

Bulmer MG (1981) Worker-queen conflict in annual social Hymenoptera. *J. Theor. Biol.* 93: 239-251.

Buschinger A (1965) *Leptothorax* (*Mychothorax*) *kutteri* n. sp., eine sozialparasitische Ameise (Hymenoptera, Formicidae). *Insectes Soc.* 12: 327-334.

Buschinger A (1966a) Untersuchungen an *Harpagoxenus sublaevis* Nyl. (Hym. Formicidae). I. Freilandbeobachtungen zu Verbreitung und Lebenweise. *Insectes Soc.* 13: 5-16.

Buschinger A (1966b) Untersuchungen an *Harpagoxenus sublaevis* Nyl. (Hym., Formicidae). II. Haltung und Brutaufzucht. *Insectes Soc.* 13: 311-322.

Buschinger A (1966c) *Leptothorax* (*Mychothorax*) *muscorum* Nylander und *Leptothorax* (*M.*) *gredleri* Mayr: zwei gute Arten. *Insectes Soc.* 13: 165-172.

Buschinger A (1968a) Untersuchungen an *Harpagoxenus sublaevis* Nyl. (Hymenoptera, Formicidae). III. Kopula, Koloniegründung, Raubzüge. *Insectes Soc.* 15: 89-104.

Buschinger A (1968b) "Locksterzeln" begattungsbereiter ergatoider Weibchen von *Harpagoxenus sublaevis* Nyl. (Hymenoptera, Formicidae). *Experientia* 24: 297.

Buschinger A (1968c) Mono- und Polygynie bei Arten der Gattung

Leptothorax Mayr (Hymenoptera Formicidae). Insectes Soc. 15: 217-226.

Buschinger A (1970) Neue Vorstellungen zur Evolution des Sozialparasitismus und der Dulosis bei Ameisen (Hym., Formicidae). Biol. Zbl. 89: 273-299.

Buschinger A (1971a) "Locksterzeln" und Kopula der sozialparasitischen Ameise Leptothorax kutteri Buschinger (Hym., Form.). Zool. Anz. 186: 242-248.

Buschinger A (1971b) Weitere Untersuchungen zum Beggattungsverhalten sozialparasitischer Ameisen (Harpagoxenus sublaevis Nyl. und Doronomyrmex pacis Kutter, Hym., Formicidae). Zool. Anz. 187: 184-198.

Buschinger A (1972) Giftdrüsensekret als Sexualpheromon bei der Ameise Harpagoxenus sublaevis. Naturwissenschaften 59: 313-314.

Buschinger A (1973a) Zum Sexualverhalten der dulotischen Ameise Harpagoxenus sublaevis (Nyl.) (Hym., Formicidae). Proc. VIIth Int. Congr. IUSSI, London, 50-55.

Buschinger A (1973b) The role of daily temperature rhythms in brood development of ants of the tribe Leptothoracini (Hymenoptera: Formicidae). In: Wieser W (ed.), Effect of Temperature on Ectothermic Organisms, Springer-Verlag, New York, Heidelberg, Berlin, pp. 229-232.

Buschinger A (1974a) Zur Biologie der sozialparasitischen Ameise Leptothorax goesswaldi Kutter (Hym., Formicidae). Insectes Soc. 21: 133-144.

Buschinger A (1974b) Experimente und Beobachtungen zur Gründung und Entwicklung neuer Sozietäten der sklavenhaltenden Ameise Harpagoxenus sublaevis (Nyl.). Insectes Soc. 21: 381-406.

Buschinger A (1974c) Monogynie und Polygynie in Insektensozietäten. In: Schmidt GH (ed.), Sozialpolymorphismus bei Insekten, Wiss. Verl., Stuttgart, pp. 862-896.

Buschinger A (1975a) Sexual pheromones in ants. In: Noirot C, Howse PE, Le Masne G (eds.), *Pheromones and Defensive Secretions in Social Insects*, University of Dijon, Dijon, pp. 225-233.

Buschinger A (1975b) Eine genetische Komponente im Polymorphismus der dulotischen Ameise *Harpagoxenus sublaevis*. *Naturwissenschaften* 62: 239-240.

Buschinger A (1978a) Genetisch bedingte Entstehung geflügelter Weibchen bei der sklavenhaltenden Ameise *Harpagoxenus sublaevis* (Nyl.) (Hym., Form.). *Insectes Soc.* 25: 163-172.

Buschinger A (1978b) Queen polymorphism in ants. *Réunion Scientifique de la Section Française IUSSI, 1978, Besançon, Bull. Intérieur* 12-22.

Buschinger A (1981) Biological and systematic relationships of social parasitic Leptothoracini from Europe and North America. In: Howse PE, Clément J-L (eds.), *Biosystematics of Social Insects*, Academic Press, London, New York, pp. 211-222.

Buschinger A (1982) Stock manipulation in leptothoracine ants. Handout prepared for genetics workshop, *IXth Int. Congr. IUSSI, Boulder, Colorado*.

Buschinger A (1983) Sexual behavior and slave raiding of the dulotic ant *Harpagoxenus sublaevis* (Nyl.) under field conditions (Hym., Formicidae). *Insectes Soc.* 30: 235-240.

Buschinger A (1984) Review of Brian MV (1983), *Social Insects. Z. Tierpsychol.* 66: 79-81.

Buschinger A (1986) Evolution of social parasitism in ants. *Trends in Ecology and Evolution* 1: 155-160.

Buschinger A, Alloway TM (1977) Population structure and polymorphism in the slave-making ant *Harpagoxenus americanus* (Emery)

(Hymenoptera: Formicidae). Psyche 83: 233-242.

Buschinger A, Alloway TM (1978) Caste polymorphism in Harpagoxenus canadensis M.R. Smith (Hym., Formicidae). Insectes Soc. 25: 339-350.

Buschinger A, Alloway TM (1979) Sexual behaviour in the slave-making ant, Harpagoxenus canadensis M.R. Smith, and sexual pheromone experiments with H. canadensis, H. americanus (Emery), and H. sublaevis (Nylander) (Hymenoptera; Formicidae). Z. Tierpsychol. 49: 113-119.

Buschinger A, Ehrhardt W, Winter U (1980) The organization of slave-raids in dulotic ants - a comparative study (Hymenoptera: Formicidae). Z. Tierpsychol. 53: 245-264.

Buschinger A, Frenz G, Wunderlich M (1975) Untersuchungen zur Geschlechtstierproduktion der dulotischen Ameise Harpagoxenus sublaevis (Nyl.) (Hym., Formicidae). Insectes Soc. 22: 169-181.

Buschinger A, Winter U (1975) Der polymorphismus der sklavehaltenden Ameise Harpagoxenus sublaevis (Nyl.). Insectes Soc. 22: 333-362.

Buschinger A, Winter U (1977) Rekrutierung von Nestgenossen mittels Tandemlaufen bei Sklavenraubzügen der dulotischen Ameise Harpagoxenus sublaevis (Nyl.). Insectes Soc. 24: 183-190.

Buschinger A, Winter U (1978) Echte Arbeiterinnen, fertile Arbeiterinnen und sterile Wirtsweibchen in Völkern der dulotischen Ameise Harpagoxenus sublaevis (Nyl.) (Hym., Form.). Insectes Soc. 25: 63-78.

Buschinger A, Winter U (1982) Evolutionary trends in the parasitic ant genus Epimyrma. In: Breed MD, Michener CD, Evans HE (eds.), The Biology of Social Insects, Westview Press, Boulder, Colorado, pp. 266-269.

Buschinger A, Winter U (1983) Population studies of the dulotic ant, *Epimyrma ravouxi*, and the degenerate slave-maker, *E. kraussei* (Hymenoptera: Formicidae). *Entomol. Gener.* 8: 251-266.

Buschinger A, Winter U (1985) Life history and male morphology of the workerless parasitic ant *Epimyrma corsica* (Hymenoptera: Formicidae). *Entomol. Gener.* 10: 65-75.

Buschinger A, Winter U, Faber W (1983) The biology of *Myrmoxenus gordiagini* Ruzsky, a slave-making ant (Hymenoptera, Formicidae). *Psyche* 90: 335-342.

Bush GL (1975) Modes of animal speciation. *Ann. Rev. Ecol. Syst.* 6: 339-364.

Cagniant H (1979) La parthénogenèse thélytoque et arrhénotoque chez la fourmi *Cataglyphis cursor* Fonsc. (Hym. Form.) Cycle biologique en élevage des colonies avec reine et des colonies sans reine. *Insectes Soc.* 26: 51-60.

Cagniant H. (1982) La parthénogenèse thélytoque et arrhénotoque chez la fourmi *Cataglyphis cursor* Fonscolombe (Hymenoptera, Formicidae) Étude des oeufs pondus par les reines et les ouvrières: morphologie, devenir, influence sur le déterminisme de la caste reine. *Insectes Soc.* 29: 175-188.

Charnov EL (1978a) Evolution of eusocial behavior: offspring choice or parental parasitism? *J. Theor. Biol.* 75: 451-465.

Charnov EL (1978b) Sex ratio selection in eusocial Hymenoptera. *Am. Nat.* 112: 317-326.

Clark PJ, Evans FC (1954) Distance to nearest neighbor as a measure of spatial relationships in populations. *Ecology* 35: 445-453.

Cole BJ (1981) Dominance hierarchies in *Leptothorax* ants. *Science* 212: 83-84.

Cole BJ (1983) Multiple mating and the evolution of social behavior in the Hymenoptera. *Behav. Ecol. Sociobiol.* 12: 191-201.

Cole BJ (1986) The social behavior of *Leptothorax allardycei* (Hymenoptera, Formicidae): time budgets and the evolution of worker reproduction. *Behav. Ecol. Sociobiol.* 18: 165-173.

Collingwood CA (1979) *The Formicidae (Hymenoptera) of Fennoscandia and Denmark*, Scandinavian Science Press Ltd., Klampenborg, Denmark.

Colombel P (1971) Étude de l'inhibition de la ponte des ouvrières d'*Odontomachus haematodes* L. (Hym. Form. Poneridae). *C. R. Acad. Sci. Paris Ser. D*. 272: 970-972.

Colombel P (1972) Recherches sur la biologie et l'éthologie d'*Odontomachus haematodes* L. (Hym. Formicoidea, Poneridae) Biologie des ouvrières. *Insectes Soc*. 19: 171-193.

Contel EPB, Kerr WE (1976) Origin of males in *Melipona subnitida* estimated from data of an isozymic polymorphic system. *Genetica* 46: 271-277.

Crawley WC (1911) Parthenogenesis in worker ants, with special reference to two colonies of *Lasius niger* Linn. *Trans. Entomol. Soc. Lond*. 59: 657-663.

Crozier RH (1974) Allozyme analysis of reproductive strategy in the ant *Aphaenogaster rudis. Isozyme Bull*. 7: 18.

Crozier RH (1975) *Animal Cytogenetics, Vol. 3: Insecta, 7: Hymenoptera*. Gebrüder Bornträger, Berlin, Stuttgart.

Crozier RH (1977) Evolutionary genetics of the Hymenoptera. *Ann. Rev. Entomol*. 22: 263-288.

Crozier RH (1980) Genetical structure of social insect populations. In: Markl H (ed.), *Evolution of Social Behavior: Hypotheses and Empirical Tests*, Weinheim: Verlag Chemie, pp. 129-145.

Crozier RH, Page RE (1985) On being the right size: male contributions and multiple mating in social Hymenoptera. *Behav. Ecol. Sociobiol.* 18: 105-115.

Dartigues D, Passera L (1979) La ponte des ouvrieres chez la fourmi *Campanotus aethiops* Latreille (Hym. Formicidae). *Ann. Soc. Entomol. Fr.* 15: 109-116.

Darwin C (1859) *The Origin of Species*, 1st ed., reprinted 1968, Penguin, Harmondsworth.

Dawkins R (1976) *The Selfish Gene*, Oxford University Press, Oxford.

Dawkins R (1979) Twelve misunderstandings of kin selection. *Z. Tierpsychol* 51: 184-200.

Dawkins R (1982) *The Extended Phenotype*, Oxford University Press, Oxford.

Dawkins R (1986) *The Blind Watchmaker*, Longmans, Harlow.

Dawkins R, Krebs JR (1979) Arms races between and within species. *Proc. R. Soc. Lond. B. Biol. Sci.* 205: 489-511.

Dejean A, Passera L (1974) Ponte des ouvrières et inhibition royale chez la fourmi *Temnothorax recedens* (Nyl.) (Formicidae, Myrmicinae). *Insectes Soc.* 21: 343-355.

Delage B (1968) Recherches sur les fourmis moissoneuses du bassin aquitain: écologie et biologie. *Bull. Biol. Fr. Belg.* 102: 315-367.

Delage-Darchen B (1974) Écologie et biologie de *Crematogaster impressa* Emery, fourmi savanicole d'Afrique. *Insectes Soc.* 21: 13-34.

Del Rio Pesado MG, Alloway TM (1983) Polydomy in the slave-making ant *Harpagoxenus americanus* (Emery) (Hymenoptera: Formicidae). *Psyche* 90: 151-162.

Diehl SR, Bush GL (1984) An evolutionary and applied perspective of insect biotypes. *Ann. Rev. Entomol.* 29: 471-504.

Dobrzański J (1966) Contribution to the ethology of Leptothorax acervorum (Hymenoptera: Formicidae). Acta Biol. Exper. (Warsaw) 26: 71-78.

Doorn A van, Heringa J (1986) The ontogeny of a dominance hierarchy in colonies of the bumble bee Bombus terrestris (Hymenoptera, Apidae). Insectes Soc. 33: 3-25.

Douwes P, Buschinger A (1983) Två för Nordeuropa nya myror. Ent. Tidskr. 104: 1-4.

Dumpert K (1981) The Social Biology of Ants, tr. C. Johnson, Pitman, Boston, London, Melbourne.

Ehrhardt H-J (1962) Ablage übergrosser Eier durch Arbeiterinnen von Formica polyctena Förster (Ins., Hym.) in Gegenwart von Königinnen. Naturwissenschaften 49: 524-525.

Eickwort GC (1975) Gregarious nesting of the mason bee Hoplitis anthocopoides and the evolution of parasitism and sociality among megachilid bees. Evolution 29: 142-150.

Elmes GW (1973) Miniature queens of the ant Myrmica rubra L. (Hymenoptera, Formicidae). The Entomologist 106: 133-136.

Elmes GW (1974) Colony populations of Myrmica sulcinodis Nyl. (Hym. Formicidae). Oecologia 15: 337-343.

Elmes GW (1976) Some observations on the microgyne form of Myrmica rubra L. (Hymenoptera, Formicidae). Insectes Soc. 23: 3-21.

Elmes GW (1978) A morphometric comparison of three closely related species of Myrmica (Formicidae), including a new species from England. Syst. Entomol. 3: 131-145.

Evans HE (1977) Extrinsic versus intrinsic factors in the evolution of insect sociality. BioScience 27: 613-617.

Evers CA, Seeley TD (1986) Kin discrimination and aggression in honey bee colonies with laying workers. Anim. Behav. 34: 924-925.

Fielde AM (1905) Observations on the progeny of virgin ants. Biol. Bull. 9: 355-360.

Fletcher DJC, Ross KG (1985) Regulation of reproduction in eusocial Hymenoptera. Ann. Rev. Entomol. 30: 319-343.

Forsyth A (1980) Worker control of queen density in Hymenopteran societies. Am. Nat. 116: 895-898.

Forsyth A (1981) Sex ratio and parental investment in an ant population. Evolution 35: 1252-1253.

Franks NR (1985) Reproduction, foraging efficiency and worker polymorphism in army ants. In: Hölldobler B, Lindauer M (eds.), Experimental Behavioral Ecology and Sociobiology, Gustav Fischer Verlag, Stuttgart, New York, pp. 91-107.

Franks NR, Scovell E (1983) Dominance and reproductive success among slave-making worker ants. Nature 304: 724-725.

Free JB (1955) The behaviour of egg-laying workers of bumble bee colonies. British J. Anim. Behav. 3: 147-153.

Free JB, Weinberg I, Whiten A (1969) The egg-eating behaviour of Bombus lapidarius L. Behaviour 35: 313-317.

Free JB, Williams IH (1974) Factors determining food storage and brood rearing in honeybee (Apis mellifera L.) comb. J. Entomol. 49: 47-63.

Freeland J (1958) Biological and social patterns in the Australian bulldog ants of the genus Myrmecia. Aust. J. Zool. 6: 1-18.

Fresneau D (1984) Développement ovarien et statut social chez une fourmi primitive Neoponera obscuricornis Emery (Hym Formicidae, Ponerinae). Insectes Soc. 31: 387-402.

Gadagkar R (1985) Kin recognition in social insects and other animals - A review of recent findings and a consideration of their relevance for the theory of kin selection. Proc. Indian Acad. Sci. (Anim. Sci.) 94: 587-621.

Gladstone DE (1981) Why are there no ant slave rebellions? *Am. Nat.* 117: 779-781.

Goodloe L, Sandwald R (1985) Host specificity in colony-founding by *Polyergus lucidus* queens (Hymenoptera: Formicidae). *Psyche* 92: 297-302.

Greene A (1979) Behavioral characters as indicators of yellowjacket phylogeny (Hymenoptera: Vespidae). *Ann. Entomol. Soc. Am.* 72: 614-619.

Greene A, Akre RD, Landolt P (1976) The aerial yellowjacket *Dolichovespula arenaria* (Fab.): nesting biology, reproductive production, and behavior (Hymenoptera: Vespidae). *Melanderia* 26: 1-34.

Hamilton WD (1964) The genetical evolution of social behaviour I, II. *J. Theor. Biol.* 7: 1-52.

Hamilton WD (1967) Extraordinary sex ratios. *Science* 156: 477-488.

Hamilton WD (1972) Altruism and related phenomena, mainly in social insects. *Ann. Rev. Ecol. Syst.* 3: 193-232.

Hamilton WD (1979) Wingless and fighting males in fig wasps and other insects. In: Blum MS, Blum NA (eds.), *Sexual Selection and Reproductive Competition in Insects*, Academic Press, New York, pp. 167-220.

Haskins CP (1978) Sexual calling behavior in highly primitive ants. *Psyche* 85: 407-415.

Haskins CP, Haskins EF (1950) Notes on the biology and social behavior of the archaic ponerine ants of the genera *Myrmecia* and *Promyrmecia*. *Ann. Entomol. Soc. Am.* 43: 461-491.

Herbers JM (1984) Queen-worker conflict and eusocial evolution in a polygynous ant species. *Evolution* 38: 631-643.

Herbers JM (1986) Nest site limitation and facultative polygyny in the ant *Leptothorax longispinosus*. *Behav. Ecol. Sociobiol.*

19: 115-122.

Hermann HR, Blum MS (1981) Defensive mechanisms in the social Hymenoptera. In: Hermann HR (ed.), Social Insects, Vol. II, Academic Press, New York, London, pp. 77-197.

Hesse B (1979) Ovarentwicklung und Eiablage bei Arbeiterinnen verschiedener Rassen von Apis mellifera L. Apidologie 10: 67-68.

Hogeweg P, Hesper B (1983) The ontogeny of the interaction structure in bumble bee colonies: a MIRROR model. Behav. Ecol. Sociobiol. 12: 271-283.

Hölldobler B (1976) Tournaments and slavery in a desert ant. Science 192: 912-914.

Hölldobler B (1979) Territoriality in ants. Proc. Am. Philos. Soc. 123: 211-218.

Hölldobler B (1984) Evolution of insect communication. In: Lewis T (ed.), Insect Communication, Academic Press, London, pp. 349-377.

Hölldobler B, Bartz SH (1985) Sociobiology of reproduction in ants. In: Hölldobler B, Lindauer M (eds.), Experimental Behavioral Ecology and Sociobiology, Gustav Fischer Verlag, Stuttgart, New York, pp. 237-257.

Hölldobler B, Haskins CP (1977) Sexual calling behavior in primitive ants. Science 195: 793-794.

Hölldobler B, Michener CD (1980) Mechanisms of identification and discrimination in social Hymenoptera. In: Markl H (ed.), Evolution of Social Behavior: Hypotheses and Empirical Tests, Weinheim: Verlag Chemie, pp. 35-57.

Hölldobler B, Wilson EO (1977) The number of queens: an important trait in ant evolution. Naturwissenschaften 64: 8-15.

Hölldobler B, Wilson EO (1983) Queen control in colonies of weaver ants (Hymenoptera: Formicidae). Ann. Entomol. Soc. Am. 76: 235-238.

Honk C van (1982) The social structure of Bombus terrestris colonies: a review. In: Breed MD, Michener CD, Evans HE (eds.), The Biology of Social Insects, Westview Press, Boulder, Colorado, pp. 196-200.

Honk C van, Hogeweg P (1981) The ontogeny of the social structure in a captive Bombus terrestris colony. Behav. Ecol. Sociobiol. 9:111-119.

Honk CJG van, Röseler P-F, Velthuis HHW, Hoogeveen JC (1981) Factors influencing the egg-laying of workers in a captive Bombus terrestris colony. Behav. Ecol. Sociobiol. 9: 9-14.

Hung A C-F (1973) Reproductive biology in dulotic ants: preliminary report (Hymenoptera: Formicidae). Entomol. News 84: 253-259.

Imms AD (1977) Imm's General Textbook of Entomology, Vol. I, 10th ed., (ed. Richards OW, Davies RG), Chapman and Hall, London.

Ishay JS (1964) Observations sur la biologie de la guêpe orientale Vespa orientalis. Insectes Soc. 11: 193-206.

Ishay JS (1976) Comb building by the oriental hornet (Vespa orientalis). Anim. Behav. 24: 72-83.

Itow T, Kobayashi K, Kubota M, Ogata K, Imai HT, Crozier RH (1984) The reproductive cycle of the queenless ant Pristomyrmex pungens. Insectes Soc. 31: 87-102.

Iwasa Y (1981) Role of sex ratio in the evolution of eusociality in haplodiploid social insects. J. Theor. Biol. 93: 125-142.

Jacobson M (1965) Insect Sex Attractants, Interscience, New York.

Jaisson P (1985) Social behaviour. In: Kerkut G, Gilbert L (eds.), Comprehensive Insect Physiology, Biochemistry, and Pharmacology, Vol. 9, Behavior, Pergamon Press, Oxford, New York, pp. 673-694.

Jay SC (1968) Factors influencing ovary development of worker honeybees under natural conditions. *Can. J. Zool.* 46: 345-347.

Jay SC (1970) The effects of various combinations of immature queen and worker bees on the ovary development of worker honybees in colonies with and without queens. *Can. J. Zool.* 48: 169-173.

Jay SC (1972) Ovary development of worker honeybees when separated from worker brood by various methods. *Can. J. Zool.* 50: 661-664.

Jay SC (1975) Factors influencing ovary development of worker honeybees of European and African origin. *Can. J. Zool.* 53: 1387-1390.

Jay SC, Jay DH (1976) The effect of various types of brood comb on the ovary development of worker honeybees. *Can. J. Zool.* 54: 1724-1726.

Jay SC, Nelson EV (1973) The effects of laying worker honeybees (*Apis mellifera* L.) and their brood on the ovary development of other worker honeybees. *Can. J. Zool.* 51: 629-632.

Jeanne RL (1980) Evolution of social behavior in the Vespidae. *Ann. Rev. Entomol.* 25: 371-396.

Jessen K (1986) Biosystematic revision of the parasitic ant genus *Epimyrma*. Handout prepared for symposium, Biosystematics of the ant tribe Leptothoracini, *Xth Int. Congr. IUSSI, Munich.*

Katayama E (1971) Observations on the brood development in *Bombus ignitus* (Hymenoptera, Apidae) 1. Egg-laying habits of queens and workers. *Kontyû* 39: 189-203.

Katayama E (1974) Egg-laying habits and brood development in *Bombus hypocrita* (Hymenoptera, Apidae) 1. Egg-laying habits of queens and workers. *Kontyû* 42: 416-438.

Kerr WE (1969) Some aspects of the evolution of social bees (Apidae). In: Dobzhansky T, Hecht M, Steere W (eds.), *Evolutionary Biology*, Vol. 3, Apppleton-Century Crofts, New York, pp. 119-175.

Korst P, Velthuis HHW (1982) The nature of trophallaxis in honeybees. Insectes Soc. 29: 209-221.

Kropáčová S, Haslbachová H (1969) The development of ovaries in worker honeybees in a queenright colony. J. Apic. Res. 8: 57-64.

Kropáčová S, Haslbachová H (1970) The development of ovaries in worker honeybees in queenright colonies examined before and after swarming. J. Apic. Res. 9: 65-70.

Kropáčová S, Haslbachová H (1971) The influence of queenlessness and of unsealed brood on the development of ovaries in worker honeybees. J. Apic. Res. 10: 57-61.

Kugler J, Motro M, Ishay JS (1979) Comb building abilities of Vespa orientalis L. queenless workers. Insectes Soc. 26: 147-153.

Kwait EC, Topoff H (1983) Emigration raids by slave-making ants: a rapid transit system for colony relocation. Psyche 90: 307-312.

Landolt PJ, Akre RD, Greene A (1977) Effects of colony division on Vespula atropilosa (Sladen) (Hymenoptera: Vespidae). J. Kans. Entomol. Soc. 50: 135-147.

Ledoux A (1950) Recherche sur la biologie de la fourmi fileuse (Oecophylla longinoda Latr.). Ann. Sci. Nat. Zool. Biol. Anim., Ser. 11 12: 314-461.

Ledoux A, Dargagnon D (1973) La formation des castes chez la fourmi Aphaenogaster senilis Mayr. C. R. Acad. Sci. Paris Ser. D. 276: 551-553.

Le Moli F (1980) On the origin of slaves in dulotic ant societies. Boll. Zool. 47: 207-212.

Levings SC, Traniello JFA (1981) Territoriality, nest dispersion, and community structure in ants. Psyche 88: 265-319.

Lewis T (1984) (ed.) Insect Communication, Academic Press, London.

Lin N, Michener CD (1972) Evolution of sociality in insects.

Q. Rev. Biol. 47: 131-159.

Lubbock J (1885) *Ants, Bees, and Wasps.* Kegan Paul, Trench and Co., London.

Menken S (1980) Allozyme polymorphism and the speciation process in small ermine moths (Lepidoptera, Yponomeutidae). Studies in *Yponomeuta* 2. J.H. Pasmans B.V., 'S-Gravenhage, The Netherlands.

Michener CD (1974) *The Social Behavior of the Bees,* Belknap Press of Harvard University Press, Cambridge, Massachusetts.

Möglich M (1978) Social organization of nest emigration in *Leptothorax* (Hym., Form.). *Insectes Soc.* 25: 205-225.

Möglich M (1979) Tandem calling pheromone in the genus *Leptothorax* (Hymenoptera: Formicidae): Behavioral analysis of specificity. *J. Chem. Ecol.* 5: 35-52.

Möglich M, Maschwitz U, Hölldobler B (1974) Tandem calling: a new kind of signal in ant communication. *Science* 186: 1046-1047.

Montagner H (1966) Sur l'origine des mâles dans les sociétés de guêpes du genre *Vespa. C. R. Acad. Sci. Paris Ser. D.* 263: 785-787.

Morgan ED (1984) Chemical words and phrases in the language of pheromones for foraging and recruitment. In: Lewis T (ed.), *Insect Communication,* Academic Press, London, pp. 169-194.

Moritz RFA, Hillesheim E (1985) Inheritance of dominance in honeybees (*Apis mellifera capensis Esch.*). *Behav. Ecol. Sociobiol.* 17: 87-89.

Morse DH (1982) Behavior and ecology of bumble bees. In: Hermann HR (ed.), *Social Insects,* Vol. III, Academic Press, New York, London, pp. 245-322.

Nei M (1972) Genetic distance between populations. *Am. Nat.* 106: 283-292.

Nonacs P (1986a) Ant reproductive strategies and sex allocation theory. Q. Rev. Biol. 61: 1-21.

Nonacs P (1986b) Sex ratio determination within colonies of ants. Evolution 40: 199-204.

Ollett DG, Morgan ED, Attygalle AB, Billen JPJ (1987) The contents of the Dufour gland of the ant *Harpagoxenus sublaevis* Nyl. (Hymenoptera: Formicidae). Z. Naturforsch. 42c: 141-146.

Orians GH, Pearson NE (1979) On the theory of central place foraging. In: Horn DJ, Stairs GR, Mitchell RD (eds.), Analysis of Ecological Systems, Ohio State University Press, Columbus.

Oster G, Eshel I, Cohen D (1977) Worker-queen conflict and the evolution of social insects. Theor. Pop. Biol. 12: 49-85.

Oster GF, Wilson EO (1978) Caste and Ecology in the Social Insects, Princeton University Press, Princeton, New Jersey.

Owen RE, Plowright RC (1982) Worker-queen conflict and male parentage in bumble bees. Behav. Ecol. Sociobiol. 11: 91-99.

Owen RE, Rodd FH, Plowright RC (1980) Sex ratios in bumble bee colonies: complications due to orphaning? Behav. Ecol. Sociobiol. 7: 287-291.

Page RE (1986) Sperm utilization in social insects. Ann. Rev. Entomol. 31: 297-320.

Page RE, Metcalf RA (1984) A population investment sex ratio for the honeybee (*Apis mellifera* L.). Am. Nat. 124: 680-702.

Pamilo P (1982a) Genetic population structure in polygynous *Formica* ants. Heredity 48: 95-106.

Pamilo P (1982b) Multiple mating in *Formica* ants. Hereditas 97: 37-45.

Pamilo P (1983) Genetic differentiation within subdivided populations of *Formica* ants. Evolution 37: 1010-1022.

Pamilo P (1984) Genetic relatedness and evolution of insect sociality. *Behav. Ecol. Sociobiol.* 15: 241-248.

Pamilo P, Crozier RH (1982) Measuring genetic relatedness in natural populations: methodology. *Theor. Pop. Biol.* 21: 171-193.

Pamilo P, Rosengren R (1983) Sex ratio strategies in *Formica* ants. *Oikos* 40: 24-35.

Pamilo P, Varvio-Aho SL (1979) Genetic structure of nests in the ant *Formica sanguinea*. *Behav. Ecol. Sociobiol.* 6: 91-98.

Passera L (1966) La ponte des ouvrières de la fourmi *Plagiolepis pygmaea* Latr. (Hym. Formicidae): oeufs reproducteurs et oeufs alimentaires. *C. R. Acad. Sci. Paris Ser. D.* 263: 1095-1098.

Pearson B (1981) The electrophoretic determination of *Myrmica rubra* microgynes as a social parasite: possible significance in the evolution of ant social parasites. In: Howse PE, Clément J-L (eds.), *Biosystematics of Social Insects*, Academic Press, London, New York, pp. 75-84.

Pearson B (1983) Intra-colonial relatedness amongst workers in a population of nests of the polygynous ant, *Myrmica rubra* Latreille. *Behav. Ecol. Sociobiol.* 12: 1-4.

Pearson B, Child AR (1980) The distribution of an esterase polymorphism in macrogynes and microgynes of *Myrmica rubra* Latreille. *Evolution* 34: 105-109.

Pisarski B (1963) Nouvelle espèce du genre *Harpagoxenus* For. de la Mongolie (Hymenoptera, Formicidae). *Bulletin de l'Académie Polonaise des Sciences.* 11: 39-41.

Plateaux L (1970) Sur le polymorphisme social de la fourmi *Leptothorax nylanderi* (Förster). I. Morphologie et biologie comparées des castes. *Ann. Sci. Nat. Zool. Biol. Anim., Ser. 12* 12: 373-478.

Plateaux L (1981) The *pallens* morph of the ant *Leptothorax* nylanderi: description, formal genetics, and study of populations. In: Howse PE, Clément J-L (eds.), Biosystematics of Social Insects, Academic Press, New York, London, pp. 63-74.

Plowright RC, Laverty TM (1984) The ecology and sociobiology of bumble bees. Ann. Rev. Entomol. 29: 175-199.

Pomeroy N (1979) Brood bionomics of Bombus ruderatus in New Zealand (Hymenoptera: Apidae). Can. Entomol. 111: 865-874.

Raignier A (1972) Sur l'origine des nouvelles sociétés des fourmis voyageuses africaines (Hyménoptères Formicidae, Dorylinae). Insectes Soc. 19: 153-170.

Regnier FE, Wilson EO (1971) Chemical communication and "propaganda" in slave-maker ants. Science 172: 267-269.

Ribbands CR (1953) The Behaviour and Social Life of Honeybees, Bee Research Association Ltd., London.

Richards KW (1977) Ovarian development of queen and worker bumble bees (Hymenoptera: Apidae) in southern Alberta. Can. Entomol. 109: 109-116.

Richards OW (1971) The biology of the social wasps (Hymenoptera, Vespidae). Biol. Rev. 46: 483-528.

Ridley M (1983) The Explanation of Organic Diversity, Clarendon Press, Oxford.

Röseler P-F, Röseler I (1977) Dominance in bumble bees. Proc. VIIIth Int. Congr. IUSSI, Wageningen, pp. 232-235.

Ross KG (1985) Aspects of worker reproduction in four social wasp species (Insecta: Hymenoptera: Vespidae). J. Zool. Ser. A. 205: 411-424.

Ross KG (1986) Kin selection and the problem of sperm utilization in social insects. Nature 323: 798-800.

Ross KG, Fletcher DJC (1985) Comparative study of genetic and social structure in two forms of the fire ant Solenopsis invicta (Hymenoptera: Formicidae). Behav. Ecol. Sociobiol. 17: 349-356.

Ruttner F (1977) The problem of the cape bee (Apis mellifera capensis Escholtz): parthenogenesis - size of population - evolution. Apidologie 8: 281-294.

Ruttner F, Koeniger N, Veith HJ (1976) Queen substance bei eierlegenden Arbeiterinnen der Honigbiene (Apis mellifica L.). Naturwissenschaften 63: 434.

Sakagami SF (1954) Occurrence of an aggressive behaviour in queenless hives, with considerations on the social organization of honeybee. Insectes Soc. 1: 331-343.

Sakagami SF (1958) The false queen: fourth adjustive response in dequeened honeybee colonies. Behaviour 13: 280-296.

Sakagami SF (1982) Stingless bees. In: Hermann HR (ed.), Social Insects, Vol. III, Academic Press, New York, London, pp. 361-423.

Sato T (1986) A brood parasitic catfish of mouthbrooding cichlid fishes in Lake Tanganyika. Nature 323: 58-59.

Schmidt GH (1982) Egg dimorphism and male production in Formica polyctena Foerster. In: Breed MD, Michener CD, Evans HE (eds.), The Biology of Social Insects, Westview Press, Boulder, Colorado, pp. 243-247.

Seeley TD (1985) Honeybee Ecology, Princeton University Press, Princeton.

Smallwood J (1982) Nest relocations in ants. Insectes Soc. 29: 138-147.

Smeeton L (1981) The source of males in Myrmica rubra L. (Hym. Formicidae). Insectes Soc. 28: 263-278.

Smeeton L (1982a) The effect of larvae on the production of reproductive eggs by workers of Myrmica rubra L.

(Hym. Formicidae). *Insectes Soc.* 29: 455-464.

Smeeton L (1982b) The effect of age on the production of reproductive eggs by workers of *Myrmica rubra* L. (Hym. Formicidae). *Insectes Soc.* 29: 465-474.

Smeeton L (1982c) The effects of the sizes of colony worker and food store on the production of reproductive eggs by workers of *Myrmica rubra* L. (Hym. Formicidae). *Insectes Soc.* 29: 475-484.

Snedecor GW, Cochran WG (1980) *Statistical Methods*, 7th ed., Iowa State University Press, Iowa.

Sokal RR, Rohlf FJ (1969) *Biometry*, W.H. Freeman and Co., San Francisco.

Sommeijer MJ (1984) Distribution of labour among workers of *Melipona favosa* F.: age-polyethism and worker oviposition. *Insectes Soc.* 31: 171-184.

Sommeijer MJ, Houtekamer JL, Bos W (1984) Cell construction and egg-laying in *Trigona nigra paupera* with a note on the adaptive significance of oviposition behaviour of stingless bees. *Insectes Soc.* 31: 199-217.

Sommeijer MJ, Velthuis HHW (1977) Worker oviposition in orphan colonies of *Melipona favosa* (F.). *Proc. VIIIth Int. Congr. IUSSI, Wageningen*, pp. 315-316.

Spradbery JP (1971) Seasonal changes in the population structure of wasp colonies (Hymenoptera: Vespidae). *J. Anim. Ecol.* 40: 501-523.

Spradbery JP (1973) *Wasps: An Account of the Biology and Natural History of Solitary and Social Wasps*, Sidgwick and Jackson, London.

Stanley SM (1979) *Macroevolution, Pattern and Process*, W.H. Freeman and Co., San Francisco.

Starr CK (1979) Origin and evolution of insect sociality: a review of modern theory. In: Hermann HR (ed.), Social Insects, Vol. I, Academic Press, New York, London, pp. 35-79.

Starr CK (1984) Sperm competition, kinship, and sociality in Aculeate Hymenoptera. In: Smith RL (ed.), Sperm Competition and the Evolution of Animal Mating Systems, Academic Press, Orlando, pp. 427-464.

Stuart RJ (1981) Abdominal trophallaxis in the slave-making ant, Harpagoxenus americanus (Hymenoptera: Formicidae). Psyche 88: 331-334.

Stuart RJ (1984) Experiments on colony foundation in the slave-making ant Harpagoxenus canadensis M.R. Smith (Hymenoptera: Formicidae). Can. J. Zool. 62: 1995-2001.

Stuart RJ, Alloway TM (1982) Territoriality and the origin of slave raiding in Leptothoracine ants. Science 215: 1262-1263.

Stuart RJ, Alloway TM (1983) The slave-making ant Harpagoxenus canadensis M.R. Smith, and its host-species Leptothorax muscorum (Nylander): slave raiding and territoriality. Behaviour 85: 58-90.

Stuart RJ, Alloway TM (1985) Behavioural evolution and domestic degeneration in obligatory slave-making ants (Hymenoptera: Formicidae: Leptothoracini). Anim. Behav. 33: 1080-1088.

Sturtevant AH (1927) The social parasitism of the ant Harpagoxenus americanus. Psyche 34: 1-9.

Taber S (1980) Bee behavior. Am. Bee J. 120: 82-83 and 135.

Taylor PD (1981) Sex ratio compensation in ant populations. Evolution 35: 1250-1251.

Thornhill R, Alcock J (1983) The Evolution of Insect Mating Systems, Harvard University Press, Cambridge, Mass.

Topoff H, LaMon B, Goodloe L, Goldstein M (1984) Social and orientation behavior of Polyergus breviceps during slave-making raids. Behav. Ecol. Sociobiol. 15: 273-279.

Torossian C (1968) Recherches sur la biologie et l'éthologie de Dolichoderus quadripunctatus (Hym. Form. Dolichoderidae). Insectes Soc. 15: 51-72.

Trivers RL (1972) Parental investment and sexual selection. In: Campbell B (ed.), Sexual Selection and the Descent of Man, Aldine Publishing Co., Chicago, pp. 139-179.

Trivers RL (1974) Parent-offspring conflict. Amer. Zool. 14: 249-264.

Trivers RL, Hare H (1976) Haplodiploidy and the evolution of the social insects. Science 191: 249-263.

Velthuis HHW (1970) Ovarian development in Apis mellifera worker bees. Entomol. Exp. Appl. 13: 377-394.

Velthuis HHW (1977) Egg laying, aggression and dominance in bees. Proc. XVth Int. Congr. Entomol., Washington, pp. 436-449.

Velthuis HHW (1985) The honeybee queen and the social organization of her colony. In: Hölldobler B, Lindauer M (eds.), Experimental Behavioral Ecology and Sociobiology, Gustav Fischer Verlag, Stuttgart, New York, pp. 343-357.

Velthuis HHW, Clement J, Morse RA, Laigo FM (1971) The ovaries of Apis dorsata workers and queens from the Philippines. J. Apic. Res. 10: 63-66.

Velthuis HHW, Verheijen FJ, Gottenbos AJ (1965) Laying worker honeybee: similarities to the queen. Nature 207: 1314.

Visscher PK (1986) Kinship discrimination in queen rearing by honey bees (Apis mellifera). Behav. Ecol. Sociobiol. 18: 453-460.

Ward PS (1983a) Genetic relatedness and colony organization in a species complex of ponerine ants. I. Phenotypic and genotypic composition of colonies. *Behav. Ecol. Sociobiol.* 12: 285-299.

Ward PS (1983b) Genetic relatedness and colony organization in a species complex of ponerine ants. II. Patterns of sex ratio investment. *Behav. Ecol. Sociobiol.* 12: 301-307.

Weir JS (1959) Interrelation of queen and worker oviposition in *Myrmica*. *Physiol. Zool.* 32: 63-77.

Wesson LG (1939) Contributions to the natural history of *Harpagoxenus americanus* Emery (Hymenoptera: Formicidae). *Trans. Am. Entomol. Soc*. 65: 97-122.

Wesson LG (1940) Observations on *Leptothorax duloticus.* *Bulletin Brooklyn Entomol. Soc*. 35: 73-83.

West-Eberhard MJ (1978) Polygyny and the evolution of social behavior in wasps. *J. Kans. Entomol. Soc*. 51: 832-856.

West-Eberhard MJ (1979) Sexual selection, social competition, and evolution. *Proc. Am. Phil. Soc*. 123: 222-234.

West-Eberhard MJ (1981) Intragroup selection and the evolution of insect societies. In: Alexander RD, Tinkle DW (eds.), *Natural Selection and Social Behavior*, Chiron, New York, pp. 3-17.

West-Eberhard MJ (1982) Introduction to the symposium on the evolution and ontogeny of eusociality. In: Breed MD, Michener CD, Evans HE (eds.), *The Biology of Social Insects*, Westview Press, Boulder, Colorado, pp. 185-186.

West-Eberhard MJ (1986) Alternative adaptations, speciation, and phylogeny (A Review). *Proc. Natl. Acad. Sci. USA* 83: 1388-1392.

Wheeler DE (1984) Behaviour of the ant *Procryptocerus scabriscutus* (Hymenoptera: Formicidae), with comparisons to other cephalotines. *Psyche* 91: 171-192.

Wheeler DE (1986) Developmental and physiological determinants of caste in social Hymenoptera: evolutionary implications.

Am. Nat. 128: 13-34.

Wheeler WM (1905) An interpretation of the slave-making instincts in ants. *Bulletin American Museum Natural History* 21: 1-16.

Wheeler WM (1910) *Ants: Their Structure, Development and Behaviour*, Columbia University Press, New York.

Wheeler WM (1916) Notes on some slave-raids of the western Amazon Ant (*Polyergus breviceps* Emery). *J. New York Entomol. Soc.* 24: 107-118.

White MJD (1978) *Modes of Speciation*, W.H. Freeman and Co., San Francisco.

Wigglesworth VB (1972) *The Principles of Insect Physiology*, 7th ed., Chapman and Hall, London.

Wilkinson GS, McCracken GF (1985) On estimating relatedness using genetic markers. *Evolution* 39: 1169-1174.

Williams GC (1966) *Adaptation and Natural Selection*, Princeton University Press, Princeton, New Jersey.

Wilson EO (1971) *The Insect Societies*, Belknap Press of Harvard University Press, Cambridge, Mass.

Wilson EO (1975a) *Sociobiology: The New Synthesis*, Belknap Press of Harvard University Press, Cambridge, Mass.

Wilson EO (1975b) *Leptothorax duloticus* and the beginnings of slavery in ants. *Evolution* 29: 108-119.

Wilson EO (1975c) Slavery in ants. *Scientific American* 232: 32-36.

Wilson EO (1976) A social ethogram of the neotropical arboreal ant *Zacryptocerus varians* (Fr. Smith). *Anim. Behav.* 24: 354-363.

Wilson EO (1985) The sociogenesis of insect colonies. *Science* 228: 1489-1495.

Winter U (1979) Untersuchungen zum Raubzugverhalten der dulotischen Ameise *Harpagoxenus sublaevis* (Nyl.). *Insectes Soc.* 26: 123-135.

Winter U, Buschinger A (1983) The reproductive biology of a slave-maker ant, *Epimyrma ravouxi*, and a degenerate slave-maker, *E. kraussei* (Hymenoptera: Formicidae). *Entomol. Gen.* 9: 1-15.

Winter U, Buschinger A (1986) Genetically mediated queen polymorphism and caste determination in the slave-making ant *Harpagoxenus sublaevis* (Hymenoptera: Formicidae). *Entomol. Gen.* 11: 125-137.

Yasuno M (1964) The study of the ant population in the grassland at Mt. Hakkôda. III. The effect of the slave-making ant, *Polyergus samurai*, upon the nest distribution pattern of the slave ant, *Formica fusca japonica*. *Science Reports of the Tôhoku University, Series IV (Biology)* 30: 167-170.