

# The Conflict over Male Production in the Vespinae Wasps

A THESIS PRESENTED TO UNIVERSITY OF SHEFFIELD DEPARTMENT OF  
ANIMAL AND PLANT SCIENCES FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Kevin R. Foster  
November 2000

SUPERVISED BY FRANCIS L.W. RATNIEKS

I dedicate my thesis to my wonderful parents, for this is clearly all their fault.

## Abstract

Conflict is inherent in social systems. The eusocial hymenoptera (bees, ants and wasps) are no exception with several reproductive conflicts affecting their societies. This thesis investigates the resolution one of these conflicts, that over male production, in a group of known phylogeny, the Vespinae wasps (hornets and yellowjackets). Extensive conflict is predicted as both the queen and workers can produce males. However, worker behaviour is expected to depend on queen mating frequency (formally effective paternity). With single paternity, workers are more related to each other's sons than the queen's sons and worker male production is expected. With paternity above two, workers are more related to the queen's sons and mutual policing (worker policing) is predicted.

To test these predictions, paternity was analysed and compared to worker reproductive behaviour in seven vespine species from the genera *Vespa*, *Dolichovespula* (five species) and *Vespula*. In line with prediction, *Dolichovespula* had mostly single paternity and worker male production in colonies with a queen. Furthermore, in *D. saxonica*, worker male production was correlated with paternity among colonies: evidence of facultative worker policing. *Vespula vulgaris* had double paternity, no worker male production and worker policing. *Vespa crabro* did not fit relatedness predictions with single paternity but worker policing. Finally, queen loss in reproductive colonies negatively correlated with paternity across species suggesting that matricide in response to reproductive conflict may occur.

Five general conclusions on the resolution of reproductive conflicts can be made. (1) Relatedness is important. (2) Other factors, such as the cost of conflict, are also important as worker policing was found at paternity below two in *V. crabro* and some *D. saxonica* and *V. vulgaris* colonies. (3) Worker policing is important. (4) Conflict resolution is evolutionarily labile, with otherwise similar species having very different resolutions to conflict. (5) Conflict is associated with low colony size, with most conflict in *Dolichovespula* which has the smallest colonies.

## Acknowledgments

So many people to thank. First of all my supervisor Francis, who was always there to help and turned my confusion into science. John Gulliver and Julie McGill for making it all possible. My labmates Adam, Steve and Thibaud and soul mates Matt, Fi, Kate and Jon who make me smile. Perttö, Cia, Niclas, Anja, Matts, Palle, Simon and Per provided light in the darkness of Uppsala and Minttu, Heikki, Katja, Kriko and Lotta helped me 'find wasps' in Finland. Thanks to Matt Sledge, for sharing Italy and stimulating discussions, Tom Wenseleers for numerous debates over beer and Steve Martin for teaching me the ways of the tropics. In Dorset, Alan Raybould helped me overcome oppressive administration and Pete, Zoe, Andy, Deanne and Becka made the most of two long hot summers. Emily, Dan, Miki, Nick, Tim, Ross, Rob and my sister Gillian have been there throughout. Finally, there is Madeline, who is my love.

This thesis was funded by a BBSRC studentship and a CASE award from the Institute of Terrestrial Ecology, Furzebrook, Dorset.

# Table of Contents

## CHAPTER 1. Introduction

1.1 THEORETICAL BACKGROUND .....	1
1.11 Kin structure and reproductive conflict .....	1
1.12 Conflict over male production .....	1
Empirical data .....	2
1.2 WHY STUDY THE VESPINAE WASPS? .....	3
1.21 A well-supported phylogeny .....	4
Similar basic biology .....	4
1.23 A few important variables: kin structure, conflict resolution and colony size .....	6

## CHAPTER 2. How the thesis evolved

2.1 THE EUROPEAN HORNET, <i>VESPA CRABRO</i> .....	7
2.11 Paternity and worker relatedness .....	7
2.12 Zombie hornets .....	7
2.13 The effect of genetics on cuticular chemistry .....	8
2.2 SPITE .....	8
2.3 DOLICHOVESPULA WASPS .....	9
2.31 New Forest collection and observation .....	9
2.32 Microsatellite analysis .....	10
2.4 THE COMMON WASP <i>VESPULA VULGARIS</i> .....	10
2.41 Microsatellite analysis .....	10
2.42 Worker policing .....	10
2.5 THE HUNT FOR <i>PROVESPA</i> .....	11
2.6 THE HUNT FOR <i>VESPULA AUSTRIACA</i> .....	12
2.7 THE EVOLUTION OF WORKER POLICING AT LOW PATERNITY .....	12
2.8 WORKER POLICING IN THE HORNET <i>VESPA CRABRO</i> .....	12

## CHAPTER 3. Methodology

3.1 DNA MICROSATELLITE ANALYSIS .....	13
3.11 DNA extraction .....	13
3.12 PCR reaction .....	13
3.13 Separation and visualisation .....	13
3.2 STATISTICAL ANALYSIS OF GENETIC DATA .....	14
3.21 Worker-worker relatedness and effective paternity .....	14
3.22 Errors in paternity estimation .....	16
3.23 Errors in worker male production estimation .....	16
3.3 COLLECTION AND STUDY OF LIVE WASPS .....	17
3.31 Finding nests .....	17
3.32 Nest collection .....	17
3.33 Nest relocation .....	17
3.34 Nest observation .....	19
3.35 Worker policing assay .....	19

## **CHAPTER 4. Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps**

4.1 ABSTRACT .....	21
4.2 INTRODUCTION .....	21
4.3 METHODS .....	22
4.31 Study organism and sample collection .....	22
4.32 Molecular methods .....	22
4.33 Statistical methods .....	23
4.34 Pedigree estimates .....	23
4.35 Non-sampling error .....	23
4.36 Non-detection error .....	23
RESULTS .....	24
4.41 Allelic diversity .....	24
4.42 Estimates of relatedness .....	24
4.43 Pedigree estimates .....	24
4.44 Statistical power of analysis .....	24
4.5 DISCUSSION .....	25
4.6 ACKNOWLEDGEMENTS .....	27
4.7 APPENDIX .....	27
4.71 Nest-level non-detection error estimates .....	27
4.72 Corrections for ambiguity in identification of paternal alleles .....	27

## **CHAPTER 5. Do hornets have zombie workers?**

5.1 ABSTRACT .....	30
5.2 INTRODUCTION .....	30
5.3 METHODS .....	32
5.31 Nest boxes .....	32
5.32 Relocation .....	32
5.33 Observation .....	32
5.34 Genetic methods .....	33
5.35 Worker-worker relatedness .....	33
5.36 Effective paternity .....	33
5.37 Male non-detection error .....	33
5.38 Male non-sampling error .....	34
5.39 Worker ovary activation .....	34
5.4 RESULTS .....	34
5.41 Queen loss in the wild .....	34
Observations .....	35
5.43 Allelic diversity .....	35
5.44 Worker-worker relatedness .....	35
5.45 Male production .....	36
5.46 Diploid males .....	36
5.47 Worker ovary activation .....	36
5.5 DISCUSSION .....	36
5.6 ACKNOWLEDGEMENTS .....	39

## **CHAPTER 6. Why workers do not reproduce: worker policing in the European hornet *Vespa crabro***

6.1 ABSTRACT .....	40
6.2 INTRODUCTION .....	40
6.3 METHODS .....	41

6.31 Relocation of hornet colonies.....	41
6.32 Study colonies .....	41
6.33 Policing assay.....	42
6.4 RESULTS.....	44
6.5 DISCUSSION.....	44
6.6 ACKNOWLEDGEMENTS.....	45

## **CHAPTER 7. Colony kin structure and male production in *Dolichovespula* wasps**

7.1 ABSTRACT.....	46
7.2 INTRODUCTION .....	46
7.3 MATERIALS AND METHODS.....	47
7.31 Study organisms .....	47
7.32 Genetic Methods.....	48
7.33 Worker-worker relatedness .....	48
7.34 Effective paternity.....	48
7.35 Male non-detection error .....	48
7.36 Worker ovary activation.....	49
7.4 RESULTS.....	49
7.41 Queen loss in reproductive nests .....	49
7.42 Allelic diversity .....	49
7.43 Worker-worker relatedness .....	49
7.44 Worker ovary activation.....	50
7.45 Male production .....	50
7.46 Effect of colony size and collection date on worker male production.....	51
7.5 DISCUSSION.....	52
7.6 ACKNOWLEDGEMENTS.....	54
7.7 APPENDIX – PRIMER SEQUENCES.....	55

## **CHAPTER 8. Facultative worker policing in a wasp**

## **CHAPTER 9. Convergent evolution of worker policing by egg eating in the honeybee and common wasp**

9.1 ABSTRACT.....	59
9.2 INTRODUCTION .....	59
9.3 METHODS.....	60
9.31 Study organism.....	60
9.32 Worker relatedness.....	61
9.33 Effective paternity .....	61
9.34 Does the queen or the workers produce the colony's males? .....	61
9.35 Worker ovary activation.....	61
9.36 Policing assay.....	62
9.37 Queen or worker policing? .....	62
9.4 RESULTS.....	62
9.41 Worker relatedness.....	62
9.42 Binomial sampling error.....	62
9.43 Does the queen or worker produce the colony's males? .....	63
9.44 Worker ovary activation.....	63
Policing assay.....	64
9.46 Queen or worker policing? .....	64
9.47 Is sex allocation biasing an alternative explanation for egg removal? .....	64
9.5 DISCUSSION.....	65

9.6 ACKNOWLEDGEMENTS .....	66
----------------------------	----

**CHAPTER 10. The effect of sex allocation biasing on the evolution of worker policing in hymenopteran societies**

10.1 ABSTRACT .....	67
10.2 INTRODUCTION .....	67
10.3 THE MODEL .....	69
10.31 Overview .....	69
10.32 Inclusive fitness equations .....	70
10.33 Model parameters .....	72
10.4 RESULTS .....	73
10.5 DISCUSSION .....	75
10.6 ACKNOWLEDGEMENTS .....	77

**CHAPTER 11. Spite in social insects**

**CHAPTER 12. Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions**

12.1 ABSTRACT .....	80
12.2 INTRODUCTION .....	80
12.3 EVOLUTION OF KIN STRUCTURE .....	82
12.4 CONFLICT OVER MALE PRODUCTION .....	83
12.5 MATRICIDE .....	86
12.6 CONFLICT OVER SEX ALLOCATION .....	87
12.7 CONFLICT BETWEEN FOUNDING QUEENS .....	88
12.8 CONCLUSIONS .....	88
12.9 ACKNOWLEDGEMENTS .....	90

## Publications

The thesis is based upon the following publications and manuscripts (in order of publication):

Foster KR, Seppä P, Ratnieks FLW, Thorén PA (1999) Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps. *Behavioral Ecology and Sociobiology* **46**, 252-257. (Chapter 4)

Foster KR, Ratnieks FLW, Raybould AF (2000) Do hornets have zombie workers? *Molecular Ecology* **9**, 735-742. (Chapter 5)

Foster KR, Ratnieks FLW (2000) Facultative worker policing in a social wasp. *Nature* **407**, 692-693. (Chapter 8)

Foster KR, Wenseleers T, Ratnieks FLW (2000) Spite in social insects. *Trends in Ecology and Evolution* **15**, 469-470. (Chapter 11)

Foster KR, Ratnieks FLW (2001) Convergent evolution of worker policing by egg eating in the honey bee and common wasp. *Proceedings of the Royal Society of London B, Series B*, in press. (Chapter 9)

Foster KR, Ratnieks FLW, Gyllenstrand N, Thorén PA (2001) Colony kin structure and male production in *Dolichovespula* wasps. *Molecular Ecology*, in press. (Chapter 7)

Foster KR and Ratnieks FLW Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions. *Behavioral Ecology and Sociobiology*, accepted (Chapter 12)

Foster KR, Gulliver J, Ratnieks FLW Why workers do not reproduce: worker policing in the European hornet *Vespa crabro*, submitted. (Chapter 6)

Foster KR, Ratnieks FLW The effect of sex allocation biasing on the evolution of worker policing in hymenopteran societies, submitted. (Chapter 10)

*Can you not understand, Winston, that the individual is only a cell? The weariness of the cell is the vigour of the organism. Do you die when you cut your fingernails?*

O'Brien of the Ministry of Love  
(George Orwell, Nineteen Eighty-Four)

## Introduction

### 1.1 Theoretical background

#### 1.11 Kin structure and reproductive conflict

The potential for conflict among group members exists at all levels of biological organisation: within genomes, organisms and societies (Maynard-Smith and Szathmáry 1995; Keller 1999; Michod 1999). While many group tasks are purely cooperative, conflicts can arise over the allocation of resources to reproduction (Ratnieks and Reeve 1992; Bourke and Franks 1995; Crozier and Pamilo 1996). Understanding how such conflicts are resolved is a major question in evolutionary biology (Keller 1999). Central to the prediction and understanding of reproductive conflict is kin structure and relatedness (Hamilton 1964; Ratnieks and Reeve 1992). Kin structure identifies parties of common and differing reproductive interest thereby mapping out the structure of conflict in a society. In single-queen hymenopteran societies, such as the Vespinae wasps, the number of males that contribute genetically to the colony (paternity) is the key determinant of kin structure and is, therefore, central to understanding reproductive conflict (reviewed by Ratnieks and Reeve 1992; Bourke and Franks 1995; Crozier and Pamilo 1996).

#### 1.12 Conflict over male production

In hymenopteran societies, there is great potential for conflict over male production (Hamilton 1964; Starr 1984; Woyciechowski and Łomnicki 1987; Ratnieks 1988). In bees, ants and wasps, sex is determined by fertilisation: fertilised eggs are female and unfertilised eggs are male. This means that an unmated worker can produce males by laying unfertilised eggs. With both the queen and the workers most related to their own sons, extensive conflict over male production is predicted. Paternity is central to understanding the expression of this conflict because multiple mating by queens favours the suppression of worker reproduction by worker policing (Starr 1984; Ratnieks 1988). When the workers in a colony are offspring of a single once-mated queen, a worker is more related to another worker's son (nephew,  $r = 0.375$ ) than to the queen's sons (brother,  $r = 0.25$ ) and queen-worker conflict is predicted (Figure 1.1). However, if the queen mates multiply (effective paternity  $> 2$ ) worker-worker relatedness is reduced making workers more related to the queen's sons than to other workers' sons ( $r < 0.25$ ). This shifts the workers' collective interest into line with the queen's interest and workers are expected to police each other's reproduction resulting in enforced cooperation (Ratnieks 1988).

Although these predictions form a sound basis for investigation, it is important to understand their nature. Being based on relatedness alone, they are necessarily oversimplistic and do not consider other potentially important factors such as the cost of conflict (Ratnieks and Reeve 1992). For example, if worker reproduction reduces colony productivity this can lead to its suppression at paternity below two by worker policing, or in more extreme situations by worker self-restraint (Ratnieks 1988). In addition, the relative power of the conflicting parties is important. A relatedness

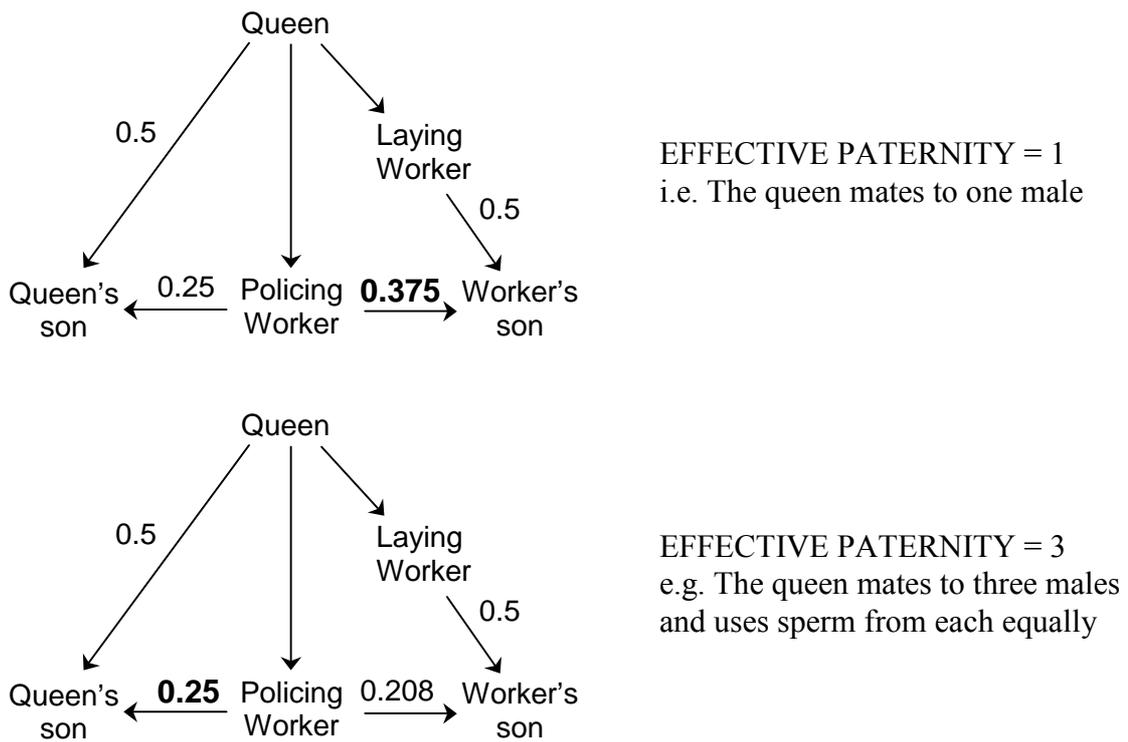


Figure 1.1 Relatedness patterns in a single-queen hymenopteran society for single and multiple paternity. Effective paternity accounts for both queen mating frequency and sperm use (Equations 3.1 and 3.2). For simplicity, laying and policing workers are shown separately although a worker could both lay and police.

prediction that workers should attempt to monopolise male production will not be fulfilled if the queen has the power to prevent their reproduction.

### 1.13 Empirical data

Worker policing occurs in the highly polyandrous (Estoup *et al.* 1994) honeybee, *Apis mellifera* in line with theory. Ratnieks and Visscher (1989) showed that workers preferentially removed worker-laid eggs when queen-laid and worker-laid eggs were introduced into the colony. In *A. mellifera* there is a dynamic conflict between laying and policing workers. Visscher (1996) showed that although around 7 % of male eggs in colonies with a queen (queenright colonies) were laid by workers only 0.12 % of adult males were worker produced (Visscher 1989). Work on the queenless ants has revealed that worker policing regulates the number of reproductive workers (gamergates) in the colony. Introduction of ovary-developed workers results in aggression from other workers (Kikuta and Tsuji 1999) causing ovary regression in the reproductive workers (Liebig *et al.* 1999). These studies provide the main direct evidence for worker policing. However, additional data comes from the correlation of paternity with worker reproduction across species.

Comparison of the honeybee with the stingless bees reveals a trend predicted by policing theory (Ratnieks 1988; Ratnieks 1990a; Peters *et al.* 1999). In contrast to *Apis*, low paternity (effective paternity > 2) appears to be the norm in stingless bees (13 species, Peters *et al.* 1999) along with ritualised queen-worker conflict (Kerr 1969) and

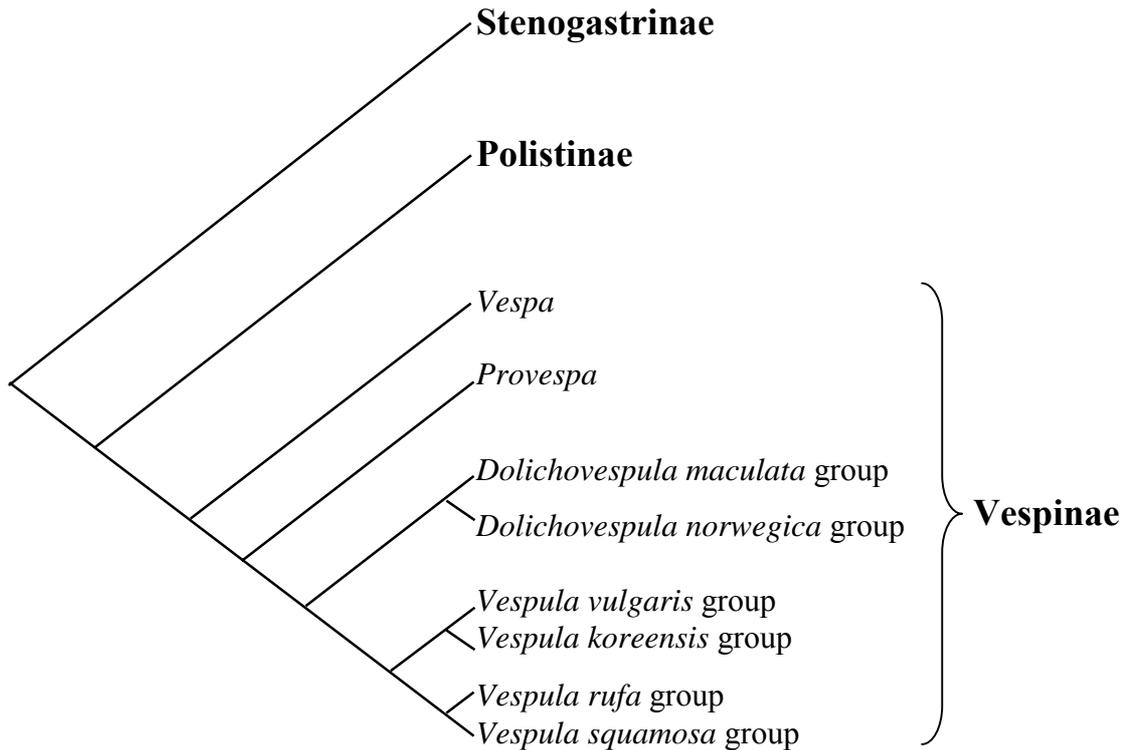


Figure 1.2 Phylogeny of the social Vespidae wasps showing the genera and species groups in the Vespinae, after Carpenter (1987).

worker laying (Sakagami 1982). In further agreement, low paternity in bumblebees (Estoup *et al.* 1995; Schmid-Hempel and Schmid-Hempel 2000) appears to be associated with intracolony conflict and worker male production (Van Honk *et al.* 1981) suggesting that worker policing is absent from the low paternity bees.

Data on worker policing in ants beyond the queenless species is very limited. However, patterns of worker reproduction across species do not follow relatedness predictions. Walin *et al.* (1998) analysed three *Formica* and one *Myrmica* ant species and showed that while relatedness predicted worker male production in all species, worker male production could only be considered a possibility in one. In addition, patterns of male production in *Leptothorax* ants do not seem to be driven by relatedness (Heinze *et al.* 1997).

## 1.2 Why study the Vespinae wasps?

The conflict over male production in the social Hymenoptera is poorly understood. However, the empirical data suggest a variety of resolutions to the conflict and a role for kin structure. More data on kin structure and colony reproduction are, therefore, required (Ratnieks 1988). The aim of this thesis is to investigate the conflict over male production in a single group, the Vespinae wasps, which are well suited as a model system for a number of reasons.

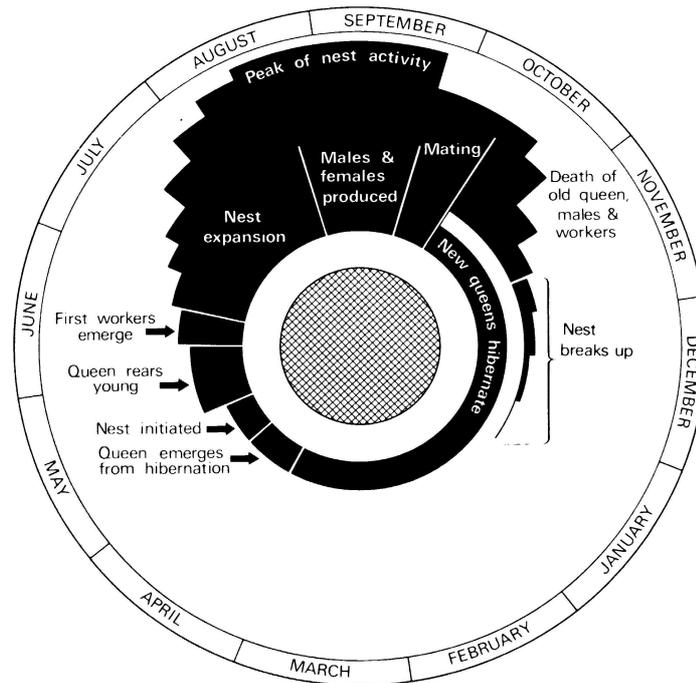


Figure 1.3 The annual life cycle of the common wasp *Vespula vulgaris*. From Edwards (1980).

### 1.21 A well-supported phylogeny

Carpenter (1987) has produced a cladogram of genera and species groups for the Vespinae, which is well supported (Figure 1.2). For example *Dolichovespula* possesses 12 autapomorphies (unique derived traits). The phylogeny provides a framework for additional data and allows evolutionary patterns and trends to be identified.

### 1.22 Similar basic biology

The vespine wasps have very similar biology. This facilitates across-species comparisons since any two species will generally differ in only a few key variables.

#### Annual life-cycle

Unlike honeybees and ants, the vespine wasps start new colonies each year (Figure 1.3, Edwards 1980). Colonies are founded by a single overwintered queen in spring. The queen performs all nest functions (founding phase) until the first workers emerge and take over work. The colony then enters a period of worker production (ergonomic phase) followed at its peak by the production of the new queens and males (reproductive phase). These leave the nest and mate. The males die and the inseminated young queens are the sole survivors to the next year. The same cycle occurs in tropical vespines although the absence of seasonality means that different colonies may not be synchronised resulting in founding and reproductive nests co-occurring (Matsuura and Yamane 1990). An exception to the annual-cycle can occur in warm temperate and subtropical areas where some *Vespula* species have occasionally been seen to overwinter. They adopt the new queens into the nest producing massive multi-queen societies (Ross and Matthews 1991; Ratnieks and Miller 1993).



Figure 1.4 Mature nest of the European hornet *Vespa crabro*

Figure 1.5 Mature nest of *Dolichovespula saxonica* with the outer envelope removed to reveal the combs containing the brood.



### The nest

Vespine wasps built characteristic paper nests (Figure 1.4) containing tiers of horizontal combs in which the young are reared surrounded by a paper envelope (Figure 1.5) (Edwards 1980). There are two sizes of cells in the combs. Early combs have small cells that are used to raise workers and small males while later combs have large cells in which new queens and larger males are reared. Nest design is highly conservative across species, although there is some variation in nest site (Table 3.3). *Vespa* and *Vespula* nest in protected cavities and produce fragile brown paper nests from rotten wood, while *Provespa* and *Dolichovespula* nest externally and use more intact woods to produce tougher weatherproof nests.

### Single distinct queen caste

The vespine wasps are unique among the social vespids (Figure 1.2) in always having morphologically distinct queen and worker castes (Ross and Matthews 1991). In addition, the vast majority of species have only a single queen in the nest. Relatedness patterns are consequently simple and determined by the number of times the queen mates (paternity). This makes the analysis of kin structure and testing of policing theory comparatively easy. Multi-queen founding occurs in *Vespa affinis* and *Vespa tropica* (Matsuura 1991). However, these species appear exceptional and whether more than one queen actually reproduces in these societies has yet to be established.

### Workers with ovaries

Although they have lost the ability to mate, vespine wasp workers possess ovaries (Edwards 1980). This enables them to lay haploid male destined eggs.

### Predatory ecology

Vespine wasps are predators or scavengers, with most opportunistically feeding on a wide variety of insects and spiders, as well as taking meat from vertebrate carcasses (Edwards 1980; Matsuura and Yamane 1990). Workers also supplement their carbohydrate intake by feeding on flowers and plant sap. There is evidence of diet specialisation among species. For example, *Vespa tropica* feeds almost exclusively by raiding polistine wasp nests and *Vespa mandarinia* commonly performs impressive group raids on honeybee colonies (Matsuura 1984).

## **1.23 A few important variables: kin structure, conflict resolution and colony size**

A major advantage of studying the vespine wasps is that, despite similar general biology, they differ in characters of central interest in the study of social evolution. Single and multiple paternity species occur (Ross 1986; F. L. W. Ratnieks and J. J. Boomsma, unpublished data) and there is variation in worker reproductive behaviour and associated queen-worker aggression (Greene *et al.* 1976; Reed and Akre 1983; Ross 1986). Colony size also varies both within and among genera (Edwards 1980). While undoubtedly a function of species life history, colony size may be an indicator of colony organisation, with low conflict societies achieving the greatest size. A link between kin structure, conflict resolution and colony size is suggested by Ross's (1986) study of two *Vespula* species which had high paternity, queen-only reproduction and large colonies.

## How the thesis evolved

### 2.1 The European hornet, *Vespa crabro*

#### 2.1.1 Paternity and worker relatedness

At the start of research in August 1997, the first challenge was to collect nests of the European hornet *Vespa crabro* for genetic analysis of colony kin structure. There was no genetic data on queen mating frequency (paternity) in *Vespa*, so this was chosen as a relatively simple starting project. The work was done in collaboration with John Gulliver of the New Forest Forestry Commission and Perttu Seppä from the Department of Genetics, Uppsala University, Sweden. John helped us get permission to collect hornets in the New Forest, which is the last stronghold for them in Britain. Despite his goal to conserve the New Forest hornets, John allowed samples to be removed for genetic analysis on the basis that our work would benefit the insect by raising its profile. He also kindly taught me how to handle the wasps, which he relocates from areas where they are a human disturbance to quieter areas of the Forest. Information on the location of the nests came courtesy of the New Forest District Council pest control unit, which passed on all hornet calls to John and later on to me. The collection was successful and highly enjoyable. The New Forest is an outstandingly beautiful area and working as a pest controller revealed a fascinating diversity of Forest people, many with wonderful tales concerning hornets and local natural history.

In January 1998, I travelled to Uppsala, Sweden with the hornet samples to the lab of Pekka Pamilo. Perttu was my mentor and introduced me to both Swedish and lab life. He patiently taught me how to perform microsatellite analysis. The markers we were using had just been designed in the same lab on another vespine wasp *Vespula rufa* by Peter Thorén (Thorén 1998). Luckily, four of the *rufo* markers amplified in *V. crabro* and were variable allowing analysis to go ahead. Six weeks later, I had completed my first study, the analysis of 20 workers from 14 nests at four microsatellite loci. We had established that hornet queens generally use the sperm of one male, so that workers are highly related (Chapter 4).

#### 2.1.2 Zombie hornets

The discovery that hornet workers were highly related led to the prediction that they should attempt to reproduce resulting in observable queen-worker conflict. I tested this in my second field season in the summer of 1998. Again with John Gulliver's help, I set about collecting hornet nests and relocating them to observation boxes which I had built in a shed in the Institute of Terrestrial Ecology, Furzebrook, Dorset, around 40 miles from the New Forest. Despite considerable mortality of the relocated nests, I was able to observe four nests and recorded all incidences of worker laying and queen worker conflict. After fifty hours, there was no evidence of worker laying or queen worker conflict – tedious but conclusive data. In addition, the collection allowed the use of microsatellites once more. This time the aim was to establish whether any males came from workers. Although the observation suggested that workers did not reproduce, the genetics allowed us to check many more colonies (15 in total). Another trip to Uppsala



John Gulliver

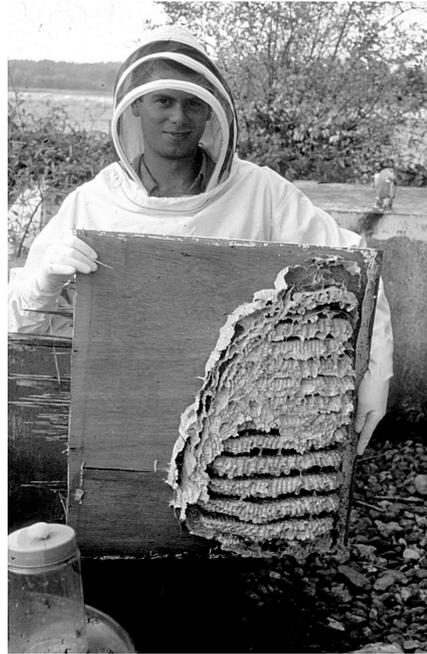
in the spring of 1999 confirmed what the behavioural data suggested. There was no evidence for worker's sons in any colonies. The hornet workers, therefore, were performing contrary to the basic predictions of kin selection theory. They appeared to be behaving as queen-controlled 'zombies' (Chapter 5).

### 2.13 The effect of genetics on cuticular chemistry

Prior to the second field season, Perttu Seppä and Francesca Dani of Florence University had asked if I could collect a sample of hornet workers to allow analysis of the surface chemistry. The aim of the project was to see if worker chemistry was correlated to their genotype. If so, then this would, in theory, allow workers to act nepotistically, discriminating and favouring their closest relatives in the colony. The discovery of facultative worker policing in response to kin structure in *D. saxonica* (below) made this study especially relevant to the thesis. The question was then could the hydrocarbons on adult workers provide the information on colony kin structure that enables facultative policing? I collected samples for the study at the same time as collecting for the male production study (above). These were genotyped while in Sweden in spring 1999 revealing nests of three types of colony kin structure: single matriline/single patriline, single matriline/double patriline and double matriline. Perttu and I then travelled to Florence to perform GCMS (Gas Chromatography Mass Spectrometry) on the samples under Francesca's guidance. Although initially promising, the data failed to show a significant effect of paternity on colony chemical diversity. Work on other aspects of this data is ongoing in the Florence lab.

## 2.2 Spite

In the spring of 1998 following my first trip to Uppsala, Tom Wenseleers of the University of Leuven, Belgium visited the Sheffield lab. After a casual conversation involving the definition of spiteful behaviours, the endosymbiont bacteria *Wolbachia*



Adam Hart, who helped to collect hornets in 1998

and reproductive conflict in social insects, we suddenly realised that several well-known behaviours could be interpreted as spite. This led to a month of intense arguments and mathematical modelling attempting to formally define spite. At the end of this time, we had a draft paper on spite which included a multitude of social behaviours from a great diversity of organisms. However, after comments from Dave Queller and Steven Frank, it became apparent that there were flaws in some of our arguments (and my maths). In the following two years, Tom and I met on several occasions to discuss spite and slowly whittled our ideas down to a solid base – reproductive conflicts in the social insects with worker policing as a central example. With this, we were able to write a new manuscript ‘Spite in social insects’ which, after a close call with *Nature*, was finally accepted in *Trends in Ecology and Evolution* in August 2000 (Chapter 11).

## 2.3 *Dolichovespula* wasps

### 2.31 New Forest collection and observation

Following a conversation with Joan Strassmann at the IUSI (International Union for the Study of Social Insects) conference in Adelaide, it became apparent that the answer to the enigma of the hornet’s zombie workers was best approached by comparative study. The field season of 1999 was, therefore, aimed at collecting samples of all four native *Dolichovespula* wasps (*media*, *saxonica*, *norvegica* and *sylvestris*) for genetic analysis of paternity and male production, and observation nest studies to check for worker laying. From my base at the I.T.E., Furzebrook, I set about collecting with the kind help of the New Forest District Council which passed on phone numbers and addresses of people who had grey nests, characteristic of the genus. Using the same observation boxes as for the previous year’s hornet work, I relocated a number of nests of *D. saxonica* and *D. media*. Being easier to move and structurally stronger, the *Dolichovespula* colonies were much more amenable to study than the hornet and yielded good data. This was particularly true of *D. saxonica*, whose workers displayed frenetic egg laying in stark contrast to the hornet.

### 2.32 Microsatellite analysis

Armed with an unprecedented number of samples from five species, in October 1999 I headed to Sweden once more, this time to analyse the kin structure and male production of *Dolichovespula* wasps. The work was again successful but pushed my organisational skills and sanity to the limit, involving the manual analysis of around 6000 alleles. All five species yielded similar results of low paternity, and worker reproduction, in queenright colonies. The coherence of the data across colonies lent itself to a single publication “Kin structure and male production in *Dolichovespula* wasps” which summarised the characteristics of the group (Chapter 7). However, whilst preparing this manuscript it came to my attention that *D. saxonica* had much greater variation in both worker relatedness and worker male production than any of the other species. This led me late one night to plot the two variables against each other. To my surprise and delight, there was a highly significant positive correlation. It seemed that in colonies with low relatedness among workers, workers were not producing males but in high relatedness colonies they were. This is as predicted by the worker policing theory. Combining this result with the observational data showing that workers were laying eggs in all colonies, this strongly suggested that workers were facultatively policing each other, the first real test of policing theory. Hence, a second chapter was born of the *Dolichovespula* sample dealing only with *D. saxonica* (Chapter 8). This one did get into *Nature* and was published in October 2000.

## 2.4 The common wasp *Vespula vulgaris*

### 2.41 Microsatellite analysis

While in Uppsala for the second time (spring 1999) analysing male production and worker relatedness for GCMS study in the hornet, I also turned my attention to the common wasp, *Vespula vulgaris*. Francis had successfully amassed a large sample of *V. vulgaris* nests in the Sheffield ultra-cold freezer leaving only the genetics to be done. Several years before, he had earmarked *Vespula* as a candidate for a study of worker policing. Work by Ken Ross on two North American species had suggested that multiple mating by queens was the norm and that workers did not reproduce, exactly the pattern expected in a social insect with worker policing. This pattern was again confirmed in *V. vulgaris*, with comparatively high effective paternity and no evidence for worker reproduction, leading the way for an empirical study to determine if worker policing occurred.

### 2.42 Worker policing

The reproductive period of *Vespula vulgaris* is conveniently a month after that of the *Dolichovespula* species. This meant that in the summer of 1999 I was able to complete my collection and study of *Dolichovespula* in the New Forest and then return to Sheffield to test for worker policing in the common wasp. Earlier in the season, Stephen Downs and Nicki Badcock had relocated a number of *V. vulgaris* nests supplied by John Allison, a local pest controller, into polystyrene boxes outside the lab. Two of these survived and appeared strong. Discarding more elaborate plans, I rehoused the nests into polystyrene boxes with the bottom cut out. The breakthrough, which seems obvious in hindsight, was to do all of the experimentation outside the lab so that any wasps that escaped from the nest while I was manipulating them would simply fly back in afterwards. Escaping wasps were always a problem with the observation nests in the

shed in Furzebrook as they had to find their way out of the shed and then round the outside to the nest entrance. I moved the two strong *vulgaris* nests to their new abodes and isolated some workers with some comb. These queenless workers laid eggs, which I then re-introduced into the colony with queen eggs in an experimental comb.

Working with the common wasp was a shock to the system. It is a remarkably aggressive and tenacious wasp whose workers when aroused, which seems their only state, will land on you and hurriedly crawl around until they find an exposed area to sting. Consequently my protective gear, which had been fine for *Vespa* and *Dolichovespula*, evolved in a matter of days to include a rain jacket with the hood up, waterproof trousers, wellington boots, washing-up gloves, a full beesuit and thick gauntlets with all joining areas taped up. Fortuitously, I was rewarded by strong evidence for worker policing. Removal of the first experimental comb led to an Archimedian moment on seeing that the worker-laid eggs had all been neatly removed, while those of the queen remained largely untouched (Chapter 9).

## 2.5 The hunt for *Provespa*

Having obtained enough data for the thesis within the first two years, I was left in my third year to pursue more risky projects. The first of these was to try to obtain a sample of the enigmatic *Provespa*. *Provespa* were the last genus of the vespine wasps I had left to study and so for completeness I set about planning a trip to find some. BBSRC provided extra funds and so with Steve Martin, the only westerner I know to have found a nest (from which he kindly gave me a sample), I headed east to Thailand which lies in the centre of their exclusively Far-Eastern range. The trip was always going to be difficult. *Provespa* are a nocturnal species with cryptic nests that do not seem to occur commonly near human habitation. This means that daytime tracking of workers and pest control information cannot be used and makes the last option of nest spotting very difficult. More fundamentally however, we had to first establish that *Provespa* occurred at all in an area. This is best done by looking at bright lights where they are often reported flying. We failed at the first hurdle, visiting multiple sites in Thailand over several weeks but failing to see a single worker. It was about five weeks into the trip, while Steve was scouring Malaysia and I was in Singapore attempting to get hornets that I had my one encounter with my, by then, tropical Nemesis. While at the Bukit Timai National Park a dull brown but unmistakably wasp-like form flitted up to the light at the park head quarters. It was indeed *Provespa*. I was delighted to see it but my joy was tempered by the fact I had spent the last three days walking around Bukit Timai failing to find nests and that collecting in the park was punishable by a 6 month prison sentence.

The trip ended on the island of Penang off the Malaysian mainland, where Steve had identified a hot spot for the tropical hornet *Vespa affinis*. Between us, we managed to obtain 8 nests to allow a basic paternity study to be performed. Leaving the Far East without *Provespa*, I was consoled by the *affinis* nests. This lasted as long as it took to go to Sweden and discover that the *Rufa* primers were entirely monomorphic in *V. affinis*. The samples are currently in the Uppsala ultra-cold freezer awaiting new vespine primers to be developed.

## 2.6 The hunt for *Vespula austriaca*

Another sparse area of data on paternity in vespine wasps was from the *Vespula rufa* group. These have relatively small nests compared to the *Vespula vulgaris* species group suggesting a potentially different ecology and so possibly mating system. In my study of British vespines, I had only ever seen one *V. rufa* worker limiting the possibilities of nest collecting. As an alternative strategy, I decided to attempt to collect spring queens and analyse the sperm in their spermatheca to get an idea of mating frequency. *Vespula rufa* has the added interest of a workerless social parasite *V. austriaca*. Having no workers of its own, the kin selection arguments regarding the effects of paternity on worker behaviour do not affect *V. austriaca*, so a comparison of paternity with that of its host held added interest. Armed with a data paper showing that both *rufa* and *austriaca* queens fly in southern Finland in late June, I headed to Tvarminne on the Baltic coast to hunt them both. Despite long walks, baits, traps and the collection of seven vespine species, the 10-day trip turned up only two *rufa* and one *austriaca* queen. The midsummer party was good though.

## 2.7 The evolution of worker policing at low paternity

Worker policing in *Vespula vulgaris* and *Dolichovespula saxonica* occurred at paternities below that predicted by theory. Using some basic maths, I sought a possible explanation. I incorporated the other major reproductive conflict in social insects, that over sex allocation, into the model. In several ant species it has been shown that workers kill male larvae in order to favour their more closely related sisters. Incorporating this factor did indeed make worker policing selectable at low paternity as policing reduces the cost of male larvae killing. The interactions of these two conflicts, therefore, may prove to be the reason behind my results (Chapter 10).

## 2.8 Worker policing in the hornet *Vespa crabro*

As a final project, I decided to test for worker policing in the hornet. With the discovery of worker policing in both *Vespula vulgaris* and *Dolichovespula saxonica*, this suddenly seemed viable. Despite being contrary to relatedness predictions it could be the reason why workers do not reproduce in hornets. In addition, there was an appealing karma in coming back to the mystery of worker sterility in my original study organism, the hornet. Testing for policing in the hornet was going to be a challenge. Their nests had proved hard to relocate successfully in my previous study and the small nest size compared to *Vespula* rendered each piece of comb precious and left little room for mistakes. John Gulliver and I relocated ten nests to observation boxes. However, it emerged that queens in all of these nests had died suggesting that the relocation procedure was particularly stressful for the queens. I got around this by studying two additional colonies. One was moved within its rabbit hutch home without any direct interference and the other was in the barn of another New Forest warden allowing me to study it without relocation. Both of these colonies remained queenright allowing the worker policing assays to be performed. In addition, I decided to study the response of the workers in the queenless colonies. To my delight the hornets obliged. In colonies with a queen, workers policed worker-laid eggs but left most of the queen-laid eggs as in *Vespula*. However, in queenless colonies the opposite happened and workers preferentially removed queen-laid eggs. These data provided a fitting end to the thesis. The mystery of hornet worker sterility was, at least in part, solved (Chapter 6).

## Methodology

The aim of this chapter is to provide an overview of the methods used in the thesis in addition to the specific descriptions made in the subsequent chapters. There were three major types of methodology: (1) DNA microsatellite analysis, (2) Statistical and mathematical analysis of genetic data, (3) Collection and study of live wasps.

### 3.1 DNA microsatellite analysis

The study of DNA microsatellites involved four stages. Extraction to obtain DNA from the tissue, the polymerase chain reaction (PCR) to amplify and radioactively label the loci of interest, separation of the PCR products by mass on an electrophoretic gel and visualisation using photographic film to allow the gel to be read.

#### 3.11 DNA extraction

1. The wasp's head was removed and a small amount of thoracic muscle scraped out with a toothpick.
2. The muscle was added to 200 $\mu$ l Chelex<sup>TM</sup> (5%), 10 $\mu$ l proteinase K (10mg/ml) and 7 $\mu$ l DDT (1M) in a 1.5ml Eppendorf tube.
3. The sample was incubated at 56°C for 1-2 hours.
4. The tube was vortexed for 10 seconds, then boiled for 8 minutes, then vortexed for 10 seconds.
5. Finally, it was centrifuged for 3 minutes at 12000 rpm.

#### 3.12 PCR reaction

PCR was used to amplify the fragments of interest in the DNA extract (Table 3.1). The primers were selected from nineteen originally designed for use on *Vespula rufa* by Thorén (1998). All nineteen were tested in each species by analysing one worker from each of ten nests. Depending on the species, 2-4 loci that were most variable were used. A touchdown PCR was used for all amplification (Don *et al.* 1991) (Table 3.2). This uses a range of annealing temperatures (56-46°C) and removes the need for conventional optimisation.

#### 3.13 Separation and visualisation

PCR products, mixed with 7 $\mu$ l of STOP solution, were run on a 6% polyacrylamide sequencing gel for 5000 volt hours and visualised using Kodak Biomax<sup>TM</sup> single emulsion film. The gel films were then hand-read and the alleles of each individual at each loci entered into a database. Control individuals were run on each gel so that the alleles across gels were comparable.

Table 3.1. PCR reaction mixture.

Reagent	Stock Concentration	Volume per reaction ( $\mu$ l)
Distilled water	-	5.7
PCR Buffer	10X	1.0
Mg <sup>2+</sup>	25mM	0.6
Forward primer	10 $\mu$ M	0.5
Reverse primer	10 $\mu$ M	0.5
dNTP mixture	2.5mM/base	0.3
BSA	1 mg/ml	0.25
Taq polymerase	5 U/ $\mu$ l	0.1
$\alpha$ - <sup>33</sup> P-dATP	0.125 $\mu$ Ci	0.05
DNA extract	-	1.0

Table 3.2. PCR thermocycler program.

Stage #	Temp ( $^{\circ}$ C)	Time (s)	Action
1	90	180	
2	90	30	
3	56	30	decrease by 0.5 $^{\circ}$ C each cycle
4	72	30	
5	-	-	Return to (2) 20 times
6	90	30	
7	46	30	
8	72	30	
9			Return to (6) 10 times
10	72	600	
11	10	$\infty$	END

### 3.2 Statistical analysis of genetic data

The statistical analysis of the genetic data is discussed at length in the relevant chapters. Here a general summary of the analyses is presented.

#### 3.21 Worker-worker relatedness and effective paternity

Two complementary methods were used to generate estimates of worker-worker relatedness, *pedigree* and *regression*.

##### Pedigree relatedness

From the microsatellite genotypes of the workers, the number of patriline (and rarely matriline) and the proportion of each are determined. Then, under the assumption of random mating (full-sister relatedness = 0.75, half sister = 0.25), the mean relatedness among workers in the nest or within a matriline is calculated. Population wide effective paternity can be directly calculated from (Starr 1984):

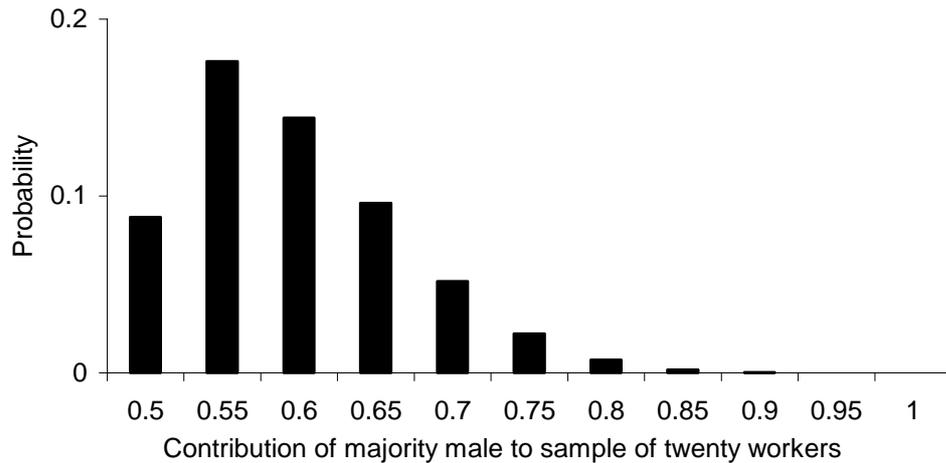


Figure 3.1. The probability distribution for the contribution of the majority male to a sample of twenty workers from a colony of two *equal* patriline. This shows clearly that the sample mean is not equal to that in the original colony (0.5). Binomial sampling error, on average, results in biased paternity in the sample (mean proportional contribution of 0.58 by the majority male).

$$M_e = \frac{n}{\sum_j \sum_i p_{ij}^2} \quad (3.1)$$

where  $p_{ij}$  is the proportional contribution of the  $i$ th male in the  $j$ th nest for  $n$  nests. Effective paternity is the evolutionarily important measure of queen mating frequency that accounts for sperm use by the queen and is directly related to colony relatedness patterns.

#### Regression relatedness

Regression relatedness is estimated directly from the worker genotype data following Queller and Goodnight (1989), using the Relatedness 4.2 program (Goodnight and Queller 1994). By comparing all combinations of pairs of individuals in a sample, this estimates the mean probability above random that two individuals carry the same allele. Regression relatedness can be used to produce a complementary estimate of effective paternity (Pamilo 1993):

$$M_e = \frac{1}{2b - 0.5} \quad (3.2)$$

where  $b$  is the regression relatedness from Relatedness 4.2.

While the two methods generally produce very similar estimates of relatedness (Chapter 4), regression relatedness is sensitive to two additional factors that pedigree relatedness is not affected by.

- (1) *Allele frequency* Because regression relatedness is the probability, *above chance*, that individuals carry the same alleles, a queen who possesses rare alleles produces workers of higher regression relatedness (more similar compared to population average) than one that carries common alleles.

- (2) *Inbreeding* Unlike the pedigree estimate which takes random mating as an *a priori* prediction, regression relatedness is sensitive to non-random mating. Mating among relatives, therefore, raises only regression relatedness.

These factors, therefore, can lead to differences in relatedness *among* colonies that pedigree relatedness does not reveal. However, in the application of worker policing theory this makes pedigree relatedness most useful. The prediction of worker policing is based upon *relative* relatedness, the comparison of worker-brother versus worker-nephew relatedness, which is what pedigree relatedness measures.

### 3.22 Errors in paternity estimation

Paternity is subject to errors that cause underestimation of the number of males that have contributed to the progeny of a queen. However, the use of multiple microsatellite loci and sampling of twenty workers per nest meant that these errors were very minor and would have no impact on the key evolutionary conclusions (e.g. whether worker policing was expected).

#### Non-detection error

If two males who have mated a queen possess the same genotype at all loci studied, their worker offspring are indistinguishable in the sample (Chapter 4). By using multiple microsatellite loci of high heterozygosity, non-detection error was shown to be negligible in all studies (Chapters 4, 5, 7, 8, 9).

#### Non-sampling error

This occurs when the worker offspring of a certain father are not present in the sample. However, by analysing 20 workers per nest, only minor patrilineages that have a small effect on effective paternity are likely to be missed. For example in a nest with one male with 90% paternity and the other male with 10% paternity, the minority male's offspring will be unsampled only 12% of the time ( $0.9^{20}$ , Chapter 4). In such nests, therefore, the estimate of effective paternity will be reduced from 1.22 to 1 (18% error), 12% of the time, a mean error of 2%.

#### Binomial sampling error

When two or more patrilineages occur in a nest, sampling only twenty workers to estimate paternity can lead to error from binomial sampling effects (Figure 3.1). The result is that paternity, on average, is more biased in the sample than in the nest. However, with twenty workers the worst case scenario effect on effective paternity is again very minor, reducing the paternity estimate by 2.5% (Chapter 9).

### 3.23 Errors in worker male production estimation

Discrimination of worker and queen-produced males using microsatellite markers is subject to error because a worker inherits half of her genes from her mother queen. Workers' sons are only detectable at a particular locus if (1) the worker's mother and father have different alleles and (2) the worker passes on her paternal allele to her son. The probability of detecting a worker-produced male can be calculated from:

$$P_j = \sum_1^n p_i (1 - 0.5^{l_i}) \quad (3.3)$$

where  $n$  is the number of patriline in the nest,  $p_i$  is the proportional representation of the  $i$ th patriline and  $l_i$  is the number of informative loci (where the queen and her mate have different alleles) analysed at the  $i$ th patriline (Chapters 5, 7, 8, 9).  $P_j$  can be used to account for the effects of male non-detection in two ways:

#### No worker produced males are detected

The probability that workers' sons occurred but none were sampled and detected can be calculated. The number of males which would be detectable as workers' sons (# assignable,  $N_a$ ) for each species' sample can be estimated from  $\Sigma(P_j N_j)$  where  $N_j$  is the number of males analysed for the  $j$ th nest. If workers produce a proportion  $x$  of the males, the probability of not sampling any worker-produced males is then  $(1 - x)^{N_a}$ .

#### If worker produced males are detected

The estimate of worker male production is adjusted upwards, to account for non-detection, by dividing the number detected by the probability of non-detection ( $P_j$ ).

### **3.3 Collection and study of live wasps**

#### **3.31 Finding nests**

The majority of nests analysed in this thesis came from the removal of nests for pest control because they were near human habitation. All New Forest samples were collected by the author using information from the New Forest District Council and Forestry Commission. The Sheffield samples were from a collection established between 1996-1999 by several members of the Laboratory of Apiculture and Social Insect Research and by John Allison, a local pest controller specialising in bees and wasps. *Dolichovespula maculata* is a North American species and was collected around Ithaca, New York by Francis Ratnieks.

#### **3.32 Nest collection**

Nests were collected wearing apicultural protective gear (veiled suit and gauntlets). The wasps were collected from a variety of locations (Table 3.3, Figure 3.2). The basic collection method was to put the nest in a thick plastic bag and then to collect foraging workers using either a net (*Vespa*, *Dolichovespula*) or a 12v vacuum cleaner pooter (*Vespula*) (Figure 3.3).

#### **3.33 Nest relocation**

For behavioural studies, several wasp nests were moved to purpose-built boxes. Some were narrow observation boxes with glass sides (Figure 3.4). Others were simple 'bird-box' design to allow collected nests to reach reproductive status and provide samples for genetics (Figure 3.5). Relocation requires more careful treatment of the nest than simple collection for freezing:

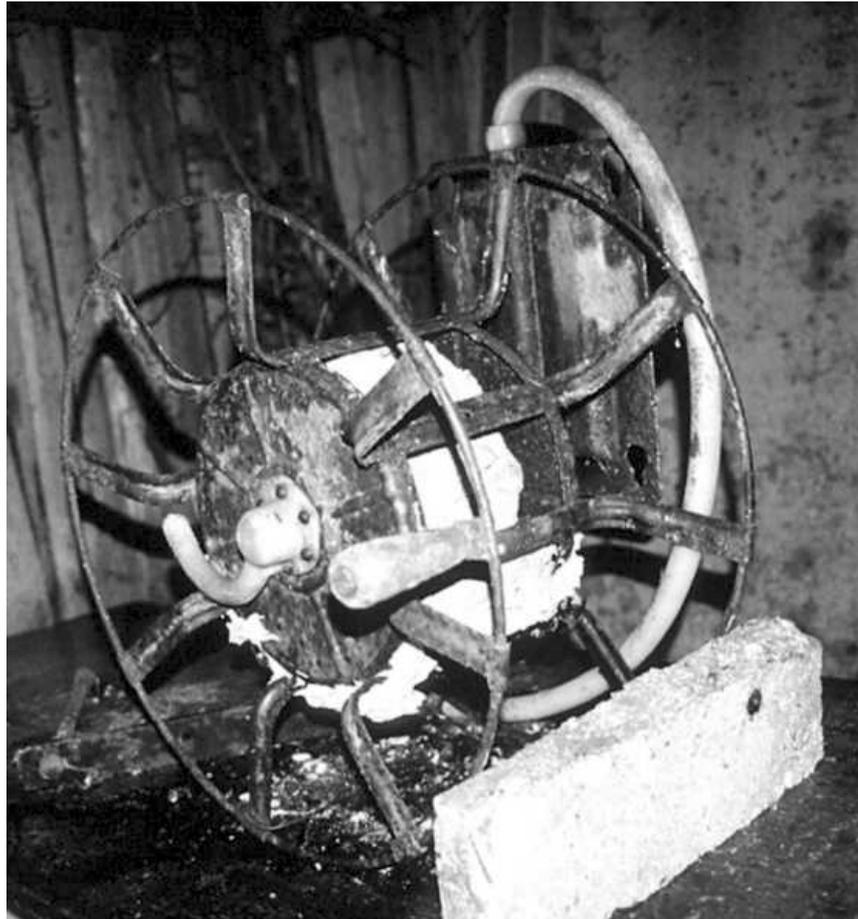


Figure 3.2 Nest of the hornet *Vespa crabro* in a garden hose reel.



Figure 3.3 Vacuum Pooter.

Table 3.3. Nest sites of colonies collected for the thesis.

Species	Nest locations
<i>Vespa crabro</i>	Cavities: bird boxes, lofts, soffit boards, sheds, barns, hay bales, wall cavities and ground cavities
<i>Dolichovespula media</i>	Exposed tree branches and loose bushes
<i>Dolichovespula sylvestris</i>	Protected tree branches, dense bushes, bird boxes, in dry stone walls
<i>Dolichovespula norwegica</i>	Exposed tree branches and bushes
<i>Dolichovespula saxonica</i>	Bird boxes and under horizontal coverings (garden tables, shed roof, animal houses, roof gable)
<i>Vespula vulgaris</i>	Cavities: lofts, soffit boards, wall cavities, ground cavities

1. Workers were collected, either with a net or vacuum pooter. Workers in the net were fed from the net into a container with air holes and a tube entrance, which acts as a one way valve as in a lobster pot. The container was then put into an ice box to calm the workers.
2. The nest, containing the queen and callows (young workers), was then carefully placed into a plastic bag or box with ice for the journey.
3. On arrival at the destination, the nest was removed and suspended in its new nest box using garden wire. The chilled workers were then added, with a dish of honey to aid recovery, and the box quickly closed.
4. After about 60 minutes, the entrance to the box was opened to allow workers to forage. This period allows the workers to recover and rejoin the nest. This is essential in making workers relocate to the new nest site, as they get lost if allowed to fly immediately.

### 3.34 Nest observation

To gather behavioural data on queen-worker conflict, observational studies of *Vespa crabro*, *Dolichovespula saxonica* and *D. media* were carried out in a modified shed at the Institute of Terrestrial Ecology, Furzebrook, Dorset, UK in 1998 and 1999. The shed contained eight wooden observation nest boxes which were 9.5cm (depth) x 30cm (horizontal) x 40cm (vertical) and faced with a hinged glass door for observation and access (Figure 3.4). A plastic pipe leading outside allowed workers to forage naturally. The shed was kept in darkness and was warmed to around 25°C using a 3kW heater.

### 3.35 Worker policing assay

To study worker policing in *Vespula vulgaris* and *Vespa crabro*, nests were relocated to special boxes. They had hinged fronts to allow removal and replacement of the experimental comb and removable bottoms for filming, as the experimental comb was the bottom comb in the nest in all experiments (Figure 3.6).

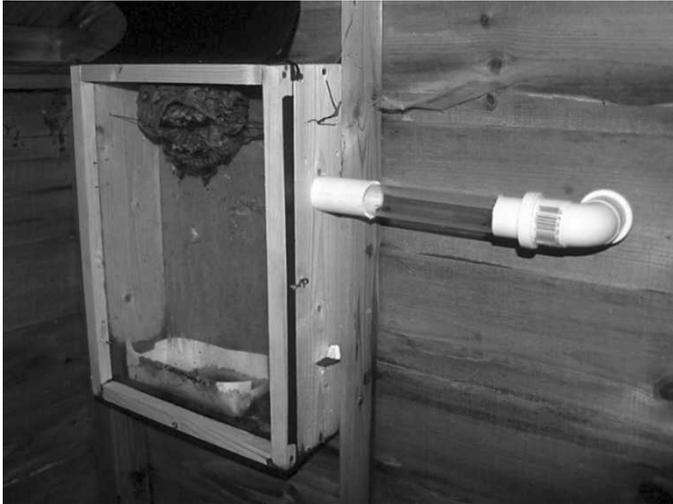


Figure 3.4. Observation nest box containing a nest of *D. saxonica*.

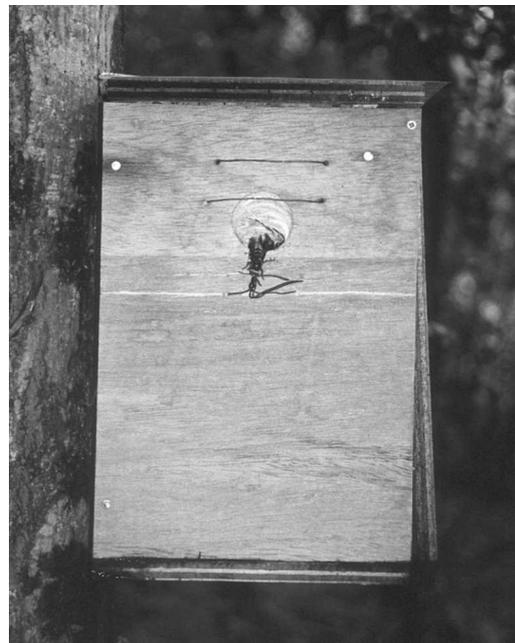


Figure 3.5. Hornet maintenance box



Figure 3.6. Worker policing box containing a nest of *V. vulgaris*. The front of the box is opened to reveal the combs.

## Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps

K.R. Foster, P. Seppä, F.L.W. Ratnieks & P.A. Thorén

### 4.1 Abstract

Queen mating frequency was studied in the European hornet, *Vespa crabro*, by analysing four DNA microsatellite loci in 20 workers from each of 14 nests. Queens were found to be predominantly singly mated (9/14), although double (4/14) and triple mating (1/14) also occurred. For most multiply mated queens, paternity was significantly biased with the majority male fathering on average 80% of the female offspring. The population-wide effective mating frequency was therefore low (1.11), and sister-sister relatedness high ( $0.701 \pm 0.023$  SE). Low effective mating frequency in *Vespa*, in combination with data from other vespines, suggests that high paternity frequency is derived in the group. Some problems with the non-detection of fathers, where the queen was not sampled or shared alleles with males are analysed.

### 4.2 Introduction

The family structure of animal societies is central to their social behaviour (Hamilton 1964; Crozier and Pamilo 1996), particularly patterns of reproductive cooperation and conflict (Trivers and Hare 1976; Pamilo 1991a, 1991b; Ratnieks and Reeve 1992). This tenet, formally kin selection theory (Maynard Smith 1964), has been used to make behavioural predictions in a wide variety of animal taxa (e.g. Emlen and Wrege 1988; Packer *et al.* 1991; Grosberg *et al.* 1996) including social insects (Crozier and Pamilo 1996).

In single-queen eusocial Hymenoptera, queen mating frequency, specifically the number of males contributing to paternity (Boomsma and Ratnieks 1996), is the key determinant of family structure. Increased paternity frequency is predicted to reduce potential queen-worker conflict over sex allocation (Trivers and Hare 1976; Benford 1978), male production (Ratnieks 1988), and queen killing by workers (Bourke 1994), but to cause potential nepotistic conflict among workers of different patriline (Getz 1981; Ratnieks and Reeve 1992; Bourke and Franks 1995; Crozier and Pamilo 1996 review the theory). Determination of queen mating frequency is, therefore, pivotal in the study of reproductive behaviour.

Although numerous studies of paternity in eusocial Hymenoptera have been made (reviewed by Page 1986; Boomsma and Ratnieks 1996; Crozier and Pamilo 1996), more data are needed. This is partly to take advantage of new techniques, particularly DNA

microsatellites, which provide greater power in detecting paternity than was typically available with protein allozymes (Queller *et al.* 1993a). In addition, more data are needed to characterise paternity adequately in the highly diverse social Hymenoptera (Boomsma and Ratnieks 1996). One specific need is for concentrations of data in specific taxa to give a better picture of the relationship between paternity frequency and reproductive behaviour in a phylogenetic context.

The vespine wasps are a taxon in which paternity data should be particularly informative (Ratnieks 1988; Boomsma and Ratnieks 1996). They have a well-supported phylogeny (Carpenter 1987) which, combined with the relatively small size of the group [*Vespa* (23 species), *Provespa* (3 species), *Dolichovespula* (13 species), and *Vespula* (22 species)], should make it possible to produce a largely complete evolutionary tree of paternity data to relate this to colony reproductive characteristics. The basic biology of the Vespinae is similar, typically with an annual monogynous paper nest, a morphologically distinct queen caste and workers that are unable to mate (Ross and Matthews 1991). However, variation in queen mating frequency between species has been recorded (Ross 1986; Thorén *et al.* 1995; Thorén 1998; F.L.W. Ratnieks and J.J. Boomsma, unpublished data). In addition, differences in key aspects of reproductive behaviour have been observed, including variation in the incidence of worker laying and associated queen-worker aggression (Greene *et al.* 1976; Reed and Akre 1983; Ross 1986), split sex ratios (Greene *et al.* 1976; F.L.W. Ratnieks and J.J. Boomsma, unpublished data), matricide (Ishay 1964), and occurrence of a queen pheromone (Ikan *et al.* 1969). This variation in mating frequency and conflict behaviour among species with otherwise similar life history traits makes comparative study of the Vespinae particularly interesting.

Hornets, *Vespa*, are an important vespine genus both in terms of the number of species and as the most basal member of the taxon (Carpenter 1987). However, there are no studies of paternity frequency. Therefore, we performed a DNA microsatellite study on the most widely distributed member of the genus, *Vespa crabro*. The results show clearly that paternity is low in *V. crabro*.

## 4.3 Methods

### 4.31 Study organism and sample collection

*V. crabro* was collected during July and August 1997 from nests located in the New Forest, Hampshire, England, where it is relatively abundant (Nixon 1982). *V. crabro* is the only hornet found in Britain and northern Europe. Nests were collected from an area of approximately 20x20 km. The entire nest was collected in four cases. For the other nests, only a sample of workers was collected. Collection of the whole nest was rarely possible due to the habit of *V. crabro* of nesting in cavities, such as hollow trees. In addition, the New Forest is a nature reserve so collecting large numbers of nests was avoided for conservation reasons. Entire nests were only removed when a nest had to be destroyed because its location caused significant human disturbance.

### 4.32 Molecular methods

Nineteen DNA microsatellite loci previously designed for *Vespula rufa* (Thorén *et al.* 1995) were screened for use in *V. crabro*. Four variable loci amplified reliably (Rufa 5,

13, 15, 18) and a further two (Rufa 2 and 6) less well. Genetic variation at the four reliable loci was studied in 20 workers from each of 14 nests. Additionally, the queen was analysed in the four nests that were collected. DNA extraction and PCR used standard methods (see Thorén *et al.* 1995). A 'touchdown' PCR was carried out from 56 to 46 °C (Don *et al.* 1991), using  $^{33}\text{P}$ -a-dATP in internal labelling. PCR products were separated in 6% polyacrylamide sequencing gels and visualised by autoradiography.

### 4.33 Statistical methods

Worker regression relatedness ( $b$ ), inbreeding ( $F$ ), and allele frequencies were estimated from the worker genotype frequency data following Queller and Goodnight (1989), using the Relatedness 4.2 program (Goodnight and Queller 1994). The program calculates standard error estimates for  $b$  and  $F$  by jackknifing across nests.

### 4.34 Pedigree estimates

Direct estimates of paternity frequency were made by inspecting worker genotypes across the four loci for each nest. This was used to produce a second estimate of relatedness ( $r$ ) assuming outbreeding and to estimate sperm bias when multiple paternity occurred. The population effective mating frequency ( $M_e$ ) was estimated after Starr (1984):

$$M_e = \frac{n}{\sum_j \sum_i p_{ij}^2} \quad (4.1)$$

where  $p_{ij}$  is the proportional contribution of the  $i$ th male in the  $j$ th nest for  $n$  nests.

### 4.35 Non-sampling error

The probability of not sampling female offspring from a father who actually has paternity was kept to acceptable levels by the analysis of 20 workers from each nest. When 20 workers are sampled, a male fathering a proportion  $p$  of the offspring will be sampled with probability  $1 - (1 - p)^{20}$ . For example, the non-sampling probabilities of 50% and 10% paternity are  $9.5 \times 10^{-7}$  and 0.12 for 20 offspring, respectively. For males with very low paternity contributions, the non-sampling probability is significant. Such males, however, have a small effect on effective mating frequency (Equation 4.1) so that not sampling them introduces only a small error in estimates of relatedness.

### 4.36 Non-detection error

Estimates were calculated at both the population ( $d_p$ ) and nest ( $d_n$ ) level. The probability of two males in a Hardy-Weinberg population having identical genotypes at all loci studied and thus having indistinguishable offspring is:

$$d_p = \prod (\sum q_{ij}^2) \quad (4.2)$$

where  $q_{ij}$  denotes the frequency of allele  $i$  at loci  $j$  (Boomsma and Ratnieks 1996). However, such an estimate assumes that paternally and maternally transmitted alleles can be distinguished. In our data set this was not always possible because the queen

Table 4.1. Genetic variation in the microsatellite marker loci studied, where  $n$  is the number of alleles detected and  $H_e$  the heterozygosity at each locus.

Locus	$n$	allele frequencies	$H_e$
5	5	0.318, 0.305, 0.265, 0.102, 0.011	0.73
13	3	0.509, 0.455, 0.036	0.53
15	6	0.5, 0.186, 0.161, 0.116, 0.036, 0.002	0.67
18	4	0.7, 0.128, 0.095, 0.076	0.48
Mean	4.5		0.60

genotype was not always known. As a result Equation 4.2 can underestimate the non-detection error. Nest-level estimates of non-detection error allow this problem to be corrected for and additionally reveal the magnitude of non-detection error per nest. Three corrections were required to account for ambiguity in paternal genotype, each corresponding to a particular situation (Appendix).

## 4.4 Results

### 4.41 Allelic diversity

Genetic variation at the four microsatellite loci studied was moderate, with three to six alleles per locus and a mean expected heterozygosity across all loci of 0.60 (Table 4.1).

### 4.42 Estimates of relatedness

Worker nestmates were related by  $b = 0.749 \pm 0.035$  (SE), exactly that expected from full sisters. However, high relatedness is partly caused by a low degree of inbreeding:  $F = 0.087 \pm 0.052$ . By adjusting for 253 inbreeding (Pamilo 1984, 1985) worker relatedness drops to 0.702 ( $b^*$ ).

### 4.43 Pedigree estimates

Genotype inspection revealed single paternity in 9 nests, double paternity in 4 nests, and triple paternity in 1 nest (Table 4.2). Single maternity gave the most parsimonious solution for all genotype arrays and in the four nests collected only a single queen was present. In the 5 multiple-paternity nests, the majority male fathered 54%, 70%, 85%, 95%, and 95% of the brood, with the latter 4 significantly different from equality (binomial probability  $< 0.05$ ). The population effective mating frequency (Equation 4.1) was 1.11. Pedigree worker relatedness ( $r$ ) was  $0.701 \pm 0.023$ . This, as expected, agrees with the inbreeding-adjusted regression estimate ( $b^*$ ).

### 4.44 Statistical power of analysis

Using Equation 4.2 and the allele frequencies observed, the population estimate of non-detection error was 0.02 suggesting that a second father is not detected only 2% of the time. The mean from the more conservative nest-based estimates was greater at  $7 \pm 2\%$  (SD). Importantly, the non-detection error is still low. Altogether 20 males were detected and the expected number of males not detected in all 14 nests was 1.0 ( $\Sigma_{dn}$ ). Thus the combination of sampling and non-detection error may lead to a slight

Table 4.2. Nest-level data on paternity, paternity bias, pedigree sister-sister relatedness ( $r$ ), and non-detection error. Non-detection error estimates include the corrections detailed in the appendix. \* indicates that the queen was analysed and so corrections 2a, 2b (Appendix) were not required.

Nest #	No. fathers detected	% contribution of majority male(s)	Sister-sister relatedness	Non-detection error ( $d_n$ )
2	1	-	0.75	0.12
3	1	-	0.75	0.04
6	2	70	0.54	0.08
7	1	-	0.75	0.05
8	2	95	0.70	0.11
12	2	95	0.70	0.07
14	2	55	0.50	0.23
15*	1	-	0.75	0.01
17*	3	85, 10	0.62	0.01
18*	1	-	0.75	0.004
19*	1	-	0.75	0.05
21	1	-	0.75	0.06
22	1	-	0.75	0.13
23	1	-	0.75	0.01

underestimate of  $M_e$  but the effect is relatively minor and does not qualitatively change the conclusion that the effective paternity is close to one.

## 4.5 Discussion

Behavioural studies on *V. crabro* have consistently shown monogyny (Matsuura and Yamane 1990). However, nest usurpation by 'piratical' queens is reported (Nixon 1983) leading to the possibility of offspring from multiple queens in one nest. Multiple matrilines were not apparent in the workers analysed suggesting, as expected, that successful nest takeovers are rare (Nixon 1983, 1986) or that usurpation occurs early in the season so that any daughters of a first queen were dead at the time of sampling.

The majority of *V. crabro* nests analysed revealed single paternity. Multiple mating occurred in 5 of 14 nests although the effective mating frequency remained low at 1.11. This is because of biased paternity with the majority male on average having 80% of paternity. However, the paternity of the majority male varied considerably, being nearly equal to that of the other male in one nest, but significantly biased in the other four. With small non-sampling and non-detection errors, the data provided by this study give a reliable estimate of paternity frequency in *V. crabro*.

In a study on *V. crabro* pheromones, Batra (1980) observed multiple copulations of queens. However, the exact copulation frequency was not recorded. Matsuura and Yamane (1990) report that queens of the Japanese hornet *Vespa mandarinia* rarely copulate more than once. These observational data are consistent with the results of this study, although observations of copulation are often an unreliable predictor of paternity (Boomsma and Ratnieks 1996). *V. crabro* fits into the single-to-multiple (s-m) category for paternity proposed by Boomsma and Ratnieks (1996), although the paternity bias

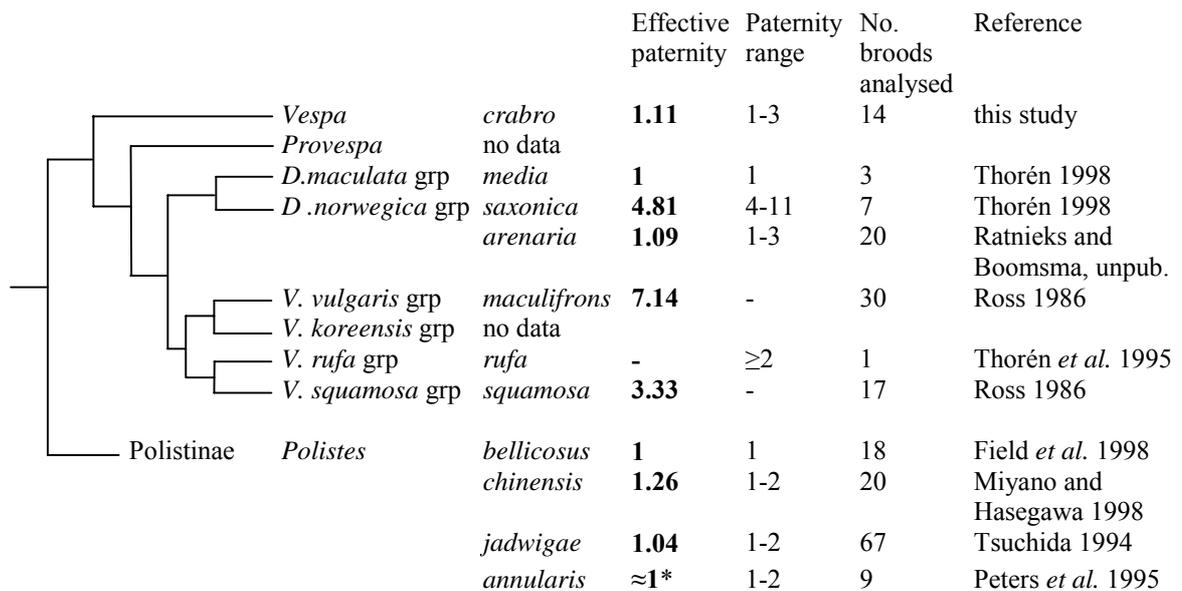


Figure 4.1. Phylogeny of paternity frequency in the Vespinae. Data from *Polistes* are shown for outgroup comparison. The phylogeny follows Carpenter (1987) and recognizes his species groups (grp) within *Dolichovespula* and *Vespa*. \*Based on sperm data.

means that the offspring relatedness of 0.7 is higher than expected for this category ( $0.6 \pm 0.65$ ). In ants, single-to-multiple represents a moderately frequent paternity category accounting for 3 out of the 19 species reviewed (Boomsma and Ratnieks 1996).

Paternity frequency data are now available for the three most diverse genera of the Vespinae, *Vespa*, *Dolichovespula*, and *Vespula*, including three of the species groups in *Vespula*. In combination with Carpenter's (1987) phylogeny, this allows some inferences to be made on the evolution of paternity frequency in the group. High effective mating frequencies ( $M_e$ ) have been shown in all three *Vespula* species groups for which there are data (Ross 1986; Thorén *et al.* 1995). *Dolichovespula* shows a mixed pattern with paternity close to one in two species (*Dolichovespula arenaria*: F.L.W. Ratnieks and J.J. Boomsma, unpublished data, *D. media*: Thorén 1998) but high paternity in *D. saxonica* (Thorén 1998). With a low mating frequency in *Vespa*, parsimony suggests that high paternity is a derived, and hence recent, character in the Vespinae (Figure 4.1). This prediction is supported by outgroup comparison with the Polistinae where studies consistently show paternity frequencies near one (Tsuchida 1994; Peters *et al.* 1995; Goodnight *et al.* 1996; Field *et al.* 1998; Miyano and Hasegawa 1998). The data suggest multiple origins to high paternity within the Vespinae. A single origin is only possible if *Dolichovespula* is polyphyletic, which seems unlikely as 12 autapomorphies unite the genus (Carpenter 1987). Using Carpenter's phylogeny, the most parsimonious solution is of two origins to high paternity, one at the base of *Vespa* and one within the *D. norwegica* group.

Several aspects of reproduction in social Hymenoptera depend on paternity frequency. Low paternity frequency should lead to queen-worker conflict over worker laying (Ratnieks 1988), sex allocation (Trivers and Hare 1976), and queen killing by workers in annual nests (Ratnieks 1988; Bourke 1994). The phylogeny (Figure 4.1) suggests that these conflicts are ancestral in the Vespinae with multiple mating leading to decreased

queen-worker conflict and increased social coherence in the more derived *Vespula*. Behavioural data seem to support this trend with the occurrence of queen killing in *Vespa* (Ishay 1964) and worker reproduction in *D. arenaria* (Greene *et al.* 1976; F.L.W. Ratnieks and J.J. Boomsma, unpublished data) in contrast with the *Vespula squamosa* and *vulgaris* groups where worker reproduction (Ross 1986) and matricide (Akre *et al.* 1976) have not been observed. High paternity in *D. saxonica* and the observation of worker reproduction in *V. consobrina* (*V. rufa* group, Akre *et al.* 1982) are potential exceptions since these are atypical of their respective genera. Further data would be valuable, particularly on worker reproduction in *D. saxonica* and paternity frequency in *V. consobrina* and other species in the *V. rufa* group. Currently the only data for this group is from eight workers from a single colony (Thorén *et al.* 1995).

The Vespinae give the clearest indication to date that high paternity is a derived trait associated with low intracolony conflict. This pattern seems to extend to the eusocial Hymenoptera in general with the highest paternity for bees found in the honeybee *Apis* (Estoup *et al.* 1994), and ants in the leafcutter, *Acromyrmex* (Boomsma *et al.* 1999), both well known for their highly derived eusociality.

## 4.6 Acknowledgements

We thank John Gulliver of the Forestry Commission for help in collecting the hornets, Pia Gertsch, Ignacio Fernandez-Escudero, Anna Lindvall, Anna Goropashnaya, and Niclas Gyllenstrand for assistance in the laboratory, and Koos Boomsma, Roger Lowe, and Pekka Pamilo for discussions. Funding for this study was obtained through the research network 'Social Evolution' of the University of Aarhus, Firenze, Keele, Sheffield, Uppsala, Würzburg and the ETH Zürich, financed by the European Commission via the Training and Mobility of Researchers (TMR) programme and a PhD studentship to K.R.F. from BBSRC, a NERC grant GR9/02676 to F. Ratnieks.

## 4.7 Appendix

### 4.7.1 Nest-level non-detection error estimates

The genotypes of the father males for each nest provide estimates of non-detection error at the nest level (Pamilo 1982a). For every male there is a specific probability that a randomly selected second male has the same genotype across all loci. This is simply the product of the frequencies of the alleles possessed by the focal male. For a haploid male with allele frequencies  $i, j, k, l$  at four loci, the probability that a second male would possess the same genotype  $d_n$  is:

$$d_n = ijkl \quad (4.3)$$

### 4.7.2 Corrections for ambiguity in identification of paternal alleles

Equation 4.3 assumes that the paternal alleles can always be identified in daughter progeny and that the only source of ambiguity in assignment of paternity is when two males share the same multilocus genotype. For the data set analysed, three additional sources of ambiguity arose.

Table 4.3. Possible paternal genotypes resulting in non-detection when the colony queen is heterozygous AB and shares an allele with the first male detected A. The first example is the basic non-detection scenario. In the second example non-detection occurs when the queen transmits her A allele as only BB progeny reveal the second male.

Queen Genotype	Male Genotypes	Male 1 progeny	Male 2 progeny
AB	A, A	AA, AB	AA, AB
AB	A, B	AA, AB	<b>BB</b> , AB

#### Correction 1: a known heterozygote queen and male share an allele

In this situation, non-detection can occur two ways: (1) the second male has the same allele as the first male (basic non-detection) or (2) the second male has the queen's unique allele (Table 4.3). Pamilo (1982a) corrected for this in estimating non-sampling and non-detection error for a single biallelic locus. Only non-detection is considered here to allow application to multilocus, multiallele systems, which would otherwise be difficult (Boomsma and Ratnieks 1996). The probability of non-detection ( $d_1$ ) at an affected locus is:

$$d_1 = \underbrace{a}_{\text{basic non-detection}} + \underbrace{b(0.5)^f}_{\text{queen-masking effect}} \quad (4.4)$$

where  $a$  is the frequency of the first male's allele (A), corresponding to  $i, j, k$ , or  $l$  from Equation 4.3, and  $b$  is the frequency of the queen allele not shared with the first male (B). Queen-masking also requires that the queen donates only her A allele. The probability of this is 0.5 for each offspring, hence the term  $(0.5)^f$  where  $f$  is the number of offspring of a B male sampled. For this study,  $f$  was set to 1, corresponding to the probability that a B male with 5% (1/20) paternity is not detected.  $d_1$  is used in place of  $i, j, k$ , or  $l$  in Equations 4.3 or 4.5 for any affected loci.

#### Correction 2: queen genotype unknown

For 10 of 14 nests, the queen genotype could only be inferred from female offspring genotypes, resulting in two novel problems in detecting a second male.

(a) With two worker genotypes present at a locus, e.g., AC, BC, there are two possible parental combinations that produce the observed pattern, one reflecting single paternity, e.g. AB x C, and the other double paternity e.g. CC x A, B (Table 4.4a) It is possible, therefore, that two males with different genotypes have mated with the queen but still remain unresolvable, contrary to the assumptions of Equation 4.3. This reduces the number of useful detecting loci by one, as any such locus is uninformative. Detection requires a second locus at which the males differ (Table 4.4b). If one such locus occurs in a worker sample, the non-detection error must be calculated without it. Several affected loci can be accounted for by calculating the non-detection error discounting one locus at a time, and taking the mean. For three affected loci in a four- locus analysis:

$$d_n = \frac{(ij + ik + jk)}{3} l \quad (4.5)$$

with  $i, j$ , and  $k$  as the allele frequencies of the male allele at the affected loci assuming single mating. This compares to a non-detection error of  $ijkl$  without the correction

Table 4.4. (a) Offspring genotype combination that could reflect single or double paternity. Possible parental genotypes: Queen BC, Male A (single mating) or Queen AA, Males B and C (double mating). (b) Resolution of a double mating, if present, is only possible if males have different alleles at an additional locus. Detection then occurs through the correlation of worker genotypes across loci (B with E, C with F) indicating the non-recombinant genotypes of haploid males.

Offspring	(a)	(b)
1	AB	DE
2	AC	DF
3	AC	DF
4	AB	DE
5	AB	DE
6	AC	DF
7	AB	DE
8	AC	DF
9	AC	DF
10	AB	DE

(Equation 4.3). Equation 4.5 assumes an equal probability that each locus will ‘detect’ a double mating, which in reality is dependent on allele frequency. However, this effect is difficult to quantify, as the probability that any one locus detects double mating interacts with the probability of double mating in the population, which for the purposes of error estimates is an unknown. Assuming that each affected locus detects a double mating with equal probability in general produces the most conservative estimate. Accounting for allele frequency would cause the loci with the most effect on non-detection to be discounted least, lowering the non-detection estimate. The exception to this occurs if applying Equation 4.4 reorders the magnitudes of non-detection ( $i, j, k, l$ ) at the loci. This is in practice rare and the resulting estimate is still conservative relative to Equation 4.3.

(b) If all workers are heterozygous at a locus, it is unknown which allele is paternally and which is maternally derived. This can be accounted for by weighting the two alternative estimates of non-detection error by the relative probability that each allele is paternally derived. For worker genotypes AB of allele frequencies  $a$  and  $b$ , the probability that the queen is AA and male is B is  $a^2b$  versus  $b^2a$  for queen BB and male A. The *relative* probability that allele B is male derived is then  $a^2b/(a^2b + b^2a)$  and so the probability of non-detection at an affected locus ( $d_2$ ) equals:

$$d_2 = \frac{a^2b}{a^2b + b^2a}b + \frac{b^2a}{a^2b + b^2a}a = \frac{2ab}{a + b} \quad (4.6)$$

where, as for  $d_1$ ,  $d_2$  is used in place of simple allele frequency ( $i, j, k$ , or  $l$ ) in Equations 3 or 5.

Non-detection error for each nest was calculated by classifying ambiguities at all affected loci and applying these corrections. In the polyandrous queen nests, corrections 1 and 2a were required. The latter is only required when the suspected queen alleles (B and C in Table 4.4a) are found in only one patriline, as the queen genotype remains ambiguous. This is likely with high paternity bias.

## Do hornets have zombie workers?

K.R. Foster, F.L.W. Ratnieks & A.F. Raybould

### 5.1 Abstract

Colonies of the European hornet, *Vespa crabro*, are typically founded by a single queen mated to a single male. From the resulting colony relatedness pattern we predicted strong worker–queen conflict over male production where both the workers and the queen attempt to produce the colony’s males. To test for this conflict, male production was studied in 15 hornet nests using a combination of DNA microsatellite analysis (282 males), worker ovary dissections (500 workers from eight nests) and 50 h of observation (four nests). In contrast to our prediction, the data show that hornet males are queens’ sons, that workers never attempt to lay eggs, rarely have activated ovaries, and that there is no direct aggression between the queen and the workers. This contrasts with other data for vespine wasps, which support relatedness predictions. *Dolichovespula arenaria* has the same kin structure as *V. crabro* and workers produce males in many colonies. The similarity between these two species makes it difficult to explain why workers do not reproduce in *V. crabro*. Self-restraint is expected if worker reproduction significantly reduces colony productivity but there is no obvious reason why this should be important to *V. crabro* but not to *D. arenaria*. Alternatively, queen control may be important. The absence of expressed queen–worker conflict rules out physical control. Indirect pheromonal control is a possibility and is supported by the occurrence of royal courts and queen pheromone in *Vespa* but not *Dolichovespula*. Pheromonal queen control is considered evolutionarily unstable, but could result from a queen–worker arms race over reproductive control in which the queen is ahead. The genetic data also revealed diploid males in one colony, the first example in the vespine wasps, and two colonies with double matriline, suggesting that occasional usurpation by spring queens occurs.

### 5.2 Introduction

Insect societies are often considered superorganisms composed of cooperating individuals (e.g. Wheeler 1911; Wilson 1985; Wilson & Sober 1989). While cooperation is fundamental, there is also the potential for conflict (Ratnieks & Reeve 1992). The eusocial Hymenoptera are particularly interesting in this respect owing to their diverse kin structures and haplodiploid genetics, which cause great diversity in potential intracolony conflicts. One major area of potential conflict is male production. Being haploid, males can be offspring of both unmated workers and queens. In a colony headed by a singly mated queen, workers should prefer rearing sons ( $r = 0.5$ ) and other workers’ sons ( $r = 0.375$ ) to their mother’s sons ( $r = 0.25$ ) (Hamilton 1964; Trivers & Hare 1976; Ratnieks 1988). Relatedness, therefore, predicts that workers will conflict

with the queen over male production, both individually and collectively.

Actual conflict over male production may be reduced in colonies with effective paternities (queen mating frequency) above two (Starr 1984; Woyciechowki & Lomnicki 1987; Ratnieks 1988), or many closely related queens (Pamilo 1991b), because workers are then more related to the queens' sons than other workers' sons. Although individuals are always most related to their own sons, the workers' collective interests are now aligned with the queens' interests. Increased cooperation can then occur through collective worker policing of individual workers resulting in queen-only male production (Ratnieks 1988). However, with low paternity common in Hymenoptera (Boomsma & Ratnieks 1996) and workers being the numerically dominant power, (Trivers & Hare 1976; Keller & Nonacs 1993) actual queen-worker conflict over male production is expected to be widespread.

Worker reproduction in colonies with a queen has been recorded several times in the eusocial Hymenoptera but its occurrence is highly variable between and within species (Bourke 1988; Bourke & Franks 1995). Assessing the role of colony kin structure was initially difficult due to the requirement for accurate data on paternity or queen relatedness (Keller & Vargo 1993). The first such data came from visible genetic markers (e.g. Owen & Plowright 1982) and allozymes (e.g. Pamilo 1982b). These are now supplemented by more powerful DNA microsatellite studies (Queller *et al.* 1993a). The potential power of relatedness predictions has been demonstrated by comparing stingless bees (Meliponinae) with the honeybee *Apis mellifera* (Ratnieks 1988; Peters *et al.* 1999). *Apis* has extremely high paternity (Estoup *et al.* 1994) and workers that police each other's reproduction (Ratnieks & Visscher 1989). This contrasts with low paternity in stingless bees (13 species, Peters *et al.* 1999), ritualized queen-worker conflict (Kerr 1969) and worker laying (Sakagami 1982). In further agreement, low paternity in bumblebees (5 species, Estoup *et al.* 1995; Thorén 1998) is associated with intracolony conflict and worker male production (Honk *et al.* 1981).

Data from the vespine wasps are consistent with relatedness predictions. Two *Vespula* species have high paternities and evidence of queen only male production (*V. maculata* and *V. squamosa* Ross 1986). This contrasts with its sister group *Dolichovespula* where effective paternities below two have been found in six species (*D. arenaria*, F. L. W. Ratnieks and J. J. Boomsma, unpublished; *D. maculata*, *D. sylvestris*, *D. norwegica*, *D. media* and *D. saxonica*, Foster *et al.* 2001, Chapter 7) and worker male production occurs in queenright colonies (*D. arenaria*, Greene *et al.* 1976; F. L. W. Ratnieks and J. J. Boomsma, unpublished).

Not all data, however, agree with relatedness predictions. Paternity below two but no worker reproduction was reported in 14 colonies of *Bombus hypnorum* (Thorén 1998). Walin *et al.* (1998) analysed three *Formica* and one *Myrmica* ant species and showed that while relatedness predicted worker male production in all, it could only be considered a possibility in one. In addition, patterns of male production in *Leptothorax* ants do not seem to be attributable to kin structure alone (Heinze *et al.* 1997). These studies demonstrate that relatedness is not the only factor affecting worker reproduction in queenright colonies. Costs of worker reproduction on colony productivity and queen control may also be important, although their precise role remains uncertain due to the difficulties in quantifying them (see Bourke & Franks 1995 for a review).

Previous research has shown low effective paternity, 1.11, in the European hornet *Vespa crabro* (Foster *et al.* 1999, Chapter 4) leading to the prediction of queen–worker conflict over male production. As in all vespines, hornet workers are smaller than the queen and unable to mate but retain the ability to lay haploid male eggs in queenless groups (Matsuura & Yamane 1990). To test for worker reproduction in the queen’s presence we used a novel combination of genetics, ovary dissection of workers and observation. In contrast to prediction, the data show an absence of any expressed conflict with the queen producing all the colony’s males.

### 5.3 Methods

Thirty-two hornet nests were collected from the New Forest, Hampshire, UK in two collections in 1998, one in July and early August (ergonomic phase of the annual life-cycle) and the other in September (reproductive phase). Colonies are founded in May and end their annual life-cycle from September to November (Archer 1993). All nests were collected from pest control calls and would otherwise have been destroyed. Twenty were relocated to nest boxes for observation or maintenance at the Institute of Terrestrial Ecology, Furzebrook, Dorset. Twelve nests unsuitable for relocation, such as those lacking a queen or collected late in the season were immediately frozen at  $-70^{\circ}\text{C}$ .

#### 5.31 Nest boxes

Wooden observation nest boxes were 9.5 cm (depth)  $\times$  30 cm (horizontal)  $\times$  40 cm (vertical) and were faced with a hinged glass door to allow observation and access. Eight were set up in a shed with plastic pipe ( $\text{\O} 3$  cm) to the outside allowing the hornets to forage in the wild. Ten wooden maintenance boxes, 20 cm  $\times$  20 cm  $\times$  40 cm with a 3-cm entrance hole in the side, were also made and nailed to trees in a wood. These allowed young colonies to become reproductive to provide samples of males.

#### 5.32 Relocation

During relocation nests were chilled with ice and the workers separated from the combs. The combs, with the queen, were placed on horizontal wires inside the nest box. The chilled workers were then replaced and given 50 ml of honey to provide food during nest re-establishment. After about an hour the entrance to the nest box was opened and the hornets allowed to fly. Ten nests were relocated to observation boxes and 10 to maintenance boxes. Four observation and two maintenance nests remained queenright and developed to reproductive status.

#### 5.33 Observation

The four observation nests were sequentially scan sampled for a total of 50 h (1956 scans), a maximum of 3 h per day, from 26 August to 28 September. Nest-envelope paper that prevented observation of the combs was gently removed at intervals by sliding a hacksaw blade behind the observation box door. In each scan, all combs were carefully examined noting queen position (comb number) and activity (oviposition, queen–worker or worker–worker aggression). An additional queenless nest was also observed to determine the duration of a worker oviposition. Five worker and five queen

ovipositions were timed to give means of 126 and 130 s, respectively. The mean time taken to scan a nest was 92 s so that each nest was not watched for an average of 276 s during each round of scanning. On average, therefore, there was a 150-second period (276–126 s) each round when worker oviposition events would be missed. Thus, the effective period during which worker oviposition could be observed was approximately 118 h ( $218/368 \times 50 \text{ h} \times 4 \text{ nests}$ ).

### 5.34 Genetic methods

Ten workers and the queen, if collected, from each of 19 nests were analysed at four DNA microsatellite loci using primers designed for *Vespula rufa* (Thorén 1998) and modified for use on *Vespa crabro* (Foster *et al.* 1999, Chapter 4). Maternity was then assessed in up to 20 males at one or two loci for 13 nests. Loci were chosen in which the workers' paternal and maternal allele were different (informative genotypes). This allows identification of a worker-produced male by its inheritance of the mother worker's unique paternal allele (see also Male non-detection error). For five nests that had male pupae, equal numbers of male pupae from each comb were analysed instead of adult males in case this could give additional data on laying location. Males from two additional nests, collected in 1997, were also analysed. One of these nests was unusual in that some workers had activated ovaries (see Results). To raise the detection probability in this nest, 50 males were analysed. For the other nest 20 males were analysed to give a total sample of 282 males from 15 nests.

### 5.35 Worker–worker relatedness

Regression relatedness among offspring females (workers) ( $b$ ), inbreeding ( $F$ ), and allele frequencies were estimated from the worker-genotype frequency data using the program Relatedness 4.2 (Goodnight & Queller 1994). The program calculates standard error estimates for  $b$  and  $F$  by jackknifing across nests. Pedigree estimates of relatedness were made by inspecting worker genotypes across the four loci for each nest. This was used to produce a second estimate of relatedness ( $r$ ), assuming outbreeding, and to estimate sperm bias when multiple paternity occurred.

### 5.36 Effective paternity

Effective paternity ( $M_e$ ) was estimated from Pamilo (1993):

$$M_e = \frac{1}{2b - 0.5} \quad (5.1)$$

where  $b$  is the regression relatedness from Relatedness 4.2.

### 5.37 Male non-detection error

Workers' sons are only detected if: (i) the queen and her mate(s) have different alleles (an informative genotype), and (ii) they inherit the worker's paternal allele. With fair meiosis, the paternal allele is transmitted with probability 0.5. Hence, even if worker genotypes are informative, 50% of worker-produced males cannot be distinguished

Table 5.1. Genetic variation in the microsatellite marker loci studied, where  $n$  is the number of alleles detected in the 19 study colonies and  $H_e$  the expected heterozygosity at each locus.

Locus	$n$	allele frequencies	$H_e$
5	7	0.133, 0.314, 0.008, 0.026, 0.147, 0.346, 0.026	0.74
13	3	0.600, 0.058, 0.342	0.52
15	5	0.108, 0.434, 0.250, 0.176, 0.032	0.71
18	4	0.060, 0.134, 0.614, 0.193	0.56
Mean	4.75		0.63

from queen-produced males at this locus. With unlinked loci, the total number of assignable males in a sample ( $N_a$ ) can be estimated from:

$$N_a = \sum_j^n \left( 1 - \prod_i^{l_j} (1 - 0.5 p_{ij}) \right) N_j \quad (5.2)$$

Where  $l_j$  is the number of loci and  $N_j$  the number of males analysed for the  $j$ th of  $n$  nests and  $p_{ij}$  is the proportion of informative worker genotypes at the  $i$ th loci of the  $j$ th nest. Weighting by  $p_{ij}$  assumes that workers of all genotypes are equally likely to reproduce.

### 5.38 Male non-sampling error

If workers produce a proportion  $x$  of the males, the probability of not sampling any worker-produced males is  $(1 - x)^{N_a}$ .

### 5.39 Worker ovary activation

The ovaries of 500 workers from eight queenright nests were examined by dissection under a binocular microscope with a graticule eyepiece. The size of the largest egg was compared to the mean size of five worker-laid eggs (taken from a queenless nest) and placed into one of three categories: less than half size, greater than half size, greater than 90% full-size.

## 5.4 Results

### 5.41 Queen loss in the wild

Four of 23 prereproductive nests collected in July and early August were queenless and, hence, unable to develop to a large size. Pre-reproductive status was defined by the presence of worker-sized cells only (the start of large cell construction signifies the start of laying of gyne-destined and the great majority of male-destined eggs, Archer 1993). Two out of the nine reproductive nests collected in late September were queenless male-producing nests with reproductive workers.



Figure 5.1. Probability of not detecting any worker produced males as a function of the percentage of all males that are worker produced, for 176 assignable males ( $N_a$ ).

#### 5.42 Observations

Eighty-five queen ovipositions but no worker ovipositions were seen during scan sampling of the four queenright observation nests. The queen was completely ignored by the workers and no aggression between the two was ever seen. There were nine cases of workers mauling each other, an enigmatic behaviour seen in several vespine species (Greene 1991). The queen in all nests spent most time on the middle combs and least on the upper. The four nests observed all produced males and built at least one new comb after relocation. One nest produced approximately 150 workers, 100 males and 20 gynes. The other three nests produced about 50 workers and 5–10 males. This is within the natural range, but small.

#### 5.43 Allelic diversity

Genetic variation at the four microsatellite loci studied was moderate, with 3–7 alleles per locus and a mean expected heterozygosity across all loci of 0.63 (Table 5.1). The allele frequencies did not significantly differ from the estimate obtained from 14 nests from the same population in 1997 (Foster *et al.* 1999, Chapter 4) ( $\chi^2$ ,  $P > 0.05$  for each locus).

#### 5.44 Worker–worker relatedness

Worker nestmates were related by  $b = 0.67 \pm 0.06$  SE across the 19 nests with an inbreeding coefficient not significantly different from zero  $F = -0.063 \pm 0.065$ . The pedigree estimate of worker nestmate relatedness gave a very similar result  $r = 0.68 \pm 0.03$ . Fourteen nests were monogynous and monoandrous, two nests had two matriline and three had two patriline. In the two patriline nests, the majority males fathered 95%, 60% and 50% of the workers analysed. In the two matriline nests, the majority matriline represented 70% and 60%. An estimate of relatedness reflecting paternity effects was only obtained by weighting all matriline equally,  $b = 0.73 \pm 0.04$ .

This gives an effective paternity ( $M_e$ ) of 1.04. Non-detection and non-sampling errors may cause relatedness to be slightly overestimated. However, the potential effects in this system are minor (Foster *et al.* 1999, Chapter 4) and do not affect the key conclusion that  $M_e$  is much less than two and the prediction that worker reproduction is expected but worker policing is not (Ratnieks 1988).

#### 5.45 Male production

All haploid male genotypes from the 14 nests were consistent with being queens' sons. The number of assignable males  $N_a$  was estimated to be 176 (Equation 5.2). This is equivalent to a probability of less than 5% of missing a worker contribution to male production greater than 2% (Figure 5.1).

#### 5.46 Diploid males

In one nest, which had a single patriline, the 10 males analysed were diploid. This nest consisted of the queen, 40 workers and 36 males and was notable in producing adult males at an early date, 8 August. These males were not counted in calculating  $N_a$  (see Methods).

#### 5.47 Worker ovary activation

In 400 workers dissected from seven nests none had full-size eggs and only two had eggs greater than half size. In one nest 6/100 workers had a single full-size egg and another six had an egg greater than half size.

### 5.5 Discussion

The discovery of diploid males in *Vespa crabro* is the first record for the Vespinae (yellowjackets and hornets). Diploid males have been reported in many hymenopteran taxa but there are only two other records for social wasps, one Polistinae, and one Stenogastrinae (J. E. Strassman, personal communication in Crozier & Pamilo 1996). In the honeybee (*Apis mellifera*) and parasitoid wasp (*Bracon hebetor*) diploid males occur as a result of matched mating at a single multiallelic sex-determination locus (see Crozier & Pamilo 1996). A single sex-determination locus is consistent with our data in that there were near equal number of workers to males (40 to 36), as expected with single paternity assuming that diploid males have similar survival to females.

The worker–worker relatedness of 0.68 is very similar to the estimate of 0.70 obtained by an analysis of 14 nests from the same population in 1997 (Foster *et al.* 1999, Chapter 4). In addition there is no significant difference in the proportion of multiple-patriline nests found (3/19 in 1998 vs. 5/14 in 1997,  $\chi^2$ ,  $P = 0.307$ ). However, the discovery of two nests with two matriline is new. Both nests had only one queen at collection, suggesting successful nest takeover by usurping queens. The data show that successful takeover is quite rare, occurring in only 2/33 nests (combining the 1997 and 1998 data). Nixon reported 'piratical' behaviour in queen hornets but found that usurped nests rapidly declined (Nixon 1983; 1986). The nests studied here had adult workers from the new queen showing that the colonies had survived at least one month since usurpation.

Queens of *V. crabro* produce all or the vast majority of the colonies' males. Genetic analysis of 272 haploid males from 14 nests ( $N_a = 176$ ) revealed that all male genotypes were consistent with being queens' sons. Observational data further revealed an absence of any behavioural conflict between the queen and the workers, nor was there any attempted worker laying during an effective observational period of 118h in which 82 ovipositions by queens were seen. This contrasts with the observation of worker laying in the queenless nest and data from a queenright *Dolichovespula arenaria* nest with 32 queen to 10 worker ovipositions (Greene *et al.* 1976). The two studies are comparable, with observations in both being made in the latter part of the reproductive period. Worker ovary dissection of *V. crabro* also suggests a general absence of worker reproduction, although one nest had some workers with activated ovaries indicating that worker laying in queenright colonies could occur. However, the 50 males analysed from this nest ( $N_a = 37.5$ ) revealed no evidence of worker reproduction showing that, as in the other colonies, it is either absent or rare. In addition, ovary-activated workers only ever had one full-size egg in their ovaries suggesting that they would have had lower fecundity than the queen. Our data agree with other *Vespa* data. There are no confirmed reports of worker laying in queenright hornet colonies despite several observational studies (notably Nixon 1985a; Matsuura & Yamane 1990). Ishay (1964) stated 'it was permissible to suppose' queenright worker reproduction occurred in the upper combs of *V. orientalis* since the queen spent most time on the lower combs, but no supporting data were given. In *V. analis*, only one out of 1062 workers from 16 queenright reproductive colonies had fully activated ovaries (Matsuura 1984). Martin (1990) dissected 600 workers from six *V. simillima* nests (500 from one nest) and found six workers with activated ovaries. However, as in this study, the activated ovaries only contained a single full-size egg contrasting with the ovaries of workers from queenless colonies, which contain several full-size eggs (SJ Martin, personal communication).

The absence of male production by workers in *V. crabro* does not fulfil our prediction arising from relatedness theory (Hamilton 1964). This contrasts with available data from *Dolichovespula*, and *Vespula*, where relatedness seems a reliable predictor of the absence or presence of male production by workers in queenright colonies (Ratnieks 1988; Foster *et al.* 1999, Chapter 4). In *D. arenaria*, which has a similar low paternity, workers in queenright colonies have activated ovaries, oviposit and succeed in producing males in queenright colonies (F. L. W. Ratnieks and J. J. Boomsma, unpublished; Greene *et al.* 1976). In addition to kin structure, *V. crabro* and *D. arenaria* also share lifecycle, ecology, queen-worker size dimorphism and colony size making it hard to explain why worker reproductive behaviour should differ. If worker reproduction is costly to colony productivity, self-restraint (possibly enforced through worker policing) can be favoured (Ratnieks & Reeve 1992). However, with *D. arenaria* and *V. crabro* being so similar, there is no obvious reason for an increased cost to worker reproduction in *V. crabro*. This suggests either that an increased cost is caused by some subtle and as yet unknown factor or that such costs are not important.

An alternative hypothesis to explain the absence of worker reproduction in *V. crabro* is that the queen controls worker reproduction (Bourke & Franks 1995). Physical queen control (queen policing) is a likely explanation for the absence of worker reproduction in the small colony vespids *Polistes bellicosus* and *P. dorsalis* (Arevalo *et al.* 1998). However, no queen aggression or oophagy has been seen in *Vespa* (this study; Nixon 1985a; Matsuura & Yamane 1990). Instead, hornet queens may exert indirect pheromonal control to cause the worker's acquiescent zombie-like behaviour. Queen pheromone (Ikan *et al.* 1969, *V. orientalis*) and royal courts (Nixon 1985a; Matsuura

1991, *V. crabro*) both occur in *Vespa* and are not recorded in *Dolichovespula* (or *Vespula*, where queens are multiply mated and worker policing may act). Although queen pheromones may yet be discovered in these genera, studies looking for pheromones have not been successful (Greene 1991) and the absence of royal courts suggests that, if queen pheromones do occur, they have a less direct effect on workers.

The idea of queen pheromonal control has been criticised (Seeley 1985; Keller & Nonacs 1993). If the queen's signal is against worker interests then workers will be selected to ignore it, rendering it simply an honest signal of the queen's presence (Seeley 1985). However, the queen may then be selected to regain control and enter an arms race with the workers (West-Eberhard 1981). Keller & Nonacs (1993) argued that this would not persist, as it would quickly become too costly for the queen to invest in new and greater quantities of chemicals to prevent worker evasion. However, an arms race need not be costly to the participants. With no memory in the system, it can proceed by alternating between a limited set of states with little innovation and no escalation (p. 67 Ridely 1993; Lythgoe & Read 1999). In addition, the queen may have an inherent advantage in the race because workers in queenright colonies should only lay during the reproductive phase of the life cycle. Hence, in annual societies, for most of the season a queen signal would be cooperative and honest. Worker counter-evolution would be constrained because worker reproduction too early in the season will reduce the total amount of reproduction by the colony and be costly. The workers require a strategy that not only blocks worker response to queen pheromone but also only does so at a particular stage in the season. A persistent arms race is therefore a possibility. Providing evidence for or against such hypotheses is extremely difficult. However, one prediction of arms race theory is that the outcome should be fairly arbitrary across lineages (Bourke & Franks 1995, p. 239). This could explain why worker reproduction is absent in *V. crabro* but present in the otherwise similar *D. arenaria*.

In annual colonies with queen control, another strategy enabling workers to reproduce is matricide (Bourke 1994). There are several anecdotal reports of queen killing by workers in *V. crabro*. Nixon (1985a) reported that at the peak of colony development workers may surround the queen and aggressively jostle her, but that the queen survived this attention. Matsuura (1984) described this as royal court behaviour, further stating that workers actually bite the queen and may kill her. Other possible accounts of matricide come from Janet (1895) who described a *V. crabro* worker biting the queen who later died and Ishay (1964) who stated that queens of *V. orientalis* are licked to death. However, only two out of nine colonies collected at the end of the season in this study were queenless nests and potential candidates for matricide. In addition, no aggression towards the queen was seen in the four observation colonies. Therefore, if matricide is a real phenomenon in *V. crabro*, it is probably restricted to a minority of colonies.

Kin selection predictions are complicated by unknown costs and constraints, which act in addition to the effects of relatedness. However, the comparison of similar species, such as *V. crabro* and *D. arenaria*, eliminates many potential variables and enables possible causal agents to be identified. This approach is important for the future of kin-selection research. It improves on the potentially anecdotal nature of single-species studies while being more specific than broad correlation (Ratnieks 1988; JE Strassmann, personal communication). Such comparisons require that the otherwise similar species differ in key reproductive traits. Several examples of this are found in

the vespine wasps. Worker reproduction has been observed in *Vespula consobrina* (Akre *et al.* 1982) and *V. acadica* (Reed & Akre 1983) but not *V. atropilosa* (Akre *et al.* 1976), which are all members of the small colony *V. rufa* species group of *Vespula* (Carpenter 1987). Relatedness itself varies between and within species in the *D. norvegica* species group with single paternity found in most *D. norvegica* and *D. sylvestris* colonies but a mix of single and multiple paternity in *D. saxonica* (Foster *et al.* 2001, Chapter 7). Finally, royal court behaviour like that found in *Vespa* has been observed in the fourth vespine genus *Provespa* (Matsuura & Yamane 1990) allowing further investigation of the queen control hypothesis. This diversity of social traits in an otherwise homogenous group makes the vespine wasps an excellent group for further study of kin selection.

## 5.6 Acknowledgements

We thank John Gulliver and Deanne Williams for help with the hornets, Perttu Seppä, Cia Olsson, Niclas Gyllenstrand and Peter Thorén for assistance in the DNA laboratory and Laurent Keller for helpful comments. Funding for this study was provided by a Biotechnology and Biological Sciences Research Council studentship to K.R.F. and the research network ‘Social Evolution’ of the Universities of Aarhus, Firenze, Keele, Sheffield, Uppsala, Würzburg and the ETH Zürich, financed by the European Commission via the Training and Mobility of Researchers (TMR) programme.

## Why workers do not reproduce: worker policing in the European hornet *Vespa crabro*

K.R. Foster, J. Gulliver & F.L.W. Ratnieks

### 6.1 Abstract

Although generally capable of producing males, workers in most hymenopteran societies (bees, ants and wasps) perform little or no reproduction in the presence of the queen. We investigated why workers do not reproduce in the European hornet *Vespa crabro*. Previous genetic and behavioural work on this species had shown that, although queen mating frequency is low (effective paternity, 1.1) causing workers to be more related to workers' sons than to the queen's sons, workers do not lay eggs and the males reared are all the queen's sons. This suggested that workers are under queen pheromonal control. Here we show that this is not the case. Using egg introduction experiments, we show that worker policing behaviour occurs. We introduced queen-laid and worker-laid eggs into four discriminator colonies in five trials. In colonies with a queen, workers removed significantly more worker-laid than queen-laid eggs (1/79 worker-laid eggs but 46/72 queen-laid eggs remained after 16 hours,  $P < 0.001$ ). In colonies without a queen, workers removed significantly more queen-laid than worker laid eggs (30/44 worker-laid eggs but 13/41 queen-laid eggs remained after 16 hours,  $P \leq 0.001$ ). The presence of worker policing in queenright hornet colonies provides a proximate explanation for the absence of worker reproduction. Workers are not under queen control but instead are collectively enforcing their own sterility. Worker policing at low paternity may have been selected for because it enhances colony productivity by eliminating costly conflicts over reproduction.

### 6.2 Introduction

Workers in many hymenopteran societies perform little or no reproduction (Bourke 1988; Bourke and Franks 1995). However, in almost all species workers are capable of reproduction. In species with morphologically distinct queens and workers, workers are generally unable to mate but possess ovaries and will lay haploid, male eggs in the absence of the queen (Wilson 1971; Bourke 1988). Furthermore, workers are generally more related to each other's sons than the queen's sons (Hamilton 1964; Starr 1984). The absence of worker reproduction in the presence of the queen, therefore, requires explanation (Bourke and Franks 1995).

Queen control is likely to be important in some species (Wilson 1971; Ratnieks 1988). Physical queen control seems to occur in small colony species (e.g. *Polistes*, Arevalo *et al.* 1998). However, in larger colonies physical control seems unlikely (Ratnieks 1988). It has been suggested that in large colonies physical queen control gives way to

pheromonal control (Wilson 1971; Hölldobler and Bartz 1985). However, this idea was criticised by Seeley (1985) and Keller and Nonacs (1993) who argued that workers should simply ignore such a queen control pheromone if it was against their fitness interests. In support of this, workers have been shown to adjust the sex ratio against the queen's interest by selectively killing brothers (e.g. Sundström *et al.* 1996).

The absence of worker reproduction may alternatively be explained by worker policing (Ratnieks 1988) or reproductive self-restraint (Bourke and Franks 1995). Worker policing is especially likely to be selected in single-queen societies when the mother queen is inseminated by multiple males (effective paternity  $> 2$ ), because workers will be more related to the queen's sons ( $r = 0.25$ ) than other worker's sons ( $r < 0.25$ ) (Starr 1984; Ratnieks 1988). However, worker policing, and also reproductive self-restraint, may be selected for at any paternity if worker reproduction significantly reduces colony productivity (Ratnieks 1988; Bourke and Franks 1995). Relatedness suggests that worker policing will be most likely to evolve from productivity effects because workers are less related to nephews ( $r = 0.375$ ) than sons ( $r = 0.5$ ).

The aim of this study was to investigate the absence of worker reproduction in the European hornet, *Vespa crabro*. Most colonies are headed by a singly mated queen and population-wide effective paternity is low (1.11 Foster *et al.* 1999, Chapter 4) leading to the prediction of worker reproduction in queenright colonies. However, in a genetic study of 282 males from 15 colonies, only queen's sons were detected (Foster *et al.* 2000, Chapter 5). This raised the possibility that hornet workers were under queen pheromonal control (Foster *et al.* 2000, Chapter 5). This study tests an alternative hypothesis: that worker reproduction is suppressed by worker policing. Using egg transfer experiments we show that workers in queenright colonies remove worker-laid eggs but leave the majority of queen-laid eggs. This suggests that workers are not under queen control but instead are collectively enforcing their own sterility.

## 6.3 Methods

### 6.3.1 Relocation of hornet colonies

We collected ten colonies of the European hornet, *Vespa crabro*, in the New Forest, Hampshire, UK from pest control calls from July to September 2000. All were removed at the request of residents as they were in close proximity to human habitation. We relocated them (as described in Foster *et al.* 2000, Chapter 5) to wooden nest boxes (30 x 30 x 40cm) attached to trees in private woodland near Ashurst, New Forest. The boxes had a 4cm diameter entrance at the front and were hinged at the top allowing experimental access. We studied two additional colonies. One we relocated by moving the rabbit hutch it was in to Ashurst and the other we studied *in situ* in a barn near Holmsley, New Forest.

### 6.3.2 Study colonies

Two of the ten nests relocated to boxes became well established and developed to reproductive status, but none remained queenright. We used these two successful nests as queenless discriminator colonies (see below) and as a source of worker-laid eggs. The rabbit hutch and barn colonies both remained queenright and developed to reproductive status. These were used as queenright discriminator colonies and as

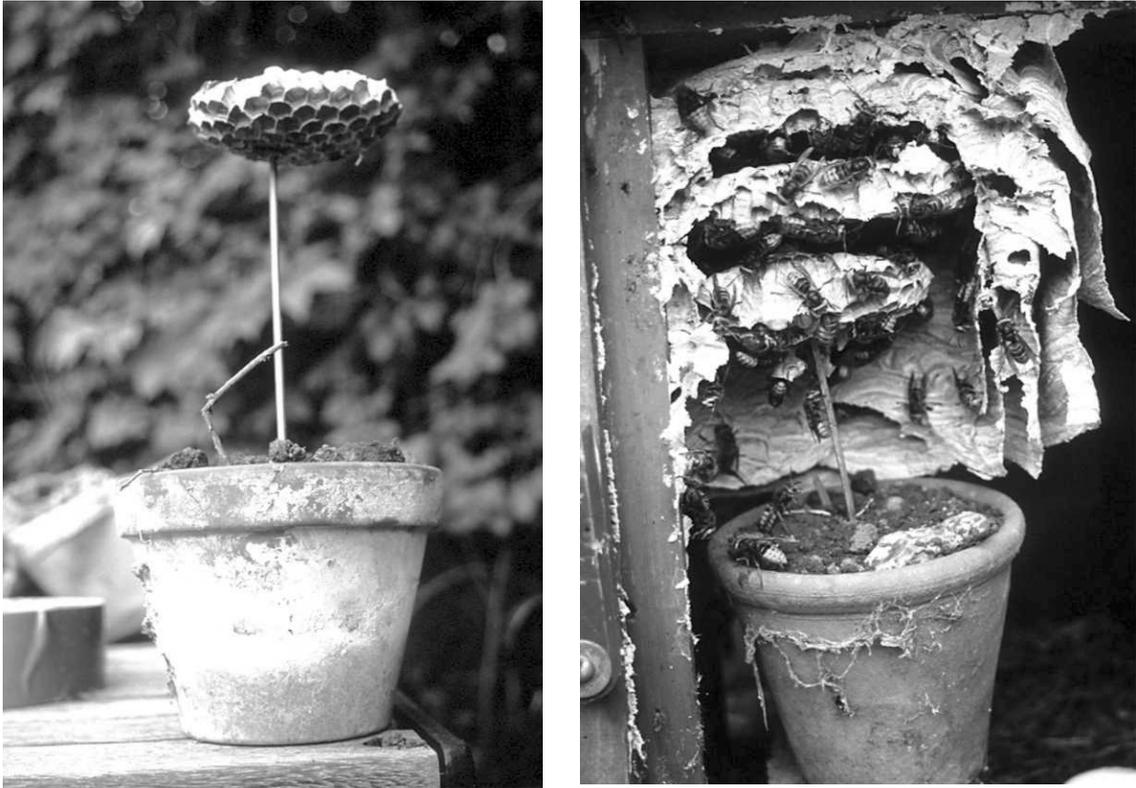


Figure 6.1. Photographs of the discriminator test comb. After the foreign queen-laid and worker-laid eggs had been glued into the comb, the comb itself was glued to a piece of dowel, which was inserted into soil in a flower pot (left). The comb could then be reintroduced into the colony in its original position (right).

sources of queen-laid eggs. Previous work had shown that workers never or very rarely lay eggs in queenright colonies (Foster *et al.* 2000, Chapter 5) so we can be confident that eggs from queenright colonies were queen-laid. An additional two colonies which failed to establish were used as sources of queen-laid (Colony 1) and worker-laid eggs (Colony 2), immediately after their collection (Table 6.1).

### 6.33 Policing assay

We investigated worker policing by introducing queen and worker-laid eggs from foreign colonies into discriminator colonies in five trials in late August/early September (Table 6.1). Each trial involved three colonies, a discriminator colony, a queen-laid eggs source colony and a worker-laid eggs source colony. By introducing only foreign eggs into discriminator colonies we controlled for all variables other than egg maternity.

A policing trial was started by removing the bottom comb from each of the three colonies: discriminator, queen egg source and worker egg source. We removed and discarded all the eggs and brood in the discriminator test comb. Next, eggs were removed from the source combs using a pair of forceps. Eggs in vespine wasps are glued to the paper comb and so were removed with a small piece of paper attached, c. 3x3mm. We then glued the eggs by their paper attachments, using water-based PVA glue, into the test comb in alternating rows of queen-laid and worker-laid eggs. Finally, we replaced the test comb now containing the foreign queen-laid and worker-laid eggs into the discriminator colony (Figure 6.1). The number of queen-laid and worker-laid eggs remaining was checked after 45 minutes and 16 hours. To exclude the possibility

Table 6.1. Removal of eggs by discriminator colonies in *Vespa crabro*. Workers selectively discriminate against worker-laid eggs in *queenright* colonies but against queen-laid eggs in *queenless* colonies. The trials are numbered in the order that they were performed. \*Indicates that a 1-tailed Fisher's exact test was used as expected values in some cells were less than five. For all others trials, a Chi-square test was used.

Trial	Discriminator colony	Worker source	Queen source	Queen	Egg type	Start	1 <sup>st</sup> check	2 <sup>nd</sup> check	Q v W at 2 <sup>nd</sup> check
1	Rabbit hutch	Colony 1	Colony 2	Yes	W	26	5	0	P = 0.000
					Q	17	14	14	
2	Rabbit hutch	Orchard	Martin's barn	Yes	W	25	0	0	P = 0.000
					Q	25	12	12	
4	Martin's barn	Oak tree	Rabbit hutch	Yes	W	28	11	1	P = 0.000
					Q	30	26	20	
3	Orchard	Colony 1	Rabbit hutch	No	W	20	12	7	P = 0.004*
					Q	20	2	0	
5	Oak tree	Orchard	Martin's barn	No	W	24	24	23	P = 0.006*
					Q	21	20	13	

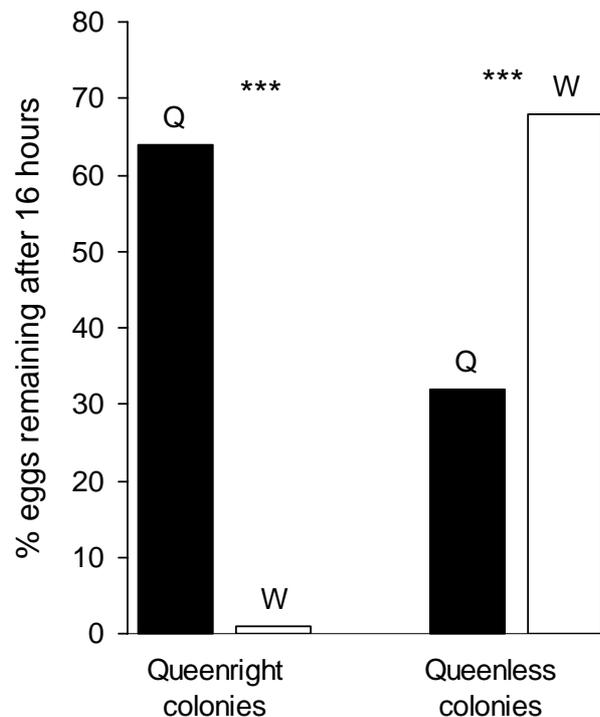


Figure 6.2. Egg removal by workers in queenright and queenless colonies of the European hornet, *Vespa crabro*. The percentages of foreign queen-laid eggs (■) and worker-laid eggs (□) 16 hours after their introduction are shown. Percentages are calculated from the frequency data summed across trials (Table 6.1). Statistics are from Chi-square tests performed on the frequency data summed across trials.

of queen policing in the queenright colonies, we checked the test comb for the queen's presence every 10 minutes prior to the first check.

## 6.4 Results

Queenright colonies removed significantly more worker-laid eggs and queenless colonies removed significantly more queen-laid eggs (Figure 6.2). This was highly significant in every trial (Table 6.1). The dissociation between queenright and queenless colonies is most strongly illustrated by trials 4 and 5 where the discriminator colonies were given eggs from the same two source colonies. Colony 5 was slower to remove eggs than the other colonies. This can be explained by its small size – approximately 20 workers at the time of testing. The other colonies all had in excess of 100 workers during the tests. In the queenright colonies, 10-15 workers were seen on the test comb at each 10-minute check, while the queen was only seen on a test comb once and this was at the time of the first check (trial 4). This strongly suggests that workers were responsible for the egg removal that had occurred at the first check. The queen, however, may have contributed to the removal of eggs between the 45 minute and 16 hour checks. Newly laid eggs could be identified as they were not on the small paper attachments that we glued in. These were found after 16 hours, in trials 3 and 4 and were not included in the data.

## 6.5 Discussion

Worker policing by egg eating occurs in queenright colonies of the European hornet, *Vespa crabro*. This provides a proximate explanation for the absence of attempted worker reproduction in hornet societies (Foster *et al.* 2000, Chapter 5). Effective policing will favour workers investing in the colony rather than in reproduction because attempted reproduction is likely to fail and so provide little benefit to the laying worker. However, with single mating by queens the norm in *V. crabro* (effective paternity 1.11, Foster *et al.* 1999, Chapter 4) worker policing is not predicted on relatedness grounds. Workers are considerably more related to the nephews that they kill ( $r = 0.35$ ) than the brothers that they spare ( $r = 0.25$ ). This suggests that other factors select for worker policing. Worker policing is expected at paternity below two if worker reproduction is costly to colony productivity (Ratnieks 1988). Alternatively, worker policing may be favoured because it reduces the cost of worker sex allocation biasing (Foster and Ratnieks submitted, Chapter 10). In some ants, workers have been shown to remove males at the larval stage to bias sex allocation towards their more related sisters (e.g. Sundström *et al.* 1996). By removing males at the egg stage, worker policing will lower the cost of this conflict (Foster and Ratnieks submitted, Chapter 10). It has yet to be established whether this male killing occurs in the Vespinae.

Worker policing has been discovered in two other genera of vespine wasps (*Vespula* and *Dolichovespula*). Egg introductions in queenright colonies of the common wasp, *Vespula vulgaris*, revealed very similar results, with 0/120 worker-laid eggs but 80/120 queen-laid eggs remaining in queenright colonies after 16 hours. However, at 1.90 effective paternity (queen mating frequency) is considerably higher in *V. vulgaris* so that workers are almost equally related to brothers and nephews. Worker policing, therefore, is not disfavoured but rather neutral with respect to relatedness in *Vespula vulgaris* (Foster and Ratnieks 2001, Chapter 9). The importance of relatedness in

worker policing in vespines has been shown in *Dolichovespula saxonica* (Foster and Ratnieks 2000, Chapter 8). *D. saxonica* workers facultatively police each other, policing more in multiple paternity colonies where worker relatedness is low than in single paternity colonies. In combination, these three studies clearly show that while relatedness is important in the evolution of worker policing, other factors are also involved. The contrast of *V. crabro* with *D. saxonica* where worker policing is absent from single paternity colonies, further shows that the relative importance of relatedness can vary greatly between closely related species. This is additionally supported by data from four other *Dolichovespula* species that have low paternity and, in line with relatedness predictions, significant queenright worker reproduction (Foster *et al.* 2001, Chapter 7).

Hornet workers in queenless nests performed the opposite discrimination to workers in queenright nests, removing significantly more queen-laid eggs than worker-laid eggs. This strengthens the conclusion that worker policing in queenright colonies is a real phenomenon by showing that the policing response is facultative. Reverse discrimination by queenless workers may represent an adaptation to intraspecific parasitism. Genetic and behavioural evidence suggest that usurpation of queenright nests by foreign queens is common in *V. crabro* (see Foster *et al.* 2000, Chapter 5). Queens, therefore, may also usurp already queenless nests. Because workers will be unrelated to such a new queen, they will benefit from removing her eggs to favour their own offspring. This contrasts with perennial societies such as the honeybee *Apis mellifera* in which workers raise a new sister queen when the old queen dies. Here queenless workers must tolerate queen-laid eggs in order to allow the new queen's offspring to be reared.

The discovery of worker policing in *Vespa crabro* that has mean paternity close to one suggests that worker policing can evolve in societies of any kin structure. This contributes to a growing body of evidence suggesting that worker policing is of widespread importance in the eusocial Hymenoptera (Foster and Ratnieks 2001, Chapter 9). Worker policing has been demonstrated in the honeybee, *Apis mellifera* (Ratnieks and Visscher 1989) and two vespine wasp genera in addition to *Vespa* (above). Furthermore, worker policing by mutual aggression occurs in queenless ants (Liebig *et al.* 1999), where it also seems to occur both in line with and against relatedness predictions (Kikuta and Tsuji 1999; Monnin and Ratnieks, submitted). Just how common worker policing is remains to be seen. However, it seems likely to prove a major explanation in the puzzle of why workers do not reproduce.

## 6.6 Acknowledgements

We thank the Forestry Commission for permission to work with hornets in the New Forest and Martin Noble of the Forestry Commission for allowing us to study the hornet colony in his barn. Funding for this study was provided by a BBSRC studentship to KRF.

## Colony kin structure and male production in *Dolichovespula* wasps

K.R. Foster, F.L.W. Ratnieks, N. Gyllenstrand & P.A. Thorén

### 7.1 Abstract

In annual hymenopteran societies headed by a single outbred queen, paternity (determined by queen mating frequency and sperm use) is the sole variable affecting colony kin structure and is therefore a key predictor of colony reproductive characteristics. Here we investigate paternity and male production in five species of *Dolichovespula* wasps. Twenty workers from each of ten colonies of each of five species, 1000 workers in total, were analysed at three DNA microsatellite loci to estimate paternity. To examine the relationship between kin structure and reproductive behaviour, worker ovary activation was assessed by dissection and the maternal origin of adult males was assessed by DNA microsatellites. Effective paternity was low in all species (*D. media* 1.08, *maculata* 1.0, *sylvestris* 1.15, *norwegica* 1.35 and *saxonica* 1.35) leading to the prediction of queen-worker conflict over male production. In support of this, workers with full size eggs in their ovaries (4/5 species) and adult males that were workers' sons (5/5 species) were found in queenright colonies. However, workers were only responsible for a minority of male production (*D. media* 7.4%, *maculata* 20.9%, *sylvestris* 9.8%, *norwegica* 2.6% and *saxonica* 34.6%) suggesting that the queen maintains considerable reproductive power over the workers. Kin structure and reproductive conflict in *Dolichovespula* contrast with their sister group *Vespula*. *Dolichovespula* is characterised by low paternity, worker reproduction, and queen-worker conflict and *Vespula* by high paternity, effective worker policing and absence of worker reproduction. The trend revealed by this comparison is as predicted by kin selection theory suggesting that colony kin structure has been pivotal in the evolution of the yellowjacket wasps.

### 7.2 Introduction

The study of reproductive conflict is central to our understanding of social evolution (Keller 1999). In non-clonal groups, the divergent genetic interests of group members can cause within-group conflicts that disrupt social organisation (Hamilton 1964; Ratnieks and Reeve 1992; Keller 1999). By identifying parties of common and differing interests (Ratnieks and Reeve 1992), kin structure is central to understanding the structure of these conflicts, and their resolution. In single-queen hymenopteran societies (ants, bees and wasps), paternity, which is determined by queen mating frequency and sperm use, is a key determinant of colony relatedness patterns. Therefore, the study of

paternity is central to the study of hymenopteran social evolution (Crozier and Pamilo 1996; Bourke and Franks 1995; Boomsma and Ratnieks 1996).

There is great potential for conflict over male production in hymenopteran societies (Hamilton 1964; Starr 1984; Woyciechowski and Łomnicki 1987; Ratnieks 1988). Although workers are generally unable to mate, because of haplodiploidy their unfertilised eggs become males. With both the queen and the workers individually most related to their own sons, extensive conflict over male production is predicted. Paternity is of importance in understanding whether this conflict will actually be expressed, because multiple mating by queens is predicted to result in the suppression of worker reproduction by worker policing (Starr 1984; Ratnieks 1988). When the workers in a colony are offspring of a single queen but several fathers, a worker is more related to the queen's sons (0.25) than to other workers' sons (<0.25). This shifts the workers' collective interest into line with the queen's interest in that workers are expected to police each other's reproduction resulting in enforced cooperation (Ratnieks 1988).

The wasp subfamily Vespinae (*Vespa*, *Provespa*, *Dolichovespula*, *Vespula*) have proved highly informative in the study of reproductive conflicts (Ratnieks 1988; Foster *et al.* 1999, Chapter 4; Foster *et al.* 2000, Chapter 5). The main reason for this is that the four genera are generally very similar in their basic biology (annual life cycle, single queen, physically distinct queen caste; Ross and Matthews 1991), yet paternity varies allowing contrasting predictions to be made concerning male production and reproductive conflict (Ross 1986; Foster *et al.* 1999, Chapter 4; Foster *et al.* 2000, Chapter 5). This has allowed fruitful comparison between taxa (Foster *et al.* 2000, Chapter 5). Because a well-supported phylogeny of the Vespinae is available (Carpenter 1987), inferences on the evolution of reproductive traits can also be made (Foster *et al.* 1999, Chapter 4).

*Dolichovespula* is the sister group of *Vespula*. Together they form the yellowjackets, the most derived clade of the Vespinae (Carpenter 1987). *Dolichovespula* wasps make characteristic ball-shaped grey paper nests, consisting of a queen and typically about 100 workers (Greene 1991). Despite extensive study of their biology (reviewed by Greene 1991), little is known of paternity or worker reproduction. Using DNA microsatellites, we studied paternity and male production in five *Dolichovespula* species. Paternity was low in all species leading to the prediction of queen-worker conflict over male production. Worker ovary dissection and genetic analysis of males showed that workers do indeed attempt to reproduce, producing a small but significant proportion of the adult males in queenright colonies.

## 7.3 Materials and Methods

### 7.31 Study organisms

*Dolichovespula sylvestris* (13 nests), *norwegica* (18 nests) and *media* (13 nests) were collected from the Sheffield area, UK in 1996-99. *Dolichovespula sylvestris* (9 nests) and *D. media* (22 nests), along with *D. saxonica* (15 nests), were also collected in the New Forest, Hampshire, UK in 1999. *D. maculata* (19 nests) were collected in and around Ithaca, New York in 1991. All nests were collected from pest control calls and would otherwise have been destroyed.

### 7.32 Genetic Methods

Twenty workers and the queen, if collected, from each of ten nests of each species (1000 workers in total) were analysed at three DNA microsatellite loci (Rufa 5, 13, 15 for *D. sylvestris*, *D. norvegica* and *D. maculata*, Rufa 5, 13, 18 for *D. saxonica*, *D. media*, see Appendix) using methods as in Foster *et al.* (1999, Chapter 4). The majority of nests of *D. sylvestris* (8/10) and *D. media* (9/10) analysed were from the New Forest sample. Eighteen to forty males from 7-10 nests of each species (1125 males in total) were analysed at all informative loci (Foster *et al.* 2000, Chapter 5 and below) to determine whether they were the queen's or workers' sons. All males analysed came from nests with female pupae. The presence of female pupae confirms that nests were queenright when the eggs that gave rise to the males were laid. In *D. sylvestris* and *D. saxonica*, male pupae were also available for a few colonies that were queenright on collection. These were analysed instead of adult males because they provide additional information on rearing location, showing in which combs workers were laying. The number of males analysed for each species was designed to give approximately 150 assignable males per species (assignable numbers ranged from 148-153, see 'Male non-detection error' below).

### 7.33 Worker-worker relatedness

The program Relatedness 4.2 (Goodnight and Queller 1994) was used to calculate regression relatedness among workers ( $b$ ), inbreeding ( $F$ ) and allele frequencies. Assuming outbreeding, pedigree worker-worker relatedness ( $r$ ) was estimated by inspection of progeny genotypes. Inspection also allows the number of fathers and their relative paternities to be determined when multiple paternity occurs.

### 7.34 Effective paternity

The population effective mating frequency ( $M_e$ ) was estimated after Starr (1984),

$$M_e = \frac{n}{\sum_j \sum_i p_{ij}^2} \quad (7.1)$$

where  $p_i$  is the proportional contribution of the  $i$ th male in the  $j$ th nest for  $n$  nests.

### 7.35 Male non-detection error

The mean probability of detecting a worker-produced male present in a sample was calculated for each nest from:

$$P = \sum_1^n p_i (1 - 0.5^{l_i}) \quad (7.2)$$

where  $n$  is the number of patriline in the nest,  $p_i$  is the proportional representation of the  $i$ th patriline and  $l_i$  is the number of informative loci analysed at the  $i$ th patriline. An informative locus is one where the queen and her mate have different alleles so that the workers carry an allele which the queen does not (Foster *et al.* 2000, Chapter 5). This equation is more general than that in Foster *et al.* (2000, Chapter 5), which although correct for the analysis performed, is not applicable when more than one locus in a colony has both informative and uninformative patrilines. The number of assignable

Table 7.1. Genetic variation and the probability of detecting a second father for the microsatellite markers studied (non-detection error,  $d_n$ ).

Species	Expected heterozygosity at <i>V. rufa</i> locus				$d_n$
	5	13	15	18	
<i>D. media</i>	0.738	0.812	-	0.733	0.010
<i>D. maculata</i>	0.900	0.865	0.826	-	0.002
<i>D. sylvestris</i>	0.860	0.792	0.841	-	0.005
<i>D. norvegica</i>	0.817	0.804	0.687	-	0.010
<i>D. saxonica</i>	0.874	0.887	-	0.821	0.003

males ( $N_a$ ) for each species is then  $\Sigma(P_j N_j)$  where  $P_j$  is the probability of detecting a worker-produced male and  $N_j$  is the number of males analysed for the  $j$ th nest.

### 7.36 Worker ovary activation

Twenty workers from all genetically analysed queenright nests of each species were dissected and their ovaries inspected under a binocular microscope using a reticule eyepiece. The mean size of five eggs taken from cells was taken for comparison and the largest egg(s) in worker ovaries was classed as < 50% full size (none), 50-90% (half) full size, > 90% full size (full).

## 7.4 Results

### 7.41 Queen loss in reproductive nests

It has been suggested that workers in reproductive nests may kill their queen to allow them to reproduce (Bourke 1994). No queen was found on collection of 5/14 *Dolichovespula sylvestris*, 12/14 *D. norvegica*, 2/10 *D. saxonica*, 6/19 *D. media* and 14/19 *D. maculata* nests at the reproductive stage of their lifecycle. The mother queen was recognised as different from new queens by her heavily worn wings (Edwards 1980).

### 7.42 Allelic diversity

Allelic diversity and heterozygosity were high in all loci studied with 5-15 alleles at each locus and a mean heterozygosity of 0.82 (Table 7.1.) This is particularly interesting in the case of *D. media* and *D. saxonica* as both have recently invaded Britain from central Europe (1980 for *D. media*, 1987 for *D. saxonica*, Else 1994). The high heterozygosity suggests either a rapid recovery of genetic diversity at the loci studied through mutation or an absence of founder effects during this colonisation, probably because Britain was colonised by many queens.

### 7.43 Worker-worker relatedness

Regression and pedigree relatedness were high and effective paternity was low in all five species (Table 7.2). Across species, the paternity contribution of the majority male in double patriline nests (0.73) was significantly greater than that expected from

Table 7.2. Colony kin structure in five species of *Dolichovespula*.  $M_e$  is the effective paternity,  $P_{major}$  is the mean proportional contribution of the majority male in multiple paternity nests.

Species	$M_e$	Paternity				$P_{major}$	# mothers detected	Worker relatedness	
		# fathers detected			Pedigree $r$			Regression $b \pm s.e.$	
		1	2	3					
<i>D. media</i>	<b>1.08</b>	9	2	0	0.73	1-2	0.71	0.72±0.04	
<i>D. maculata</i>	<b>1.00</b>	10	0	0	-	1	0.75	0.74±0.02	
<i>D. sylvestris</i>	<b>1.15</b>	7	4	0	0.68	1-2	0.68	0.62±0.06	
<i>D. norwegica</i>	<b>1.08</b>	8	2	1	0.83	1-2	0.71	0.65±0.06	
<i>D. saxonica</i>	<b>1.35</b>	4	5	1	0.68	1	0.62	0.57±0.04	

sampling twenty workers from a nest with two equal patriline (0.58), calculated using the binomial expansion ( $H_0 = 0.58$ , t-test,  $P < 0.01$ , Foster and Ratnieks 2001, Chapter 9):

$$P_r = \frac{n!}{r!(n-r)!} p^r (1-p)^{(n-r)} \quad (7.3)$$

where  $P_r$  is the probability of sampling  $r$  of patriline A and  $n-r$  of patriline B in a sample of  $n$  workers, and  $p$  is the proportional representation of patriline A in the actual colony. The probability of each combination of paternities (20A, 19A:1B, 18A:2B, etc...) can then be calculated and averaged to generate the expected contribution of the majority male to the sample. The probability that any two randomly chosen males are identical at all loci studied (non-detection error,  $d_n$ ) was calculated from expected heterozygosity. This estimate of  $d_n$  gives an approximate but reliable indication of the degree of error in estimating paternity (Boomsma and Ratnieks 1996; Foster *et al.* 1999, Chapter 4). For all species  $d_n$  is extremely low, so that very few or likely no cases of multiple mating will be missed (Table 7.1). The effects non-sampling error was kept to low levels by the analysis of twenty workers per nest (Boomsma and Ratnieks 1996; Foster *et al.* 1999, Chapter 4). Two matriline were detected in a single nest each for *D. sylvestris*, *D. norwegica* and *D. media*, with the majority matriline representing 0.65, 0.7 and 0.85 of workers sampled for each species respectively.

#### 7.44 Worker ovary activation

Workers with full-size eggs in their ovaries were found in all species except *D. maculata* (Table 7.3). However, only two colonies of this species were analysed as ovary dissections were only performed on queenright colonies. This is because worker ovary activation is likely to be affected by the queen's presence (Edwards 1980; Bourke 1988).

#### 7.45 Male production

Adult or pupal male production by workers was detected in some nests of each species (Table 7.4). Analysis of male pupae in the two *D. sylvestris* colonies studied revealed that all worker-produced males came from only one comb. However, in the single *D. saxonica* colony worker-produced males came from two of three combs (Table 7.5). In the colonies of *D. sylvestris* and *D. norwegica* that had two matrilines, all queen-

Table 7.3. Worker ovary activation and number of adult workers in five species of *Dolichovespula*. The three categories of ovary activation are defined in the text.

Species	# workers analysed	# colonies analysed	Worker ovary activation (% workers)			Mean # workers in each colony	Estimated # reproductive workers per colony
			None	Half	Full		
<i>D. media</i>	140	7	94	1	5	74	4
<i>D. maculata</i>	40	2	100	0	0	181	-
<i>D. sylvestris</i>	140	7	91	2	7	76	5
<i>D. norvegica</i>	20	1	90	0	10	44	4
<i>D. saxonica</i>	160	8	88	1	11	69	8

Table 7.4. Adult male production by workers in five species of *Dolichovespula*. Percentage of total males produced by workers is estimated as  $(\# \text{ detected}/N_a) \times 100$ , see *male non-detection error* in Methods.  $N_a$  is the number of assignable males. ‘% colonies’ is the percentage of colonies in which at least one workers’ son was detected.

	worker male production			
	# colonies analysed	% total males	$N_a$	% colonies
<i>D. media</i>	8	7.4	148.0	12.5
<i>D. maculata</i>	7	20.9	153.3	57.1
<i>D. sylvestris</i>	10	9.8	153.5	40.0
<i>D. norvegica</i>	8	2.6	151.8	12.5
<i>D. saxonica</i>	9	34.6	150.1	77.8

Table 7.5. Male production by workers on different combs in two nests of *D. sylvestris* and one of *D. saxonica*. In both *D. sylvestris* nests the difference in worker male production between the combs where male production was detected and those where it was not is highly significant (Fisher’s exact test,  $P < 0.01$ ). The data shown is the number worker-produced males detected over the total number of males analysed for each comb.

Species	Comb #		
	Top	Middle	Bottom
<i>D. sylvestris</i>	6/10	0/10	-
<i>D. sylvestris</i>	0/7	0/7	5/6
<i>D. saxonica</i>	6/9	2/4	0/1

produced males analysed came from the majority queen. No males were analysed in the two matriline *D. media* colony.

#### 7.46 Effect of colony size and collection date on worker male production

The percentage of males that were workers’ sons did not correlate with date of nest collection or colony size. Spearman’s rank correlation tests were performed for ‘% workers’ sons versus date of collection’ and ‘% workers’ sons versus number of

workers'. These two tests were performed for each species individually and upon the entire data set by combining the within-species ranks ( $N = 42$ ). For all tests  $P > 0.10$ .

## 7.5 Discussion

Our data show that the majority of *Dolichovespula* queens mate just once and that even when they mate multiply, most sperm comes from a single male. Across the four species in which multiple mating was detected paternity was biased with the majority male fathering an average of 73% of female offspring. As a result there was high relatedness among workers in all five species. Paternity is significantly more biased in these *Dolichovespula* than in a comparable data set of the common wasp, *Vespula vulgaris* (majority male contribution in double paternity colonies = 0.64, Foster and Ratnieks 2001, Chapter 9), (t-test comparing the proportional majority male contribution in 11 double paternity *Dolichovespula* colonies with 10 double paternity *V. vulgaris* colonies,  $P = 0.037$ ). This suggests that the high worker relatedness in *Dolichovespula* is due not only to the lower frequency of multiple mating, but also more biased paternity when multiple mating occurs. In *D. maculata* all queens were singly mated resulting in the highest possible worker relatedness, 0.75, without inbreeding. In the other species paternity and worker relatedness varied somewhat between colonies but only in one colony of the fifty studied (*D. saxonica*) did worker relatedness fall below 0.5.

Two matrilineages were found in one colony each of *D. sylvestris*, *D. norwegica* and *D. media*. Because only one adult queen was found in each of these colonies, this suggests a successful nest take-over by a second queen. Although observations of nest usurpation are numerous in *Vespula* (Greene 1991), this is the first evidence suggesting that take over successfully occurs in *Dolichovespula*. Molecular evidence for queen take-overs (2/33 nests) has also been found in the hornet *Vespa crabro* (Foster *et al.* 2000, Chapter 5) and *Vespula vulgaris* (Foster and Ratnieks 2001, Chapter 9). It appears, therefore, to be a widespread strategy of vespine wasp queens.

Low paternity in all five *Dolichovespula* species supports the conclusion made by Foster *et al.* (1999, Chapter 4) that high paternity is derived in the vespine wasps. Foster *et al.* (1999, Chapter 4) also suggested that there were two origins of multiple paternity in the vespine wasps, once at the base of the genus *Vespula* and once in *D. saxonica*. However, the more extensive analysis presented here reveals that although moderate multiple mating does occur in *D. saxonica*, extreme multiple mating (effective paternity  $> 2$ ) is restricted to *Vespula* (Ross 1986). The conclusion that multiple mating is a derived trait in the Vespinae is, therefore, still strongly supported.

With their high relatedness, *Dolichovespula* workers are more related to each other's sons than to the queen's sons (with the exception of one *D. saxonica* colony). Therefore, they are not expected to police each other's male production and conflict with the queen is expected (Starr 1984; Ratnieks 1988). In line with this prediction, reproductive workers were found in queenright colonies in four out of five species. Observation nest studies of *D. media* (23 queen-laid and 12 worker-laid eggs observed across 3 colonies, Foster pers. obs.), *D. saxonica* (101 queen-laid and 164 worker-laid eggs observed across 4 colonies, Foster and Ratnieks 2000, Chapter 8) and *D. maculata* (Balduf 1954; Greene 1979) have also shown that worker laying is common in queenright *Dolichovespula* colonies. Importantly, the genetic data also show that workers in all five

species successfully produce adult males in queenright colonies. Actual queen-worker conflict over male production, therefore, occurs in *Dolichovespula* as predicted by kin structure. Interestingly, in the two *D. sylvestris* colonies where pupae were available workers only produced males on one comb suggesting that the nest was divided into areas of queen and worker control.

In spite of their numerical advantage *Dolichovespula* workers only produce a minority of the males. How can the queen's power over male production be explained? Traditionally queen pheromonal control has been invoked to explain the reproductive primacy of queens in insect societies. This has been criticised because such control is evolutionarily unstable because workers will be selected to ignore the pheromone (Seeley 1985; Keller and Nonacs 1993). Recent work on the hornet *Vespa crabro*, which also has low paternity (1.11), showed that workers do not activate their ovaries, lay eggs or aggress the queen, indicating a possible role for pheromonal control (Foster *et al.* 2000, Chapter 5). However, additional work has shown that workers preferentially remove worker-laid over queen-laid eggs (Foster *et al.* submitted, Chapter 6). This suggests that worker policing and not queen pheromonal control causes worker sterility.

An alternative explanation for the queen's near monopolisation of male production in *Dolichovespula* is that worker reproduction is costly and reduces colony reproduction. This could cause workers to police each other at low paternity (Ratnieks 1988; Franks 1995) as occurs in *Vespa crabro*, although if policing is occurring it is less effective than in *Vespa*. Alternatively, such a cost to worker reproduction may be causing workers to exercise reproductive self-restraint. The cost may arise simply because reproducing workers work less (Ratnieks 1988; Bourke and Franks 1995). Additionally, worker reproduction may interfere with colony female reproduction. If unable to tell the gender of eggs, reproducing workers risk killing their more valuable sisters as well as their brothers (Nonacs and Carlin 1990). This seems likely in vespine wasps where males and females are often reared in the same cells.

The cost of worker reproduction in queenright colonies is likely to be further exacerbated by the occurrence of queenless colonies. In queenless colonies, workers develop their ovaries and produce many males (Bourke 1988). The large proportion of queenless colonies in *Dolichovespula* means that this extra male production is likely to be significant. This will reduce the mating success of males further reducing the benefits of worker reproduction, particularly in queenright colonies if it is detrimental to female production. With so many colonies without a queen, queen killing by workers (matricide) is a possibility in *Dolichovespula* wasps. Matricide is predicted in low-paternity annual societies after sufficient workers have been raised and queen-destined eggs laid, as it allows workers to monopolise male production (Ratnieks 1988; Bourke 1994). Without observation of workers killing the queen, however, other causes of mortality cannot be excluded.

The ultimate cause of the low level of worker reproduction in *Dolichovespula* societies is not yet clear, but what proximate mechanisms are involved? It appears that both self-restraint by workers and egg removal are important. Our ovary dissections show that the majority of workers have inactive ovaries suggesting that only a few are actually reproducing (Table 7.3). This may be established by a dominance hierarchy among the workers (Greene 1991). Removal of workers eggs by queens (queen policing) has been observed in *D. maculata* (Balduf 1954), *D. saxonica* and *D. media* (Foster pers obs)

and, with worker policing found in *D. saxonica* (see below) worker removal of other workers eggs is also potentially important.

*Dolichovespula saxonica* is exceptional and is the subject of a separate paper (Foster and Ratnieks 2000, Chapter 8). It has the greatest range of worker relatedness and significant male production by workers in several colonies. Most importantly worker relatedness is positively correlated with worker male production. In line with theory, this suggests that workers are policing each other's reproduction in the multiple-paternity colonies but not in the single-paternity colonies (Foster and Ratnieks 2000, Chapter 8). In the other species, less worker reproduction occurs despite worker relatedness comparable to the *D. saxonica* colonies with high worker-relatedness where workers dominate male production. This suggests that the conflict over male production has been resolved in a unique way in *D. saxonica*. Whether worker policing also reduces worker reproduction in the other four species is unknown. Testing for the existence of worker policing in other *Dolichovespula* is a key future step in investigating the nature of sociality in the group.

Despite the difference between *D. saxonica* and the other species, as a genus *Dolichovespula* is a sharp contrast with its sister genus *Vespula*. *Dolichovespula* is characterised by low paternity, significant worker reproduction, and queen-worker conflict over male production while *Vespula* displays high paternity, absence of worker reproduction (Ross 1986) and worker policing (Foster and Ratnieks 2001, Chapter 9). This comparison reveals a trend exactly as predicted by kin selection suggesting that kin structure, specifically paternity frequency, has been pivotal in the social evolution of the yellowjacket wasps.

## 7.6 Acknowledgements

Many thanks to John Gulliver of the New Forest Forestry Commission for help finding the wasps and to Perttu Seppä for help and friendship in Uppsala. Funding for this study was provided by a BBSRC studentship to KRF and the research network "Social Evolution" of the Universities of Aarhus, Firenze, Keele, Sheffield, Uppsala, Würzburg and the ETH Zürich, financed by the European Commission via the Training and Mobility of Researchers (TMR) programme.

## 7.7 Appendix – Primer sequences

The primers in this study were designed using *Vespula rufa* as described in Thorén *et al.* 1995. However, the sequences have not yet been published and so are included below. Amplification success in one species from each vespine genus is shown. Ten workers, each from a different nest, were analysed for each species except for *Provespa anomola* for which all ten workers came from a single nest (N = no or non-specific amplification).

Locus	Direction	Sequence 5'-3'	# alleles detected in 10 workers			
			<i>Vespa crabro</i>	<i>P. anomola</i>	<i>D. sylvestris</i>	<i>Vespula vulgaris</i>
1	F	TTCTGAACTGCAATAATCATTTC	N	N	N	N
	R	GTTCTGAATAATCTTTCATA				
2	F	GATCGACTTAAGCAGGAATG	2	N	N	N
	R	TCGTTCGTAATTCGTTGATTC				
3	F	GTCTGCAGATTAGGGAACG	N	N	N	1
	R	CTCCATGACCGAGAATAAAG				
4	F	TTCAATGCTATTACATTATAATATTC	N	N	N	2
	R	GTCCAAGGAAGTTTCGAC				
5	F	GAGGCAAATTTTACGACGTAGG	4	2	6	1
	R	CTGCCAATCGCATGTCG				
6	F	GGACACGTTTACGTAGAAGGATG	3	N	N	N
	R	CGCAGTGACGAGTTTCCAC				
7	F	CGTTGCTCGTGAACTGTTAAC	1	2	1	1
	R	CCATCTTCGTTTCATCTTCGTTTC				
8	F	CCTGTCATCGTGTCCACG	N	N	N	N
	R	TCCGATCGTTTGAATCCTG				
9	F	GACGAGATGTATGTAGTAGTGACG	N	N	N	N
	R	GTGATGCTATGCATTCGG				
10	F	GATCGAACCTTAATTACACGATTC	N	N	N	N
	R	CAGGAAACAGCTATGACCATG				
11	F	GCATCGACGGATGAATTG	N	N	N	2
	R	GATGGAAACAAGGAAGCATG				
12	F	CTTTCTAGGAGATACTTCGTATAAAG	N	N	N	1
	R	AAGATGGAAACAAGGAAGC				
13	F	GATCTGTGTACGTAATTCTCTCC	3	2	2	1
	R	GAAAATCGGGAACGATG				
14	F	TGATGCTCGTTAGTTGCAC	N	N	N	N
	R	GATCAGATAATCAAAGAGACAGAC				
15	F	GATCAGAAATCTGATTAAGTCGAG	5	N	5	4
	R	GGTCGATTCGTTTGAAAATAG				
16	F	GTACAGTATACGTAAAACGCATAAGG	N	N	N	1
	R	GAGTGTCGGTTCGCTCTTATG				
17	F	GAGCAGGAGGGATGTGAG	N	N	1	1
	R	GAGATATCGATAAATGTGATTCG				
18	F	CGAATTTTCAACAGCAATATTG	4	2	N	6
	R	GATCGAAGTGGCTGATACTTTG				
19	F	CTCCATTTCGGAACCTCTCG	N	N	N	3
	R	CTGAGGGATTTTATGGTGG				

## Facultative worker policing in a wasp

K.R. Foster & F.L.W. Ratnieks

Kin-selection theory predicts that in social-insect colonies where the queen has mated multiple times, the workers will enforce cooperation by policing each other's reproduction (Starr 1984; Ratnieks 1988; Frank 1995; Keller 1999). We have discovered a species, the wasp *Dolichovespula saxonica*, in which some queens mate once and others mate many times, and in which workers frequently attempt reproduction, allowing this prediction to be tested directly. We find that multiple mating by the queen leads to mutual policing by workers, whereas single mating does not.

Workers in most species of social Hymenoptera (bees, ants and wasps) cannot mate but can produce unfertilised, male eggs. Workers and the queen therefore compete over male production. If the queen mates only once, workers are more closely related to the sons of other workers ( $r = 0.375$ ) than to those of their mother queen ( $r = 0.25$ ) and, in conflict with the queen, should prefer to rear other workers' sons. But if the queen mates multiple times, workers are more related to the queen's sons than to other workers' sons. This is expected to lead to worker policing, where workers attempt to stop each other from reproducing (Starr 1984; Ratnieks 1988; Frank 1995).

Mutual policing by egg eating occurs in the honeybee *Apis mellifera* (Ratnieks and Visscher 1989) and in the common wasp *Vespula vulgaris* (Foster and Ratnieks 2001,



Figure 8.1 Worker egg-laying (left) and policing (right) in *Dolichovespula saxonica*. Workers police other workers' reproduction more in colonies where the queen has mated multiple times than in those where she has mated only once.

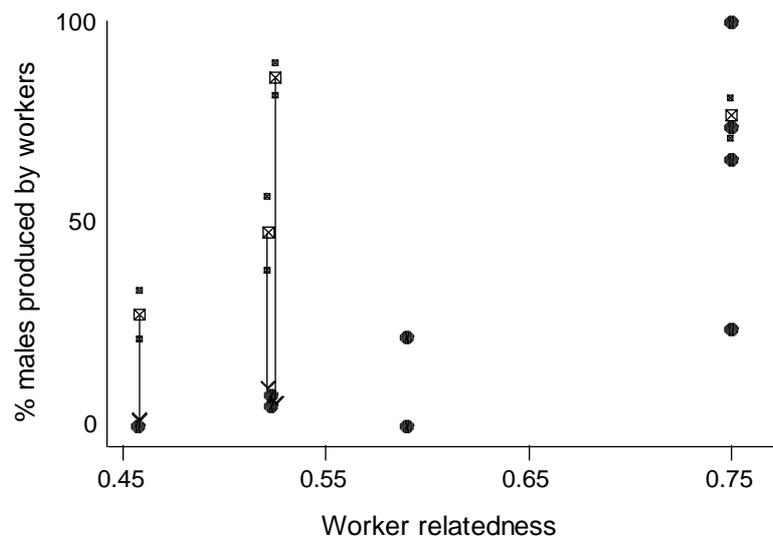


Figure 8.2 Pedigree relatedness among workers versus adult male production by workers in nine colonies of the wasp *Dolichovespula saxonica* (circles). Estimates of the percentage of the male eggs that are laid by workers in four of the colonies are also shown (squares). Nests were collected from the New Forest, UK, in 1999. Pedigree relatedness was estimated by inspection of 20 worker genotypes from each nest at three DNA microsatellite loci (Rufa 5, 13, 18, Thorén *et al.* 1995) to determine the number of fathers and their relative paternity. Male production by workers was estimated by genotyping 17–30 males from each nest at all informative loci. The percentage of worker-produced adult males detected for each nest was adjusted according to the probability of detecting workers' sons  $\sum^n p_i (1-0.5^{li})$ , where  $n$  is the number of patriline in the nest,  $p_i$  is the proportional representation of the  $i$ th patriline and  $li$  is the number of informative loci (Foster *et al.* 2000, Chapter 5) analysed at the  $i$ th patriline. This calculates the probability that a worker-produced male will inherit an allele from the queen's mate, thereby making him distinguishable from the queen's sons. The percentage of male eggs laid by workers is based on 80 h observation of four free-flying nests housed in glass-sided boxes at the Institute of Terrestrial Ecology, Furzebrook, Dorset, UK (101 queen-laid and 164 worker-laid eggs in total). Because the precise sex ratio of queen-laid eggs is unknown, three points (squares) corresponding to a range of estimates, 2M:1F (bottom), 1M:1F (middle) and 1M:2F (top), are shown. The first produces the lowest estimate of worker reproduction and is highly conservative as *Dolichovespula* rear about twice as many females as males so that the queen probably lays more female eggs than male. The estimates of the percentage of male eggs laid by workers for the singly mated colony ( $r = 0.75$ ) lie on top of the corresponding adult male production data.

Chapter 9), whose queens are multiply mated, but not in the stingless bees (Peters *et al.* 1999) and bumblebees (Estoup *et al.* 1995) whose queens typically mate only once. A direct within-species test is critical, however, because these taxa differ in many ways apart from kinship and, with only a few independent data points, the trend is not statistically significant.

In the vespine wasp *Dolichovespula saxonica* (Figure 8.1), the single queen may be either singly or multiply mated, leading to societies that differ only in kinship. We used DNA microsatellites (Thorén 1995) to analyse worker relatedness and male production in nine colonies. There was a strong positive correlation between worker relatedness and male production by workers (Figure 8.2; Spearman's rank correlation,  $P < 0.004$ ). Observation revealed that this was caused not by differences in worker laying - workers in colonies with singly and multiply mated queens laid nearly identical proportions of male eggs ( $\chi^2$ ,  $P > 0.86$ , for all three estimates of queen-laid sex ratio) - but by removal of worker-laid eggs in the nests with multiply mated queens. In the observation

colonies, worker egg production was significantly different from adult male production in the colonies of multiply mated queens ( $\chi^2$ ,  $P < 0.001$ ), but not in those of singly mated queens ( $\chi^2$ ,  $P > 0.39$ , for all three estimates of queen-laid sex ratio) (Figure 8.2). All eight nests sampled contained reproductive workers, with 1–4 of the 20 workers examined in each nest possessing full-size eggs, also indicating that the amount of worker laying is comparable in all colonies. Interestingly, the relatedness at which policing occurs is slightly higher than that predicted from relatedness alone, suggesting that costs associated with worker male production might also favour worker policing (Ratnieks 1988).

To our knowledge, our results are the first direct evidence that multiple mating of queens causes mutual policing by workers. Policing favours workers' cooperation by preventing their reproduction (Ratnieks 1988; Frank 1995; Keller 1999), but as not all *D. saxonica* colonies have policing, here it is only partially effective in countering worker reproduction. Worker policing and cooperation have progressed further in the honeybee *Apis mellifera*, which has policing in all colonies, negligible worker reproduction and large colonies (Ratnieks and Visscher 1989). *D. saxonica* may represent an intermediate stage in the evolution of enforced cooperation. By discouraging policing in some colonies, the close kinship caused by single mating of the queen may paradoxically retard social evolution in this species and others like it.

## Convergent evolution of worker policing by egg eating in the honeybee and common wasp

K.R. Foster & F.L.W. Ratnieks

### 9.1 Abstract

Mutual policing, where group members suppress each others' reproduction, is hypothesised to be important in the origin and stabilisation of biological complexity. In social insects, mutual policing among workers can reduce within colony conflict. However, there are few examples. We tested for worker policing in the common wasp, *Vespula vulgaris*. Workers rapidly removed worker-laid eggs but left most queen-laid eggs (4/120 worker versus 106/120 queen eggs remained after 1 hour). Ovary dissection (1150 workers from 6 colonies) revealed that a small but significant number of workers have active ovaries (4%) equivalent to about 5-25 workers per colony. Consistent with effective policing of worker reproduction, microsatellite analysis of males (270 individuals from 9 colonies) detected no workers' sons. Worker policing by egg eating has convergently evolved in the common wasp and the honeybee suggesting that worker policing may have broad significance in social evolution. Unlike the honeybee, relatedness patterns in *V. vulgaris* do not explain selection for policing. Genetic analysis (340 workers, 17 nests) revealed that workers are equally related to the queen's and other worker son's (worker-worker relatedness was  $0.51 \pm 0.04$  (95% c.i.)). Worker policing in *V. vulgaris* may be selected due to the colony-level benefit of conflict suppression.

### 9.2 Introduction

Explaining the regulation of individual replication and reproduction is key to understanding the major transitions in evolution, including the origin and maintenance of sociality (Maynard Smith and Szathmary 1995; Keller 1999; Michod 1999). The potential for individual selfishness exists in all non-clonal groups, which can lead to reproductive conflicts that are detrimental to group-level function. Mechanisms of conflict suppression are, therefore, central in explaining the rise and stabilisation of biological complexity (Keller 1999). Theory suggests that mutual policing, where group members invest in the suppression of each other reproduction, is an important mechanism of conflict suppression (Frank 1995; Keller 1999). Mutual policing may be particularly important in insect societies, because their unique kin structure can favour the evolution of policing among workers to suppress worker male production (Hamilton 1964; Starr 1984; Ratnieks 1988; Ratnieks and Reeve 1992; Bourke and Franks 1995; Crozier and Pamilo 1996). In addition, the mobility of individuals in animal societies may facilitate the many-against-one interactions fundamental to policing more than at other levels of organisation such as the genome.

Workers in most eusocial Hymenoptera (bees, ants, wasps) cannot mate yet can lay unfertilised, male, eggs. This leads to potential reproductive conflict among the workers, and between the workers and the queen, over male production (Hamilton 1964; Woyciechowki and Łomnicki 1987; Ratnieks 1988; Ratnieks and Reeve 1992). However, in single-queen colonies, if the queen mates multiply and the workers are the daughters of more than two males, workers are more related to the sons of their mother queen (0.25, brothers) than to their sister workers' sons (<0.25, nephews). In this situation, workers benefit from policing one another (worker policing), thereby reducing male production by workers (Starr 1984; Ratnieks 1988).

There are few data on worker policing. Worker policing by egg eating occurs in the honeybee, *Apis mellifera* (Ratnieks and Visscher 1989), a derived eusocial species with multiply-mated queens and low worker relatedness (Estoup *et al.* 1994). Workers rapidly kill worker-laid eggs but leave queen-laid eggs (Ratnieks and Visscher 1989) and despite a significant amount of worker laying very few workers' sons survive the egg stage (Ratnieks 1993, Visscher 1989; Visscher 1996). In queenless ants, experimentally introduced ovary-activated workers are selectively aggressed by non-reproductive workers suggesting that worker policing regulates the number of reproductives (Gobin *et al.* 1999; Liebig *et al.* 1999; Kikuta and Tsuji 1999). Although policing occurs in both the honeybee and queenless ants, the mechanism is different. Queenless ant workers prevent worker laying by direct aggression that causes ovary regression while honeybee workers remove the product of worker reproduction. It is possible, however, that both mechanisms are important in the honeybee. Sakagami (1954) and Visscher and Dukas (1995) found that ovary-activated worker honeybees were aggressed by other workers, although to what extent this reduces their reproduction is unclear.

We investigated the possibility that worker policing by selective egg eating occurs in the common wasp, *V. vulgaris*. We chose *Vespula* because it is a derived genus (Carpenter 1987; Foster *et al.* 1999, Chapter 4) comparable to *Apis*, where multiple mating by queens and queen-only male production have previously been shown (*V. maculifrons*, *V. squamosa*, Ross 1986). Using a combination of genetic analysis of kinship, dissection of workers' ovaries, and egg-eating bioassays, here we show that worker policing by egg eating occurs in *V. vulgaris* and that despite some worker ovary activation the queen is responsible for all or the vast majority of male production. From a non-social common ancestor, *A. mellifera* and *V. vulgaris* have convergently evolved eusociality and remarkably similar worker policing systems.

## 9.3 Methods

### 9.31 Study organism

Nests of *V. vulgaris* were collected from pest control calls in the Sheffield area, UK during late summer in 1996-99. Policing assays were performed on free-flying colonies relocated to polystyrene nest boxes at the Laboratory of Apiculture and Social Insects, Sheffield in September 1999.

### 9.32 Worker relatedness

Twenty workers from each of seventeen *V. vulgaris* nests were analysed at two microsatellite loci, Rufa 18 and 19 (Thorén 1998) using the protocol of Foster *et al.* (1999, Chapter 4). Inspection of worker genotypes for each nest was used to estimate pedigree relatedness ( $r$ ) and to estimate sperm bias when multiple paternity occurred. The expected heterozygosities at the two loci were estimated from allele frequency estimates using the program Relatedness 4.2 (Goodnight and Queller 1994).

### 9.33 Effective paternity

The population effective mating frequency ( $Me$ ) was estimated after Starr (1984),

$$Me = \frac{n}{\sum_j \sum_i p_{ij}^2} \quad (9.1)$$

where  $p_i$  is the proportional contribution of the  $i$ th male in the  $j$ th nest for  $n$  nests.

### 9.34 Does the queen or the workers produce the colony's males?

To determine whether males were the queen's sons or workers' sons, 30 adult males from each of nine queenright colonies were analysed at one or both of the loci depending on whether both were informative. Loci are informative if the worker's paternal and maternal alleles differ allowing a worker's son to be distinguished from the queen's sons when he receives the distinct paternal allele from his worker mother (Foster *et al.* 2000, Chapter 5). The probability of detecting a worker's son, that is the probability that a worker's son possesses at least one distinct paternal allele ( $P_j$ ), was then calculated for each nest from:

$$\sum_1^n p_i (1 - 0.5^{l_i}) \quad (9.2)$$

where  $n$  is the number of patriline in the nest,  $p_i$  is the proportional representation of the  $i$ th patriline and  $l_i$  is the number of informative loci analysed at the  $i$ th patriline. This is a more general equation than the equation presented in Foster *et al.* (2000, Chapter 5) since it can include more than one locus in which not all worker patriline have informative genotypes. The number of assignable males ( $Na$ ) for each species sample is then  $\sum (P_j \cdot N_j)$  where  $P_j$  is the probability of detecting a worker produced male and  $N_j$  is the number of males, analysed for the  $j$ th nest. If workers produce a proportion  $x$  of the males, the probability of not sampling any worker-produced males is  $(1 - x)^{Na}$ .

### 9.35 Worker ovary activation

1150 randomly selected workers from six mature queenright nests (150-200 per nest) were examined by dissection under a binocular microscope with a graticule eyepiece. The size of the largest egg was compared to the mean size of five eggs taken from cells and placed into one of three categories: <50%, 50-90%, >90% full-size.

### 9.36 Policing assay

Policing of worker-laid eggs was investigated by transferring worker-laid and queen-laid eggs into a test comb. This comb was then placed into a queenright test colony in the reproductive phase of the annual lifecycle. A total of 120 queen-laid and 120 worker-laid eggs were introduced into two test colonies housed in polystyrene boxes in three trials of 80 eggs each. The number of eggs remaining was checked after 1 and 16 hours.

The worker-laid eggs were obtained from four groups of around 30 queenless workers, taken from the two test colonies. Each queenless group was isolated in a 15x15x40cm wire mesh cage with a piece of comb and food (honey and honeybee pupae) *ad libitum*. The queenless workers activated their ovaries and began to lay unfertilised eggs after approximately 10 days. The existence of queenless male producing colonies in *V. vulgaris* shows that worker-laid eggs are viable (Edwards 1980). The queen-laid eggs came from the test colonies themselves. Because wasp eggs are glued to the paper comb, we cut out small pieces of comb, *c.* 3x3mm, each with an adhering egg and glued them individually into cells in the test comb with water-based polyvinyl acetate glue. The test combs were the lowest combs from each test colony and had large cells which are used to rear both males and queens.

### 9.37 Queen or worker policing?

To exclude the possibility that the eggs were removed by the queen, the first hour of the last two trials was recorded through the clear plastic base of the nest box using an infrared video-camera.

## 9.4 Results

### 9.41 Worker relatedness

Mean effective paternity ( $Me$ ) in the 17 colonies was 1.90 giving a worker-worker relatedness ( $r$ ) of  $0.51 \pm 0.04$  (95% c.i.). One nest was found to contain two matriline, which were treated separately for all paternity analyses. One queen was single-mated, eleven double-mated, four triple-mated and one quadruple-mated. In multiple paternity nests, one male contributed to on average 59% of the workers. The effects of non-detection and non-sampling error, which can cause relatedness to be overestimated (Boomsma and Ratnieks 1996; Foster *et al.* 1999, Chapter 4), were low due to the high heterozygosity of the two loci, 0.9 and 0.74, which gave a non-detection error of 0.03, and the sampling of twenty workers from each nest (Pedersen and Boomsma 1999; Foster *et al.* 1999, Chapter 4). Non-detection error is the probability that two males share the same genotype by chance at the all loci studied. Because hymenopteran males are haploid, this is equal to the probability that a diploid individual is homozygous at all loci:  $\prod(1-H_i)$ , where  $H_i$  is the expected heterozygosity at the *ith* loci of *n*.

### 9.42 Binomial sampling error

In species where two patriline are common, using a sample of twenty workers to estimate paternity can lead to error from binomial sampling effects. The binomial expansion allows this error to be estimated:

$$P_r = \frac{n!}{r!(n-r)!} p^r (1-p)^{(n-r)} \quad (9.3)$$

where  $P_r$  is the probability of sampling  $r$  of patriline A and  $n-r$  of patriline B in a sample of  $n$  workers, and  $p$  is the proportional representation of patriline A in the actual colony. The probability of each combination of paternities (20A, 19A:1B, 18A:2B, etc...) can then be calculated and averaged to generate the expected contribution of the majority male to the sample. For a sample of twenty workers from a colony with two equal patrilines, this predicts that the majority male will contribute to 0.58 of the workers. In our data, the paternity contribution of the majority male in the doubly mated nests (mean = 0.64) is not significantly different from this binomial estimate (1 tailed t-test,  $P = 0.08$ ). Therefore, the paternity bias found could be an artifact of sampling. *Effective* paternity estimates can also, therefore, be affected. However, this effect is minor, particularly if actual paternity is skewed (Figure 9.1). But even in the worst-case scenario of sampling from two equal patrilines, which estimates paternal contributions as 0.58/0.42, effective paternity is estimated as 1.95 (Equation 9.1), very close to the actual value of 2. Therefore, the key conclusion that effective paternity in *V. vulgaris* is close to two is unaffected.

#### 9.43 Does the queen or worker produce the colony's males?

No workers' sons were detected. The number of assignable males ( $Na$ ) was estimated at 171. This means that there is a probability of less than 5% of missing a worker contribution to male production greater than 2% (Foster *et al.* 2000, Chapter 5).

#### 9.44 Worker ovary activation

Five out of 1150 workers, from 3 out of the 6 nests examined, had fully-activated ovaries. A further 7 workers from 4 of the nests had eggs greater than half size in their ovaries. This is a low but biologically significant proportion of ovary activation given that mature *V. vulgaris* nests contain 1000-5000 workers (Wilson 1971; Edwards 1980).

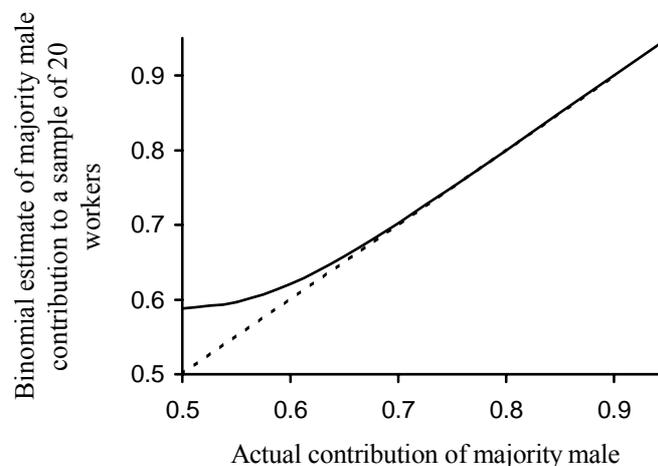


Figure 9.1. The effect of binomial sampling on the paternal contribution of the majority male to a sample of twenty workers, as a function his actual paternal contribution in the colony. As paternity bias increases, the binomial sampling effect rapidly becomes negligible.

Table 9.1. Worker policing in *Vespula vulgaris*. Workers removed all worker-laid eggs and left the majority of queen-laid eggs in each cell. For each trial, the number of queen-laid versus worker-laid eggs remaining after 16 hours is significantly different, Chi-squared,  $P < 0.001$ .

Trial	Egg Source	Test Colony	Egg Source Colony	# of eggs in cells		
				Start	1 hour	16 hours
1	Queen	1	1	40	30	24
	Workers	1	1	40	1	0
2	Queen	2	2	40	39	34
	Workers	VESPOIDEA	1	40	2	0
3	Queen	2	1	40	37	22
	Workers	CHRYSIDOIDEA	1	40	1	0
Combined	Queen	1,2	1,2	120	106	80
	Workers	1,2	1,2	120	4	0

### 9.45 Policing assay

Worker-laid eggs were rapidly removed but the majority of queen-laid eggs were spared (Table 9.1). Summing all three trials, after one hour only 4/120 worker-laid eggs but 106/120 queen laid eggs remained (Chi-squared test,  $P < 0.001$ ). Following the rapid egg removal in Trials 1 and 2, Trial 3 was checked after 15 minutes by which time 75% of the worker-laid eggs had been removed. Trial 3 also showed that egg environment was not the basis for discrimination since all eggs came from a different colony to the test colony.

### 9.46 Queen or worker policing?

The queen was never seen on the test comb during the first hour of trials 2 and 3 showing that workers were responsible for egg removal. Up to five workers at a time visited the 40 test cells. Workers made more and longer visits to cells containing worker-laid eggs (265 visits versus 172 visits,  $P < 0.001$ ,  $\chi^2$  test, median visit duration 4.1s versus 1.0s,  $P < 0.001$ , Mann-Whitney U test, analysis based on the first 15 minutes of the two videos).

### 9.47 Is sex allocation biasing an alternative explanation for egg removal?

In some social insect species, workers have been shown to selectively kill males to favour their more related sisters (Sundström *et al.* 1996). Because all worker-laid eggs are male, it is possible, therefore, that such sex allocation biasing could produce results similar to worker policing. In order to test this possibility, 200 queen-laid eggs from the two colonies were sexed. The sex ratio was approximately even with 85 males and 83 females (the polymerase chain reaction failed for 32 eggs). The removal of all worker-laid eggs but only 33% of queen-laid eggs (Table 9.1) cannot, therefore, be explained by workers selectively removing male eggs. Furthermore, genetic analysis of 10 queen-laid eggs that remained after the trials showed that both male and female eggs had been left by workers, 6 were male. Finally, the test combs had large cells which are used to rear both males and females, and the test colonies were rearing both sexes at the time of study, giving no *a priori* reason why workers would discriminate eggs on the basis of sex.

## 9.5 Discussion

Our data shows that worker policing by egg eating occurs in the common wasp, *Vespula vulgaris*. Workers rapidly remove worker-laid eggs but leave the majority of queen-laid eggs. Ovary dissections suggest that such egg removal occurs naturally because a low but significant (c.a. 5-25) number of reproductive workers are present in reproductive-phase colonies (0.4% of 1000-5000 workers). The genetic analysis of adult males further suggests that policing is effective because we detected no worker's sons. Worker policing may be a general characteristic of *Vespula*. Like *V. vulgaris*, *V. squamosa* and *maculifrons* have multiple-mated queens and queen-only male production (Ross 1986) and low levels of reproductive workers occur in *V. maculifrons*, *flavopilosa*, *germanica* and *vidua* (0.6-2.6% of workers, Ross 1985).<sup>(bees)</sup> In addition, workers in a colony of *V. atropilosa* killed an introduced ovary-activated worker but did not kill non-reproductive workers (Landolt *et al.* 1977) raising the possibility that worker policing by aggression also occurs in *Vespula*. We suggest, therefore, that the following reproductive characteristics are found throughout *Vespula*: multiple mating, a small number of

Figure 9.2. Convergent evolution of worker policing in the common wasp and honeybee illustrated by the phylogeny of the aculeate Hymenoptera (Brothers and Carpenter 1993). Formicidae = ants, Vespidae includes vespine wasps, Apidae includes honeybees. Families containing eusocial species are shown by circles. Large circles denote families containing 'highly' eusocial species (with large colonies and significant queen-worker dimorphism). Family branches within the Vespoidea and Apoidea crown groups are shown.

reproductive workers, males primarily the offspring of the queen, and, we hypothesise, worker policing. Worker policing appears to be widespread in vespines in general. Since this study, worker policing has also been found in *Vespa crabro* (Foster *et al.* submitted, Chapter 6) and *Dolichovespula saxonica* where policing is facultative in response to queen mating frequency (Foster and Ratnieks 2000, Chapter 8).

Worker policing by mutual egg eating in *V. vulgaris* is strikingly similar to that found in the honeybee, *Apis mellifera* (Ratnieks and Visscher 1989). This is not due to common ancestry since the vespine wasps and honeybees belong to lineages that have independently evolved eusociality (Brothers and Carpenter 1993) (Figure 9.2). From solitary origins, both lineages have evolved large complex societies, strong queen-worker dimorphism (Wilson 1971) and a worker policing system that appears to be highly efficient at reducing worker reproduction. The two species further share a low level of worker ovary activation (1 in 10000 worker honeybees have full size eggs in their ovaries, Ratnieks 1993) suggesting that the majority of workers in both species exercise reproductive self-restraint. Self-restraint is probably due to effective policing. When policing is effective, workers receive little benefit from egg laying, and may benefit more by working to increase total colony reproduction (Ratnieks 1988; Ratnieks and Reeve 1992).

Honeybee queens are highly polyandrous, causing very low worker-worker relatedness. This results in workers being more related to their mother queen's sons (0.25) than other workers' sons (c.0.15, Estoup *et al.* 1994), and so selects for worker policing (Ratnieks 1988). Worker-worker relatedness is also low in *V. vulgaris* when compared to most other single-queen hymenopteran societies (Boomsma and Ratnieks 1996). However, the observed relatedness between workers of approximately 0.5 means that workers are

equally related to the queen's sons and other workers' sons. Relatedness, therefore, cannot explain selection for worker policing in *V. vulgaris*. This makes the *V. vulgaris* system particularly interesting because it suggests that worker policing is selected for due to other factors, such as the colony-level benefits of reducing reproductive conflict (Ratnieks 1988; Frank 1995; Keller 1999). Such apparently costly conflict over male production occurs in *Dolichovespula*, the sister group of *Vespula*, where male production by workers and physical queen-worker conflict have been recorded (Ross and Matthews 1991; Foster *et al.* 2001, Chapter 7).

Mutual policing is thought to be important in the evolution of more complex biological organisation because it suppresses conflict among lower-level units (Frank 1995; Maynard Smith and Szathmáry 1995; Keller 1999). The convergent evolution of worker policing in *Apis* and *Vespula*, and the suggestion that in *V. vulgaris* worker policing is selected for because it enhances colony performance, support this hypothesis. The honeybee and the common wasp are both well studied, common, and geographically widespread social insects. But in neither was worker policing observed until suitable experiments were carried out. We suggest that worker policing by egg eating should be deliberately looked for in other eusocial Hymenoptera, particularly in the ants where large complex societies have evolved independently (Figure 9.2). Studies of queenless ants suggest that worker policing by aggression regulates the number of reproductives (Gobin *et al.* 1999; Liebig *et al.* 1999; Kikuta and Tsuji 1999), but data from the largest ant societies, such as the leafcutter, wood, and weaver ants, are lacking. Mutual policing may also be important at other levels of organisation. A recent model of the transition from single to multi-celled organisms predicts that mutual policing among cells should evolve once organisms reach a critical cell number (Michod 1997). At the intragenomic level, recombination (Haig and Grafen 1991) and meiosis (Hurst and Pomiankowski 1991) have been suggested to reflect the policing of selfish genetic elements within a 'parliament of genes' (Leigh 1977; 1991). Although these mechanisms are unlikely to prove formally identical to worker policing (Hurst *et al.* 1996), they reflect a similar many-against-one suppression of reproductive conflict. Mutual policing may prove to be a widespread and important answer to what Leigh (1999) has referred to as the "fundamental problem in ethology" - how conflict is prevented in cooperative groups.

## 9.6 Acknowledgements

We thank Perttu Seppä and Niclas Gyllenstrand for help with the genetics, Nicki Badcock and Stephen Downs for help with the wasps and Tom Wenseleers, Koos Boomsma and Lotta Sundström for helpful comments. Funding for this study was obtained through the research network "Social Evolution" of the University of Aarhus, Firenze, Keele, Sheffield, Uppsala, Würzburg and the ETH Zürich, financed by the European Commission via the Training and Mobility of Researchers (TMR) programme and a Ph.D. studentship to KRF from BBSRC.

## The effect of sex allocation biasing on the evolution of worker policing in hymenopteran societies

K.R. Foster and F.L.W. Ratnieks

### 10.1 Abstract

Mutual policing is thought to be important in conflict suppression at all levels of biological organisation. In hymenopteran societies (bees, ants and wasps), multiple mating by queens favours mutual policing of male production among workers (worker policing). However, worker policing of male production is proving to be more widespread than predicted by relatedness patterns. It occurs in societies headed by single-mated queens in which, paradoxically, workers are more related to the workers' sons that they kill than the queen's sons that they spare. Here we develop an inclusive fitness model to show that a second reproductive conflict, that over sex allocation, can explain the evolution of worker policing contrary to relatedness predictions. In ants, and probably other social Hymenoptera, workers kill males to favour their more related sisters. Importantly, males are killed at the larval stage, presumably because workers cannot determine the gender of queen-laid eggs. Sex-allocation biasing favours worker policing because policing removes some males (the workers' sons) at low cost at the egg stage rather than at higher cost at the larval stage. Our model reveals an important interaction between two reproductive conflicts, in which the presence of one conflict (sex allocation) favours the suppression of the other (male production by workers).

### 10.2 Introduction

Mutual policing, where group members suppress each others' reproduction, is thought to have played an important role in the rise of biological complexity (Frank 1995, Maynard Smith and Szathmary 1995; Keller 1999). Multiple mating by queens in the eusocial Hymenoptera (bees, ants and wasps) is predicted to result in mutual policing of reproduction among workers (worker policing: Starr 1984; Ratnieks 1988; Crozier and Pamilo 1996). Although typically unable to mate, workers in most species can compete with the queen over male production by laying unfertilised eggs, which are male. However, in a colony with more than two worker patriline (effective paternity  $> 2$ ) workers are more related to the queen's sons (brothers,  $r = 0.25$ ) than to other workers' sons (nephews,  $r < 0.25$ ) and so worker policing is selectively favoured. Consistent with this theoretical prediction, worker policing by egg eating has been found in the multiple-mated honeybees *Apis mellifera*, *cerana* and *florea* (Ratnieks and Visscher 1989; Barron *et al.* submitted), while in the typically single-mated stingless bees and bumble bees, worker policing appears absent because many workers' sons are reared (Peters *et al.* 1999, Van Honk *et al.* 1981, Estoup *et al.* 1995). In addition, evidence for worker policing has been found in multiple paternity but not single paternity colonies of the wasp *Dolichovespula saxonica* (Foster and Ratnieks 2000, Chapter 8). However,

non-equal paternity in the offspring of some multiple-mated *D. saxonica* queens makes effective paternity less than two so that workers are in fact slightly more related to nephews than brothers. Worker policing at paternity below two has also been found in the common wasp *Vespula vulgaris* (effective paternity = 1.90, Foster and Ratnieks 2001, Chapter 9) and the European hornet *Vespa crabro* (effective paternity = 1.11, Foster *et al.* 1999, Chapter 4; Foster *et al.* submitted, Chapter 6). In many colonies, workers are paradoxically causing the replacement of their more related nephews ( $r > 0.25$ ) by less related brothers.

In addition to conflict over male production, queen-worker conflict over sex allocation also occurs in hymenopteran societies (Hamilton 1964, Trivers and Hare 1976). Workers typically favour a more female-biased sex ratio than the queen. This is because workers are more related to the queen's daughters (sisters) than the queen's sons (brothers) while the queen is equally related to both sexes ( $r = 0.5$ ) (Hamilton 1964; Trivers and Hare 1976). Consistent with worker-control of sex-allocation biasing, sex allocation in most ant species is female-biased (Trivers and Hare 1976; Boomsma 1989; Pamilo 1990; Bourke and Franks 1995). In the few species where the mechanism of sex-allocation biasing by workers has been investigated, biasing occurs by the killing of males. Male larvae killing has been demonstrated in the wood ant *Formica exsecta* (Sundström *et al.* 1996; Chapuisat *et al.* 1997) and the Argentine ant, *Linepithema humile* (Passera and Aron 1996). In addition, evidence for the removal of males between the egg and pupae stages has been found in *Formica truncorum* (Sundström pers. comm.), the fire ant, *Solenopsis invicta* (Aron *et al.* 1995) and the ant *Myrmica tahoensis* (Evans 1995). It has been suggested that workers remove male larvae rather than eggs because they are unable to identify the sex of eggs (Nonacs 1993). Evidence that workers have difficulty identifying the sex of young brood was provided by Nonacs and Carlin (1990) who showed that workers of the Florida carpenter ant, *Camponotus floridanus* failed to discriminate the sex of brood until the pupal stage.

Here we consider the effect of the queen-worker conflict over sex allocation on the evolution of mutual policing among workers. We model a situation in which workers kill male larvae to bias sex allocation and examine the conditions under which worker policing, that is the killing of worker-laid male eggs, is selected. We show that sex allocation manipulation can select for worker policing when the queen is singly mated because policing reduces the cost of male killing by removing some of the males at very low cost as eggs.

## 10.3 The Model

### 10.31 Overview

We consider a situation in which workers remove excess males to cause a female-biased sex-allocation ratio (Figure 10.1). They can either start killing males at the egg stage or in the larval stage. Consistent with the empirical data (above), we assume that in the egg stage the only eggs which they know to be male are those laid by workers. That is, workers cannot recognise the gender of queen-laid eggs but they can recognise whether an egg is queen-laid or worker-laid. In the larval stage, we assume that workers can recognise the gender of larvae but not their maternal origin. The latter is supported by Ratnieks and Visscher (1989) who showed that honeybees accepted both workers' and queen's sons in the larval stage, but preferentially killed workers' sons at the egg stage.

Table 10.1 Parameters and variables used in the model.

Value	Description
$V_{np}, V_p$	allocation component of fitness in non-policing and policing colonies
$g_f, g_b, g_n$	relatedness of focal worker to sisters, brothers and nephews
$v_f, v_m$	sex-specific reproductive values of females and males
$x, y$	proportions of colony sex allocation to females and males
$X, Y$	proportions of population sex allocation to females and males
$W_c$	proportion of males that are worker's sons in the colony
$W_{pop}$	proportion of males that are worker's sons in the population
$y_{change}$	reduction in the allocation to males by male killing as a proportion of all the brood
$c$	the proportion of the energy invested in an adult male that is lost if he is killed
$B_{policing}$	difference in inclusive fitness of a worker in a policing versus a non-policing colony
$C_p, C_{np}$	proportional reduction in total colony productivity resulting from male killing
$k$	effective paternity in colonies and the population
$w$	proportion of eggs that are laid by workers
$x_q, y_q$	proportion of queen-laid eggs that are female and male
$x_1, y_1$	proportion of females and males before sex allocation biasing
$x_2, y_2$	proportion of adult females and males after sex allocation biasing
$P$	frequency of policing colonies versus non-policing colonies
$X_{opt}$	stable sex allocation ratio for workers

We assume that there is a negligible cost in killing eggs but that larvae that are killed incur a cost of  $c$ . That is, only  $1-c$  of energy in males can be reallocated by killing. The reinvested resources can be reallocated either to reproductives of both sexes (males and queens, as in Figure 10.1) or only to females (queens).

Our model reveals a novel benefit to worker policing. In colonies with worker policing, the cost of sex-allocation biasing is reduced because some males are removed at low cost as eggs rather than at higher cost as larvae (Figure 10.1,  $C_p < C_{np}$ ).

### 10.32 Inclusive fitness equations

The model follows Pamilo's (1991a,b) formulation of inclusive fitness, and compares the 'allocation component of fitness' ( $V$ ) of a non-laying worker in a colony with worker policing to a non-laying worker in a colony without worker policing. All parameters used in the model are shown in Table 10.1. The allocation component of fitness is the sum of the fitness gained from each class of offspring or relative. With worker reproduction, there are three classes of reproductive relatives of the focal non-laying worker: queen's daughters (the focal worker's sisters), queen's sons (the worker's brothers) and workers' sons (the worker's nephews) so that:

$$V = g_f v_f x / X + (1 - W_c) g_b v_m y / Y + W_c g_n v_m y / Y$$

or

$$V = g_f v_f x / X + [(1 - W_c) g_b + W_c g_n] v_m y / Y \quad (10.1)$$

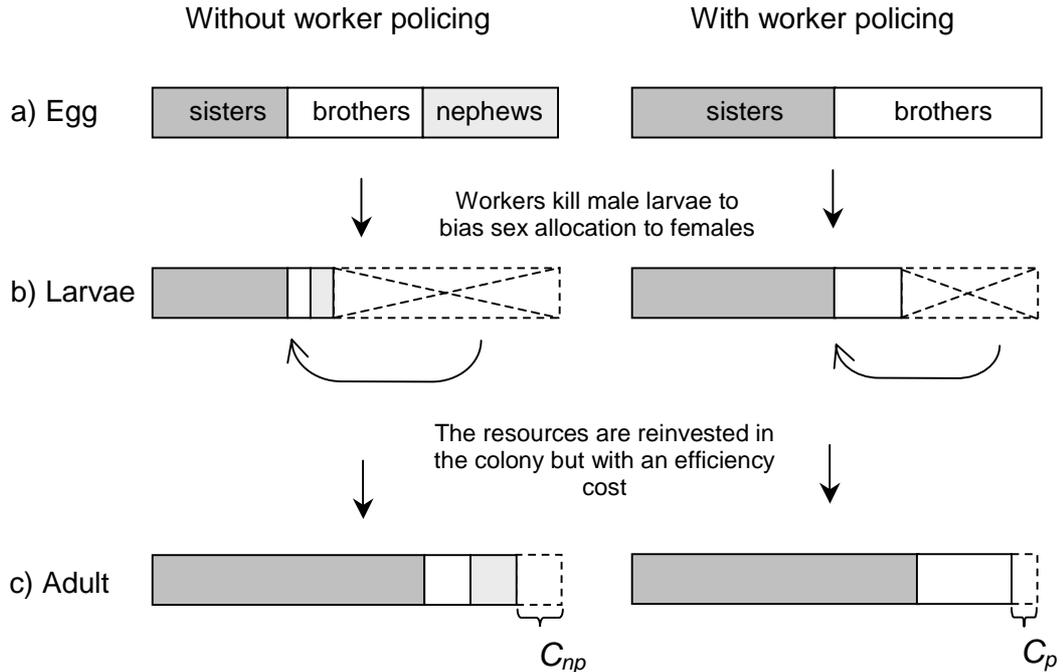


Figure 10.1 How worker policing reduces the cost of sex allocation biasing i.e.  $C_p < C_{np}$  (Equation 10.5). Three stages are shown a) egg stage – when worker policing occurs and worker-laid eggs are targeted, b) larval stage – when sex allocation biasing is performed by killing a proportion of the male brood, the final sex ratio shown is 3F:1M, c) adult stage –workers reinvest the resources from the killed male larvae into the remaining brood but at an efficiency cost of 20%.

where  $v_f$  and  $v_m$  are the sex-specific reproductive values of females and males,  $x$  and  $y$  are the proportional allocations to each sex in the focal colony and  $X$  and  $Y$  in the whole population,  $W_c$  is the proportion of males in the colony that are workers' sons, and  $g_f$ ,  $g_b$  and  $g_n$  are the regression relatednesses of the focal worker to sisters, brothers and nephews. Worker policing will be selected if the inclusive fitness of the focal worker in a policing colony is greater than that of a focal worker in a non-policing colony.

First, we calculate the allocation component of fitness for a focal worker in policing ( $V_p$ ) and non-policing colonies ( $V_{np}$ ):

$$V_{np} = g_f v_f + [(1 - W_c)g_b + W_c g_n] v_m \quad (10.2)$$

$$V_p = g_f v_f + g_b v_m \quad (10.3)$$

We assume that all colonies in the population have the same sex allocation ratio so that  $x = X$  and  $y = Y$  in Equation 10.2 and 10.3, which cancel (Equation 10.1). (We discuss the effect of relaxing this assumption below.) Next, we incorporate an efficiency cost of sex allocation  $C$ :

$$C = y_{change} c \quad (10.4)$$

where  $c$  is the inefficiency of energy transfer between the killed larval males and the colony, and  $y_{change}$  is the change in the proportional allocation to males (change in male allocation as a proportion of all the brood, Figure 10.3). We can now compare the

inclusive fitness of non-laying workers in policing versus non-policing colonies ( $B_{policing}$ ):

$$B_{policing} = (1-C_p)V_p - (1-C_{np})V_{np} \quad (10.5)$$

where  $C_p$  and  $C_{np}$  are the costs of sex allocation in policing and non-policing colonies and conversely  $1-C_p$  and  $1-C_{np}$  are the relative productivity of each colony type. Worker policing is selected when  $B_{policing}$  is positive.

### 10.33 Model parameters

We now have an equation which calculates the fitness effect of worker policing in colonies where sex allocation biasing occurs. We are interested in the effect of six key factors on  $B_{policing}$ , which need to be incorporated into equations 10.2-10.5:

- $k$  effective paternity; the basis of the original worker policing predictions (Ratnieks 1988)
- $c$  cost of recycling male larvae
- $w$  amount of worker laying, as this varies greatly between species (Bourke and Franks 1995)
- $x_q$  primary sex ratio of queen-laid eggs (the proportion of eggs that are female)
- $x_2$  final allocation to females, after workers have performed sex-allocation biasing
- $P$  frequency of policing colonies in the population

We now define the variables in Equations 10.2-10.5 in terms of these parameters.

#### 1) Regression relatedness (Hamilton 1970, Grafen 1985).

We base the model on single-queen societies as colony kin structure can be defined in terms of a single variable ‘effective paternity’, itself determined by queen mating frequency and sperm use (Boomsma and Ratnieks 1996). However, the method is also applicable to multiple-queen societies where relatedness patterns that favour policing or disfavour policing also occur dependent on the number and relatedness of queens (Pamilo 1991b).

$$g_f = 0.25 + 0.5(1/k) \quad (10.6a)$$

$$g_b = 0.5 \quad (10.6b)$$

$$g_n = 0.25 + 0.5(1/k) \quad (10.6c)$$

#### 2) The proportion of the males in the population that are worker’s sons $W_{pop}$ .

This is dependent on two factors.

(a) The proportion of males that are worker’s sons in the non-policing colonies  $W_c$  (Figure 10.2),

$$\begin{aligned} W_c &= w/[w+(1-w)y_q] \\ &= w/[w+(1-w)(1-x_q)] \end{aligned} \quad (10.7a)$$

(b) The frequency of policing colonies ( $P$ ), where we assume that no workers' sons are reared, versus non-policing colonies ( $1-P$ ).

$$W_{pop} = (1-P)W_c \quad (10.7b)$$

### 3) Reproductive value (Fisher 1930).

When all males in the population are queens' sons females have twice the reproductive value of males because males only contribute genes to females of the next generation. With worker reproduction, however, father males also contribute genes to males, thereby raising male reproductive value (see Bourke and Franks 1995; Crozier and Pamilo 1996, for a summary of reproductive value and relatedness).

$$v_f = 1 \quad (10.8a)$$

$$v_m = 1/(2-W_{pop}) \quad (10.8b)$$

### 4) The amount of male killing needed

To change the primary sex ratio to the sex ratio after sex allocation biasing ( $y_{change}$ , Equation 10.4). The primary sex allocation to females is (Figure 10.2).

$$x_1 = (1-w) x_q \quad (10.9)$$

where  $w = 0$  in policing colonies. We consider the two extreme cases of male reinvestment: into reproductives of both sexes or only into queens.

a) If resources are reinvested into reproductives of both sexes (Figure 10.3a):

$$\begin{aligned} (y_1 - y_{change})/x_1 &= y_2/x_2 \\ y_{change} &= 1 - x_1/x_2 \end{aligned} \quad (10.10a)$$

b) If resources are reinvested only into queens (Figure 10.3b):

$$\begin{aligned} (y_1 - y_{change})/(x_1 + (1-c)y_{change}) &= y_2/x_2 \\ y_{change} &= (x_2 - x_1)/(1 + cx_2 - c) \end{aligned} \quad (10.10b)$$

### 5) The stable sex-allocation ratio for workers ( $X_{opt}$ ).

This is determined by the relative kin value of males versus females (Fisher 1930; Trivers and Hare 1976; Benford 1978; Pamilo 1991a).

$$X_{opt} = gf \cdot vf / [gf \cdot vf + (v_m(1 - W_{pop})g_b + W_{pop}g_n)] \quad (10.11)$$

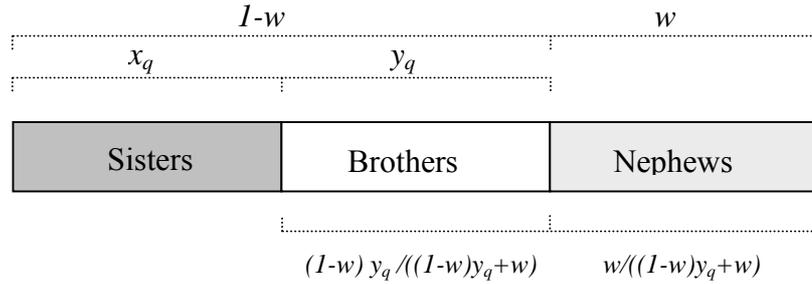


Figure 10.2 The proportion of males that are workers' sons if the workers lay a proportion  $w$  of the total eggs and  $x_q$  of the queen's eggs are female and  $y_q = 1 - x_q$  are male ( $W_c$ , Equation 10.7a).

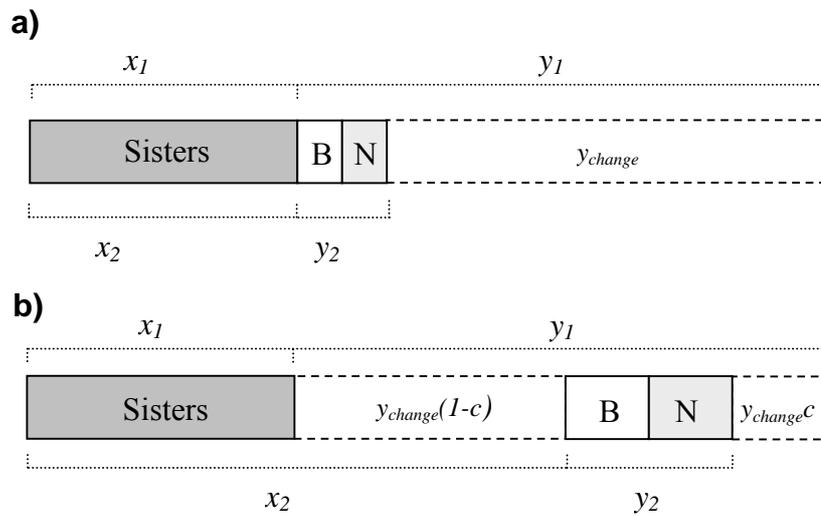


Figure 10.3 Calculation of  $y_{change}$ : the amount of males that must be killed to produce a given secondary sex allocation ratio.  $x_1$  and  $y_1$  are the proportions of females and males before, and  $x_2$  and  $y_2$  after sex allocation biasing. (a) Sex allocation with reinvestment back into the colony as a whole (Equation 10.10a). (b) Sex allocation with reinvestment back into females (Equation 10.10b). Reinvestment of a proportion  $y_{change}$  of the males results in the production of  $(1-c)y_{change}$  new brood (females and males in (a) and females only in (b)) and a waste of  $y_{change}c$ .

### 10.4 Results

The equations were combined and  $B_{policing}$  was evaluated using the Mathematica 3.0 (Figure 10.4). Our aim was to determine the effect of the cost of sex allocation biasing  $c$  on the paternity frequency at which worker policing is selected (when  $B_{policing} > 0$ ) and to see how robust the results are with respect to parameters  $w$ ,  $x_2$ ,  $x_q$  and  $P$ . Unless otherwise stated, the results are for:

$c$	0.1	10% of the killed males' energy is lost
$w$	0.5	half of all eggs are laid by workers
$x_q$	0.5	the queen lays eggs at an equal sex ratio
$x_2$	$X_{opt}$	Colony (and population) sex allocation is at the worker optimum
$P$	0	Worker policing is invading
$y_{change}$		Killed male larvae are reinvested into both females and males

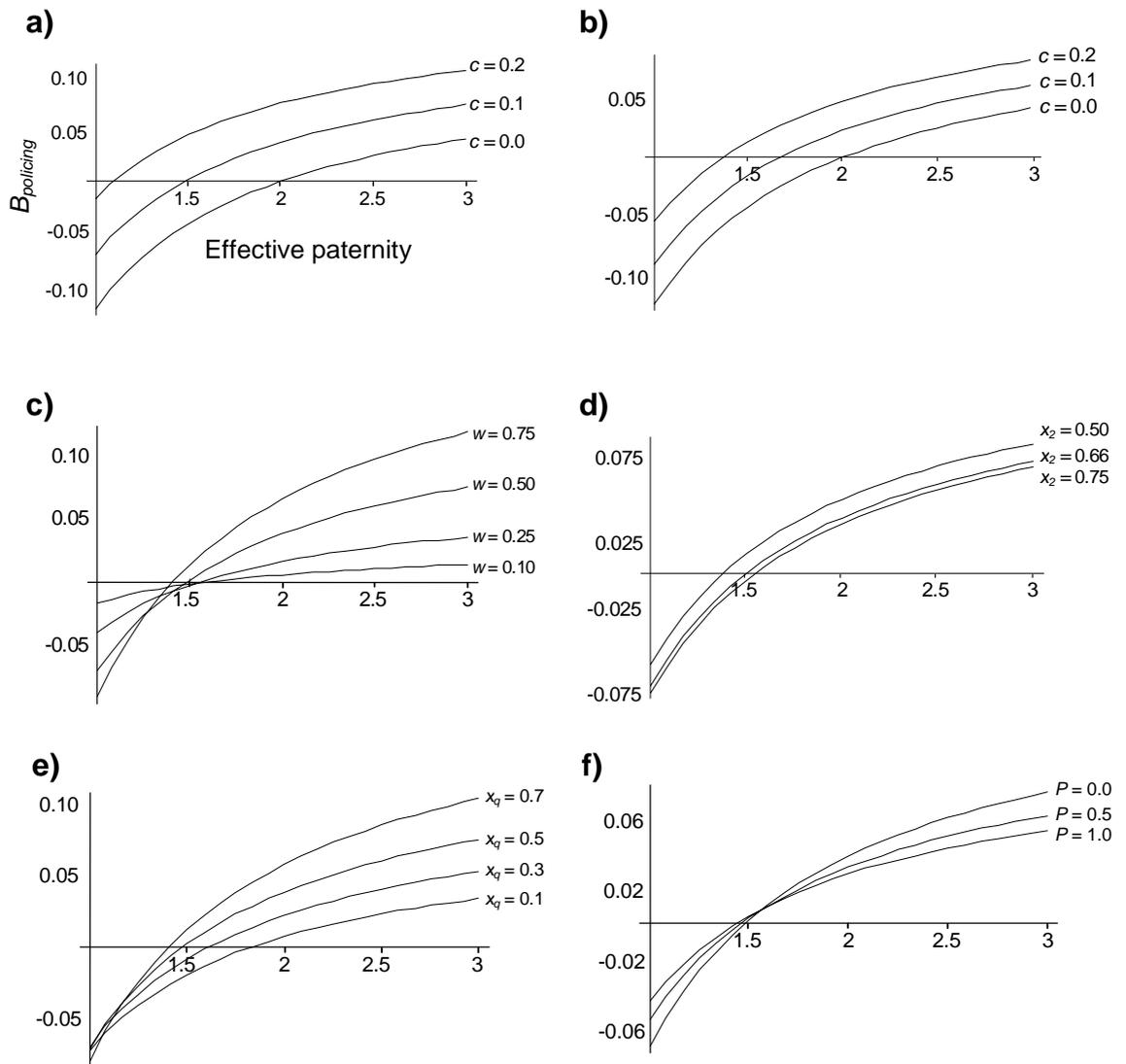


Figure 10.4 a) Effect of an efficiency cost of male killing,  $c$ , on the paternity frequency at which worker policing is selected. Positive values of  $B_{\text{policing}}$  indicate a benefit to non-laying workers from worker policing. With no cost ( $c = 0$ ), the classic worker policing result that workers will police at effective paternity  $> 2$  is recovered (Ratnieks 1988). b) When resources are re-invested directly into new queens. c) Effect of the amount of worker laying. d) Effect of the final sex-allocation ratio e) Effect of the queen-laid sex ratio. f) Effect of the frequency of colonies with worker policing in the population. Unless otherwise stated killed males are reinvested into both sexes,  $c = 0.1$ ,  $w = 0.5$ ,  $x_2 = X_{\text{opt}}$ ,  $x_q = 0.5$  and  $P = 0$ .

#### Figure 10.4a) Cost of sex-allocation biasing $c$

Introducing an efficiency cost of 10 or 20% to the recycling of males killed during sex allocation has a large effect on the threshold at which worker policing is favoured. An efficiency cost of  $>20\%$  results in policing being favoured at all paternities. With no efficiency cost, the classic result is recovered (Starr 1984, Ratnieks 1988) that worker policing is selected when effective paternity is above two.

Figure 10.4b) Reinvestment directly into females

If killed males are reinvested directly into new queens the benefit of worker policing is reduced. That is, for a given efficiency cost ( $c$ ), the paternity at which worker policing is favoured is higher than in Figure 10.4a). This is expected because reinvestment directly into queens makes sex allocation biasing by male larvae killing more efficient, and therefore reduces the benefit from worker policing.

Figure 10.4c) The amount of worker laying

Worker policing is favoured by increased worker egg laying. This is because more worker-laid eggs results in more male larvae to kill which raises the cost of sex allocation biasing in non-policing colonies. Importantly, worker policing is always selected for at paternities below two at all levels of worker reproduction. This is logical because worker policing of eggs can only reduce the cost of killing male larvae.

Figure 10.4d) The proportion of females raised by the workers

The final sex-allocation ratio slightly affects the paternity frequency at which policing is favoured. The more female biased the final sex-allocation ratio (closer to the worker optimum), the more difficult it is for worker policing to evolve. This is because a female bias requires more male killing in both policing and non-policing colonies which decreases the difference in male larvae killing in non-policing versus policing colonies. That is  $C_{np}-C_p$  is reduced (see Figure 10.1), which reduces the relative benefit of worker policing.

Figure 10.4e) The proportion of female eggs laid by the queen

Figure 10.4e shows that if the queen lays a male-biased sex ratio, the benefit of worker policing is reduced. As with a female-biased final sex ratio (Figure 10.4d), a male-biased primary sex ratio causes more killing of male larvae in both policing and non-policing colonies thereby decreasing the difference between the two colony types.

Figure 10.4f) The frequency of worker policing in the population

The frequency of policing colonies in the population has little effect on the paternity at which policing is selected. There is, however, a marked difference in the gradient of the curves in Figure 10.4f. This is because worker policing ( $P = 0.5, 1$ ) reduces male production by workers, which lowers the reproductive value of males (Equation 10.8). This reduces the inclusive fitness of workers in all colonies and decreases the magnitude of  $B_{policing}$  at all paternities.

## 10.5 Discussion

The results show that worker policing can be selected at low paternity when worker sex allocation biasing by the killing of male larvae occurs. That is, worker policing is favoured even when workers are more related to other worker's sons than to the queen's sons. Worker policing is favoured because it reduces the cost of sex-allocation biasing by removing males at low cost in the egg stage. This conclusion is robust for both

reinvestment of killed males into both sexes or into young queens alone, for any amount of worker laying, at both the queen and worker sex-allocation optima, when policing is either rare or common, and for all but extremely male-biased primary sex ratios of queen-laid eggs ( $x_q < 0.1$ ) (Figure 10.4).

The paternity frequency at which policing is favoured is critically dependent on the amount of investment into males that can be recovered by killing. In our model, a loss of greater than 20% results in worker policing being favoured at all paternities i.e. irrespective of relatedness. The amount of energy lost through male killing is dependent on four key factors. (1) Metabolic efficiency, which is the percentage of energy consumed that is incorporated into new biomass. (2) The amount of energy in males at the time of their execution, with less energy lost the earlier that males are killed. (3) The labour cost of rearing males, which is permanently lost. (4) Accuracy of worker recognition of the sex of larvae. If workers sometimes mistakenly kill females during sex allocation biasing, the cost of sex allocation biasing will be raised. The importance of factors 3 and 4 are unknown. However, given that metabolic efficiency in carnivorous insects is around 45% (Begon *et al.* 1990, Chapuisat *et al.* 1997) and male killing sometimes occurs late in the larval stage (Chapuisat *et al.* 1997), a loss greater than 20% seems realistic.

This extension of the theory of worker policing was inspired by the Vespinae wasps where worker policing by egg eating occurs at paternities below two (Foster and Ratnieks 2000, Chapter 8; Foster and Ratnieks 2001, Chapter 9; Foster *et al.* submitted, Chapter 6) and female-biased sex allocation occurs (Edwards 1980). However, with female-biased sex allocation common in eusocial Hymenoptera (Pamilo 1990), the potential for the interaction between worker sex allocation biasing and policing is widespread.

Our model assumes that all colonies have the same sex allocation. This is not always true. With colonies in the same population varying in paternity, it is predicted that workers in low paternity colonies benefit from specialising in females, while worker in higher paternity colonies benefit by investing in males, resulting in split sex ratios (Boomsma and Grafen 1990; 1991, Ratnieks 1991). This pattern has been found in the ants *Formica truncorum* (Sundström 1994a; 1994b) and *Formica exsecta* (Sundström *et al.* 1996) and the wasp *Dolichovespula arenaria* (F.L.W. Ratnieks and J.J. Boomsma unpublished data). Facultative sex allocation biasing also occurs when colonies vary in the number of queens in the ants *Myrmica tahoensis* (Evans 1995) and *Leptothorax acervorum* (Chan and Bourke 1994) and five epiponine wasp species (Queller *et al.* 1993b, Hastings *et al.* 1998), in line with relatedness predictions. Facultative sex-allocation biasing in response to variation in paternity frequency or queen number will also favour the evolution of worker policing against relatedness predictions. In high paternity colonies, biased allocation to males occurs and, because these colonies have high paternity, worker policing of workers' sons is more favourable due to relatedness benefits. Meanwhile, in low paternity colonies workers favour females over all males, whether queen or worker's sons. In these latter colonies, as in our model, the cost of killing male larvae will also favour the evolution of worker policing despite the low paternity.

This study shows the value of considering reproductive conflicts simultaneously as actually occurs in nature, rather than one at time. Our model reveals a condition under which sex allocation and male production cannot be treated separately. In combination

with sex allocation there is a novel benefit arising from worker policing because policing reduces the cost of killing male brood, which is carried out to cause a female-biased sex ratio. Interestingly, the presence of one conflict favours the suppression of another. A further lesson from this study is the importance of considering specific details of reproductive biology such as the timing of male killing, which is itself caused by limitations in the ability of workers to recognise the gender of brood (otherwise they would kill the queen's male eggs). Although general inclusive fitness models are very important (Hamilton 1964), the theory should be applied with care, and must often be combined with specific knowledge of the study species and the constraints on the recognition mechanisms needed to manipulate colony reproduction (Keller 1997).

Mutual policing is thought to be an important mechanism in the suppression of reproductive conflict at all levels of biological organisation (Ratnieks 1988, Frank 1995). The discovery of worker policing in several hymenopteran species against relatedness predictions supports this (Foster and Ratnieks 2001, Chapter 9). Worker policing can evolve at low paternity if worker reproduction is costly, because policing can increase group productivity (Ratnieks 1988; Frank 1995). Here we show that worker policing also reduces the cost a second reproductive conflict, that over sex allocation, further favouring its evolution.

## **10.6 Acknowledgements**

We thank Tom Wenseleers for helpful discussions on the model. Funding for this study was provided by a BBSRC Ph.D. studentship to KRF.

---

## Spite in social insects

K.R. Foster, T. Wenseleers & F.L.W Ratnieks

Gadagkar (1993) asked the question ‘can animals be spiteful?’; that is, do they ever harm another without a gain in personal reproduction. The cited examples, the killing of chicks in gulls and egg cannibalism in sticklebacks, were later shown to be better interpreted as plain selfishness (Keller *et al.* 1994). This led Keller *et al.* (1994) to conclude that ‘spiteful animals are still to be discovered’. Here, we draw attention to recent work on conflict in insect societies, which reveals several clear examples of spiteful actions.

The first class of examples are behaviours that Wilson (1975) termed spiteful. He proposed that harmful behaviour could, in the absence of personal benefits, be favoured through benefits to a third party (Figure 11.1). Consider sex-ratio biasing in ants (Sundström *et al.* 1996), where workers kill their brothers to increase the production of more valuable sister queens (fratricide; Figure 11.1). Fratricide, although detrimental to the male recipients, is not carried out to benefit the personal reproduction of the worker because workers are effectively sterile. Instead, the gain to the actor comes indirectly via benefits to sister queens. Worker sex-allocation biasing conforms to Wilson’s (1975) concept of spite: a harmful interaction that is offset by an indirect benefit to related individuals. Wilson’s definition separates two distinct processes. Sex-ratio biasing could also occur through preferential feeding of sister larvae – nepotistic altruism, whereas it actually involves harm to males (Sundström *et al.* 1996) – spite. Worker policing in honey bees (Ratnieks and Visscher 1989), where workers invest time in the destruction of worker-laid male eggs, thus facilitating their replacement with more valuable queen-laid male eggs (Ratnieks and Visscher 1989; Visscher 1996), is similarly spiteful (Figure 11.1).

But, does Hamilton’s more stringent view of spite ever occur (Hamilton 1970, 1971)? He argued that spite could evolve with only two parties, but required highly specific conditions (Hamilton 1970, 1971). Amazingly, the recently discovered ‘green-beard’ matricide in the fire ant *Solenopsis invicta* (Keller and Ross 1998; Hurst and McVean 1998) has all the conditions Hamilton predicted (Figure 11.1): (1) kin discrimination – a ‘green beard’ gene that enables workers to identify nongene carrier queens; (2) a low cost to the actor – in fact, no cost because fire ant workers are sterile; and (3) negative relatedness, from the perspective of the green beard locus, because the killed queens are less likely than random to possess the green beard gene (Hurst and McVean 1998). As the antithesis of altruistic behaviour, the harm to non-kin is sufficient to cause the spread of spite, without requiring any indirect benefits (Hamilton 1970, 1971) (Figure 11.1). However, there might also be indirect benefits to ‘green-beard’ carrier queens, meaning spite is also favoured by Wilson’s condition (1975).

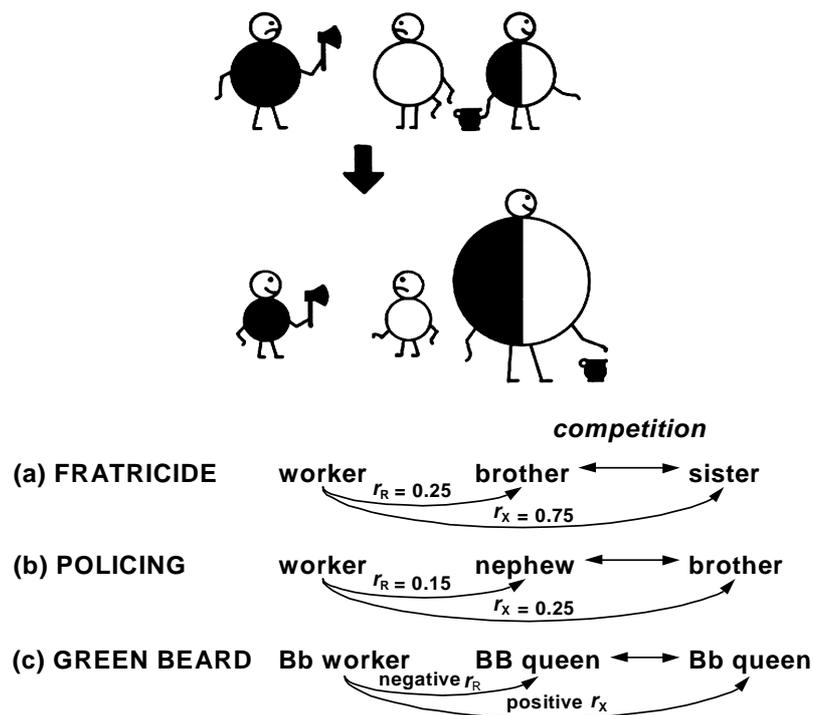


Figure 11.1. Wilson's (1975) view of spite from Sociobiology: 'The spiteful individual lowers the fitness of a competitor while reducing that of his own or at least not improving it; however, the act increases the fitness of the brother to a degree that more than compensates.' This is selected for when  $c_A + c_R r_R < b_X r_X$ , a three party extension of Hamilton's rule. Here,  $c$  and  $b$  denote costs and benefits and  $r$  denotes the actor's (life-for-life) relatedness to the recipient  $R$  and the third party  $X$ . In insect societies, the cost to the actor  $c_A$  is negligible because of worker sterility. Three examples of spite in social insects are shown: (a) worker-biasing of the sex ratio via fratricide (Sundström *et al.* 1996), (b) worker policing (Ratnieks and Visscher 1989) and (c) matricide in the fire ant (*Solenopsis invicta*), where Bb 'green-beard' workers eliminate noncarrier BB queens in the colony (Keller and Ross 1998). Relatedness can be written as  $(p_R - p)/(p_A - p)$ , where  $p_R$ ,  $p_A$  and  $p$  denote gene frequency in recipients, actors and the population at large (Grafen 1985). For 'green-beard' spite in haploid animals,  $p_A = 1$  (gene carriers perform the killing) and  $p_R = 0$  (noncarriers are eliminated), thus  $r_R = -p/(1-p)$ . For two-way inter-actions this recovers Hamilton's result (1970; 1971) that such a gene would spread when  $p > c_A/(c_A - c_R)$ . Cartoon reproduced, with permission, from Harvard Press.

In the fire ant, spite has evolved as Hamilton predicted, but is it widespread in the animal kingdom? It will be interesting to look for but, as Hamilton himself pointed out, spite is likely to be rare due to the stringent conditions for its evolution (Hamilton 1970, 1971). It is no coincidence that the famously altruistic insect societies provide the most compelling examples of spite. In the course of social evolution, insect workers have become actually or effectively sterile, thus overcoming the major obstacle in the evolution of spite – cost to personal reproduction (Hamilton 1970, 1971).

## **Paternity, reproduction and conflict in vespine wasps: a model system for testing kin selection predictions**

K.R. Foster & F.L.W. Ratnieks

### **12.1 Abstract**

The Vespinae wasps (*Vespa*, *Provespa*, *Dolichovespula* and *Vespula*) are an excellent group for testing kin selection predictions. There is considerable variation in kin structure and conflict resolution, in a group of known phylogeny where all species have a similar basic biology: morphologically distinct queens and workers, annual nests headed by a single queen. Vespine kin structure can be described by a single variable *effective paternity*, which is defined by queen mating frequency and sperm use. Low effective paternity, which causes high worker relatedness, is basal in the group (*Vespa*, *Dolichovespula*) with high paternity (c. >2) restricted to *Vespula* whose queens mate more and use sperm more equally. Paternity variation correlates well with observed patterns of reproduction and conflict. As predicted by theory, male production by workers is common in *Dolichovespula* but not *Vespula* where worker policing occurs (*V. vulgaris*). The theory is also supported by intercolony variation in *Dolichovespula* with facultative worker policing (*D. saxonica*) and sex allocation biasing by workers (*D. arenaria*) occurring in response to paternity. In addition, queen loss in reproductive colonies of 9 species is negatively correlated with paternity ( $p < 0.01$ ) in line with prediction that matricide is only expected at low paternity. However, paternity does not explain the reproductive characteristics of *Vespa crabro*. Although paternity in *V. crabro* is only 1.1, the same as the mean in six *Dolichovespula* species, its reproductive behaviour is different from *Dolichovespula*. More like *Vespula*, *V. crabro* has worker policing, workers that rarely have active ovaries, and little queen loss in reproductive phase colonies.

### **12.2 Introduction**

In non-clonal societies there is potential for conflict over reproduction between group members (Hamilton 1964; Ratnieks and Reeve 1992; Maynard-Smith and Szathmary 1995). A major question in the study of sociality is how and if reproductive disputes are resolved without too great a disruption to the group (Keller 1999). The study of conflicts and their resolution has been central to our current understanding of kin selection theory (Hamilton 1964; Bourke and Franks 1995; Crozier and Pamilo 1996; Keller and Reeve 1999) and natural selection (Leigh 1999).

The eusocial Hymenoptera (bees, ants, wasps) are particularly interesting in the study of reproductive conflict. Their haplodiploid genetics and social structure cause diverse and unusual colony kin structures and the potential for a wide range of conflicts (Ratnieks

		Effective Paternity	# broods	% males	% workers	Ref	
		range	analyzed	worker sons	active ovaries		
Polistinae (outgroup)	<i>Polistes sp.</i>	<b>&lt; 1.05</b>	1-2	-	-	1,2	
<i>Vespa</i>	<i>crabro</i>	<b>1.11</b>	1-3	14	0	3,4	
<i>Provespa</i>	<i>anomola</i>	<b>1.00</b>	1	1	-	5	
<i>D. maculata</i> grp	<i>maculata</i>	<b>1.00</b>	1	10	21	6	
	<i>media</i>	<b>1.08</b>	1-2	10	7	6	
<i>D. norwegica</i> grp	<i>arenaria</i>	<b>1.09</b>	1-4	20	17	7	
	<i>sylvestris</i>	<b>1.15</b>	1-2	10	10	6	
	<i>norwegica</i>	<b>1.08</b>	1-3	10	3	10	
	<i>saxonica</i>	<b>1.35</b>	1-3	10	35	11	
<i>V. vulgaris</i> grp	<i>germanica</i>	<b>2.35</b>	1-7	55	-	0.6	8,9
	<i>maculifrons</i>	<b>7.14</b>	-	30	0	2	9,10
	<i>vulgaris</i>	<b>1.90</b>	1-4	17	0	0.4	11
<i>V. koreensis</i> grp	no data	-	-	-	-	-	
<i>V. rufa</i> grp	<i>rufa</i>	-	≥2	1	-	-	12
<i>V. squamosa</i> grp	<i>squamosa</i>	<b>3.33</b>	-	17	0	-	10

Figure 12.1 Paternity in the Vespinae wasps, including representative data from their sister group, the Polistinae, for outgroup comparison. *Effective* paternity is the evolutionarily important value. This takes into account the relative contribution of each male to the female progeny and is proportional to worker relatedness. No study reports significant inbreeding in vespines suggesting that paternity is indeed the key factor determining kin structure. ‘% males worker sons’ and ‘% workers active ovaries’ are for queenright colonies. Diploid males appear to be rare in the Vespinae, and were reported in only one out of the ten vespine species where male production has been studied (*Vespa crabro*, 1/15 colonies had adult diploid males, Foster *et al.* 2000). Active ovaries contain at least one full-sized egg. The phylogeny is from Carpenter (1987). References: (1) Field *et al.* 1998; (2) Peters *et al.* 1995; (3) Foster *et al.* 1999; (4) Foster *et al.* 2000; (5) Foster unpublished data; (6) Foster *et al.* 2001; (7) F. L. W. Ratnieks and J. J. Boomsma unpublished data; (8) Goodisman *et al.* submitted; (9) Ross 1985; (10) Ross 1986; (11) Foster and Ratnieks 2001; (12) Thoren *et al.* 1994.

and Reeve 1992; Bourke and Franks 1995; Crozier and Pamilo 1996). Conflict between the queen and the workers over sex allocation (Trivers and Hare 1976) and the production of males (Starr 1984) appear to be particularly important. However, our understanding of these conflicts and the extent to which they depend on kin structure is still at an early stage. This is at least partly due to powerful techniques for determining kin structure only recently becoming widely available (Queller *et al.* 1993a).

One group that has been particularly neglected in the study of reproductive conflict is the Vespinae (hornets and yellowjackets), the sister group of the more extensively studied Polistinae (paper wasps, Turillazzi and West-Eberhard 1996). The Vespinae are a relatively small group of four genera with a well-supported phylogeny (Figure 12.1, Carpenter 1987): *Vespa* (23 species), *Provespa* (3 species), *Dolichovespula* (13 species) and *Vespula* (22 species). They have a similar basic biology, typically with an annual monogynous colony, enclosed paper nest, a morphologically distinct queen caste and workers that are unable to mate, facilitating across-species comparison based upon differences in kin structure (Foster *et al.* 1999, Chapter 4). Here, we discuss a number of studies on vespine wasps which provide new insight into the evolution of kin structure and reproductive conflicts.

Table 12.1 Paternity bias in double paternity nests of the Vespinae. Estimates are based on samples of twenty workers from each colony. Species are the same as in Figure 12.2. The majority male contribution is significantly different to an unbiased binomial estimate (0.58) in *Vespa* ( $P = 0.021$ ) and *Dolichovespula* ( $P = 0.002$ ) but not in *Vespula* ( $P = 0.085$ ) (1-sample, 1-tailed t-tests)

Genus	# species	# colonies	Majority male contribution
<i>Vespa</i>	1	8	0.76
<i>Dolichovespula</i>	5	11	0.73
<i>Vespula</i>	1	10	0.64

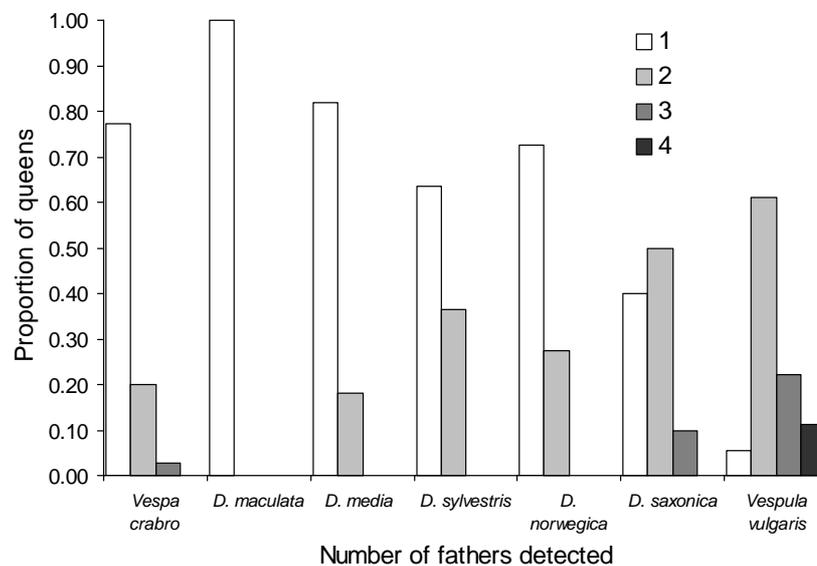


Figure 12.2. Number of mates of the queen in seven vespine species. Values are estimated from DNA microsatellite analysis of 20 workers from each of 10-17 colonies of each species. References as in Figure 12.1.

### 12.3 Evolution of kin structure

Kin selection theory predicts that relatedness will be extremely important in reproductive behaviour because, by favouring the rearing of close relatives, helping individuals can increase the transmission of their genes (Hamilton 1964). Knowledge of kin structure, which determines relatedness patterns, is therefore central to the study of reproductive conflict. Vespine wasps have a relatively simple kin structure that is highly amenable to study because societies are nearly always headed by a single outbred queen. This makes queen mating frequency (specifically effective paternity) the key variable causing variation in colony kin structure. Effective paternity is the evolutionarily important measure of queen mating frequency that takes into account sperm use (Starr 1984). Observational data of queen mating frequency have proved an unreliable predictor of paternity (Boomsma and Ratnieks 1996; compare Starr 1984 and Page 1986 with Figure 12.1). However, DNA microsatellites have made accurate paternity assessment routine and a number of vespine species have now been analysed.

In combination with Carpenter's (1987) phylogeny of the Vespinae, this allows inferences on the evolution of kin structure to be made (Figure 12.1).

Cladistic analysis suggests that high effective paternity ( $>2$ ) is a derived trait, restricted to *Vespula* (Boomsma and Ratnieks 1996; Foster *et al.* 1999). A partial exception is *Dolichovespula saxonica*, where initial paternity estimates were high (Thorén 1998). However, further analysis has shown that, while multiple paternity is common, it does not occur in all colonies (Foster *et al.* 2001). High paternity also appears to be derived in the corbiculate bees (Apidae) and fungus-growing ants, where it seems to be restricted to the honeybees *Apis* (Estoup *et al.* 1994; Peters *et al.* 1999) and the genera, *Atta* (Villesen *et al.* 1999) and *Acromyrmex* (Boomsma *et al.* 1999; Bekkevold *et al.* 1999) respectively. The increase in effective paternity in *Vespula* seems to have occurred by two mechanisms. *Vespula* queens probably engage in more matings because more fathers are detected (Figure 12.2). In addition, paternity bias seems to be lower than in *Dolichovespula* and *Vespa* (Table 12.1). The contribution of majority males in double paternity families in *Vespula vulgaris* is significantly lower than in *Dolichovespula* (t-test,  $p = 0.04$ ), borderline significant in comparison to *Vespa* ( $p = 0.07$ ) and significantly lower than in *Dolichovespula* and *Vespa* combined ( $p = 0.02$ ). Why *Vespula* queens mate more and use sperm more evenly is not yet known. However, observation suggests that the opportunity for multiple mating by queens exists in all three genera, with male mating aggregations described in species from each genus (*Vespa mandarinia*, *Vespula flaviceps*, Matsuura and Yamane 1990; *Vespula rufa*, *Vespula vulgaris*, *Vespula germanica*, *D. sylvestris* Edwards 1980 and references therein; *Vespa crabro*, K. R. Foster *pers. obs.*). This suggests that the higher mating frequency in *Vespula* reflects a difference in queen behaviour rather than increased opportunity to mate.

## 12.4 Conflict over male production

As in most species of social Hymenoptera, worker vespine wasps are unable to mate but can lay haploid male-destined eggs (Wilson 1971; Bourke 1988). This leads to potential conflict between the queen and the workers and among workers over male production. Paternity is pivotal in the prediction of the outcome of this conflict (Starr 1984; Woyciechowki and Łomnicki 1987; Ratnieks 1988). Under single paternity, workers are more related to each others' offspring (nephews,  $r = 0.375$ ) than the queen's sons (brothers,  $r = 0.25$ ). The rearing of workers' sons, therefore, is expected. This changes with effective paternity above two because the relatedness between workers is reduced such that each worker is more related to brothers (queen's sons) than nephews (worker's sons,  $r < 0.25$ ). The collective interests of the workers is now the same as the queen and workers are predicted to police each others' reproduction resulting in little male production by workers (Ratnieks 1988). In line with this prediction, worker policing occurs in the highly polyandrous honeybee *Apis mellifera* (Ratnieks and Visscher 1989) where a dynamic conflict exists between worker egg laying and policing (Visscher 1989; Ratnieks 1993; Visscher 1996).

The comparison of *Vespula* with *Dolichovespula* provides further support for these predictions (Foster *et al.* 2001, Chapter 7). *Dolichovespula* societies are characterised by low paternity and queen-worker conflict. Although workers only succeed in producing a minority of the males (Figure 12.1), many have active ovaries with full-size eggs (Foster *et al.* 2001, Chapter 7) and lay eggs in the presence of the queen (*D.*

*maculata*, Baldulf 1954; Greene 1979; *D. arenaria*, Greene *et al.* 1976; *D. media*, Foster *et al.* 2001, Chapter 7). In contrast, high paternity in the *Vespula vulgaris* and *squamosa* groups (Figure 12.1) is associated with very few ovary-activated workers (Ross 1985; Foster and Ratnieks 2001, Chapter 9), an absence of worker laying (*V. pennsylvanica*, Akre *et al.* 1976) and queen-only male production (*V. squamosa*, *V. maculifrons*, Ross 1986; *V. vulgaris*, Foster and Ratnieks 2001, Chapter 9). Furthermore, work on *V. vulgaris* has shown that the absence of worker reproduction in *Vespula* is probably due to worker policing (Foster and Ratnieks 2001, Chapter 9). In egg introduction experiments into two queenright discriminator colonies, all worker-laid eggs were removed by workers whilst the majority of queen-laid eggs remained (0/120 worker-laid versus 80/120 queen-laid eggs remained after 16 hours).

An apparent exception to the comparison of *Dolichovespula* and *Vespula* is *D. saxonica* which like *Vespula vulgaris* has worker policing (Foster and Ratnieks 2000, Chapter 8). Consideration of paternity within individual colonies, however, reveals that this is predicted by worker policing theory. Single paternity colonies have considerable worker male production, while multiple paternity colonies have very little (Figure 12.3). Observational data and ovary dissections further show that this pattern is not explained by differences in worker laying with if anything more worker laying in multiple paternity colonies. In single paternity colonies, workers laid 32/51 eggs (1 colony) and 8% (5/60) of workers had full sized eggs in their ovaries (3 colonies) while in multiple-paternity colonies 132/204 eggs were laid by workers (3 colonies) and 14% (14/100) of workers had activated ovaries (5 colonies). Egg *removal* in the multiple but not in the single-paternity colonies is therefore required to explain the absence of male production in the multiple paternity colonies. *D. saxonica* workers seem to facultatively police each other, only policing if they find themselves among low related siblings caused by multiple paternity (Foster and Ratnieks 2000, Chapter 8). The conflict over male production has been resolved uniquely in *D. saxonica*. Paternity in the other five *Dolichovespula* species is most similar to the *D. saxonica* colonies in which the workers dominate male production. However, in the other *Dolichovespula* species workers

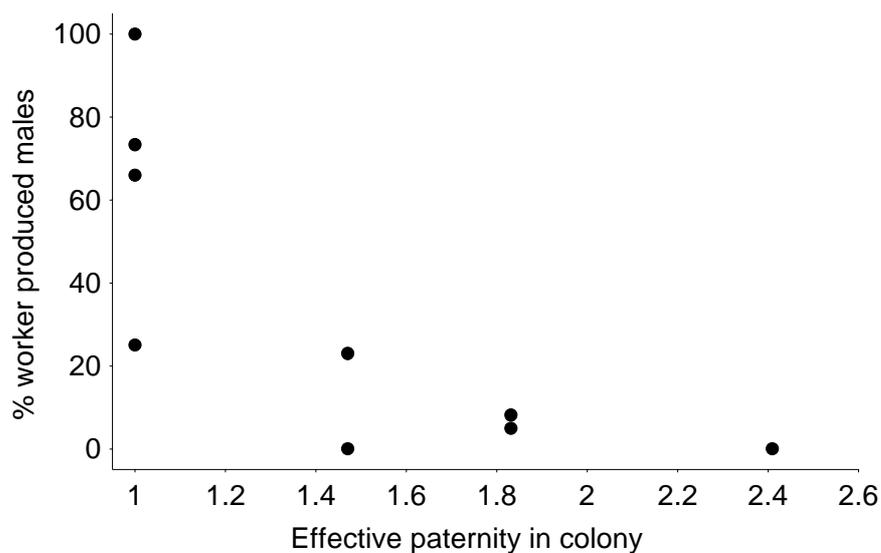


Figure 12.3. Paternity against worker male production in nine colonies of *Dolichovespula saxonica*, Spearman's rank correlation,  $P = 0.004$  (adapted from Foster and Ratnieks 2000, Chapter 8).

produce a minority of the males (Figure 12.1), more in line with the queens' interest. It is not clear why this should be the case. The effectiveness of queen policing may be an important variable. Queens remove worker eggs in *D. maculata* (Balduf 1954; Greene 1979), *D. arenaria* (Greene 1979), *D. saxonica* and *D. media* (Foster *et al.* 2001, Chapter 7) although there is not yet evidence that they remove less in *D. saxonica*. Worker policing may also be important if it has evolved in the other *Dolichovespula* species at a lower paternity than in *D. saxonica* and *V. vulgaris* (*c.f.* *Vespa crabro* below). Worker policing should now be tested for more widely in *Dolichovespula*.

Another exception to the contrast of *Dolichovespula* and *Vespula* is the *Vespula rufa* group (Figure 12.1) where worker laying in queenright colonies has been reported. Akre *et al.* (1982) observed several worker ovipositions in both of their observation colonies of *V. consobrina*. In addition, worker ovary activation (1% of workers), worker laying and overt queen-worker aggressions have been reported in *V. acadia* (Reed and Akre 1983). This may represent a different resolution to the conflict over male production than in the *Vespula vulgaris* and *squamosa* groups. However, no worker laying was seen in three colonies of *V. atropilosa* (Akre *et al.* 1976) which is a close relative of *V. consobrina* and *acadia*. Conflict resolution and paternity therefore may also vary *within* the *V. rufa* group. Paternity data from this group is currently restricted to one colony of *V. rufa*, which had multiple paternity (Figure 12.1).

In contrast to *Vespula* and *Dolichovespula*, data from the European hornet *Vespa crabro* are not consistent with relatedness predictions. Despite low paternity, there was no genetic evidence of workers' sons in 272 males from 14 queenright colonies or evidence of worker laying in 50 hours observation of four nests housed in glass-fronted boxes (Foster *et al.* 2000, Chapter 5). Furthermore, ovary dissection data from *V. crabro* (6/500 workers from 8 colonies had full sized eggs, Foster *et al.* 2000, Chapter 5) and *V. analis* (1/1062 in 16 colonies, Matsuura 1984) suggest that the level of worker ovary activation is similar to *Vespula* (Figure 12.1). Foster *et al.* (2000, Chapter 5) suggested that hornet queens might be exerting pheromonal control over the workers. This is supported by the occasional observation of royal courts in hornets (Nixon 1985a; Matsuura 1991), a phenomenon not seen in either *Dolichovespula* or *Vespula*. Recently, however, another explanation has emerged. Egg introduction experiments into queenright colonies have shown that as in *Vespula vulgaris*, *V. crabro* workers remove worker-laid eggs but leave the majority of queen-laid eggs (1/79 worker-laid eggs but 46/72 queen-laid eggs remained after 16 hours) (Foster *et al.* submitted, Chapter 6). This suggests that, rather than being under queen pheromonal control, workers are prevented from reproduction by mutual policing. Interestingly, this response was found to be facultative with workers in *queenless* colonies removing significantly more queen-laid eggs than worker-laid eggs (30/44 worker-laid eggs but 13/41 queen-laid eggs remained after 16 hours). This may represent an adaptation to counter queen parasitism (Foster *et al.* submitted, Chapter 6). The discovery of worker policing in the hornet with its very low paternity is extremely interesting because workers are killing their closer relatives (nephews) to favour more distant relatives (brothers). This apparent paradox can be explained if worker reproduction significantly reduces colony productivity (Ratnieks 1988; Ratnieks and Reeve 1992) or if worker policing lowers the cost of the conflict over sex allocation by reducing the energy lost through worker killing of males (Foster and Ratnieks submitted; see conclusions). However, it remains unclear why this should especially affect *Vespa* but not *Dolichovespula* where workers lay eggs and succeed in producing males in many colonies (Figure 12.1).

Table 12.2 Queen absence in reproductive vespine wasp nests. Only reproductive nests are considered because queen death prior to reproduction is assumed non-adaptive to workers as they receive no benefit from producing males prior to the production of gynes. A reproductive nest is defined as a nest with the large cells that are used to rear the majority of sexuals and/or sexual pupae. Data are referenced in Figure 12.1 except for 39 of the *V. vulgaris* colonies and the *V. germanica* colonies (Spradbery 1971).

Species	Effective paternity	Reproductive nests with no queen (%)	# colonies
<i>Vespa crabro</i>	1.11	14	14
<i>Dolichovespula maculata</i>	1.00	74	19
<i>Dolichovespula media</i>	1.08	32	19
<i>Dolichovespula sylvestris</i>	1.15	36	14
<i>Dolichovespula norvegica</i>	1.08	86	14
<i>Dolichovespula saxonica</i>	1.35	20	10
<i>Dolichovespula arenaria</i>	1.09	54	13
<i>Vespula germanica</i>	2.35	0	25
<i>Vespula vulgaris</i>	1.90	6	50

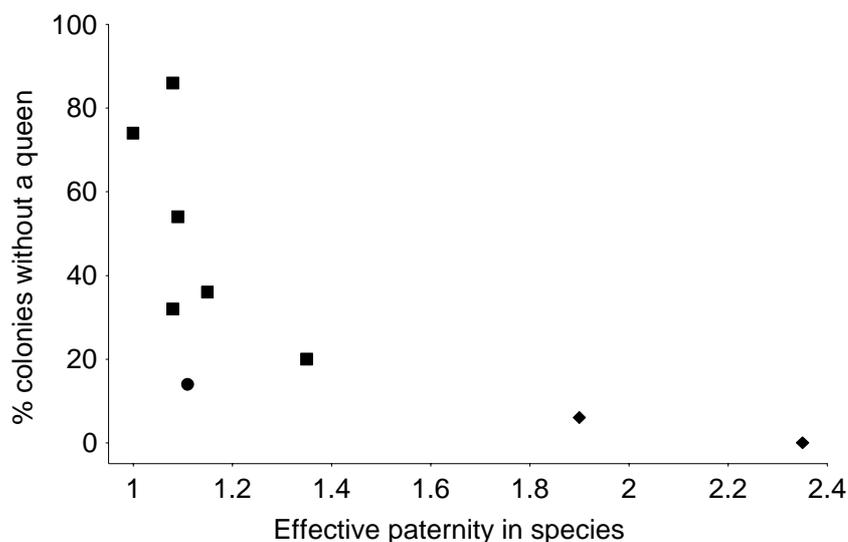


Figure 12.4. Effective paternity against queen loss in reproductive nests of nine vespine wasp species. Spearman's rank correlation,  $P = 0.005$ . The three genera are shown by different points: ● *Vespa*, ■ *Dolichovespula*, ◆ *Vespula*.

## 12.5 Matricide

Where the queen's presence reduces their reproduction, workers of annual societies may gain by killing the queen when she has produced enough workers and gynes (Ratnieks 1988; Bourke 1994) (if unmated, workers are unable to lay female eggs). There are several anecdotal reports of worker aggression towards the queen just preceding her death (Bourke 1994; Foster *et al.* 2000, Chapter 5). Matsuura (1984) reported that the royal court behaviour of *Vespa crabro* involves workers biting the queen which may kill

her. Janet (1895) also described a *V. crabro* worker biting the queen just before the queen's death and Ishay (1964) suggested that *V. orientalis* workers 'licked' the queen to death. Finally, *D. maculata* workers have been reported to kill their queen by stinging her (Akre *et al.* 1982). Despite such reports, there is no direct evidence that matricide is a general phenomenon in any one species. However, interspecific comparison in the vespine wasps suggests that matricide may be important. Kin selection theory predicts that matricide is most likely in low paternity species where the conflict between the queen and the workers over male production is strongest (Bourke 1994). This prediction is supported by a highly significant positive correlation between the percentage of queenright reproductive nests and paternity (Spearman's rank correlation,  $P = 0.005$ , Table 12.2, Figure 12.4). Furthermore, the data suggest a link between queen loss and male production conflict. Comparable levels of queen loss were found in *Vespa* and *Vespula*, which both have effectively sterile workers (Fisher's exact test on queen loss frequency between in *Vespula* versus *Vespa*,  $P = 0.17$ ). While in *Dolichovespula* where queenright worker reproduction is common, queen loss is also more common (*Dolichovespula* has significantly more queen loss than *Vespa*,  $P < 0.01$  and *Vespula*  $P < 0.001$ , Fisher's exact test.). This link between paternity, queen-worker conflict and queen death suggests that matricide may prove to be an important aspect of the conflict over male production in vespine species with low paternity.

## 12.6 Conflict over sex allocation

Increased paternity lowers the relatedness of workers to their sisters ( $0.75 > r > 0.25$ ) but not their brothers ( $r = 0.25$ ) and, therefore, is important in determining the optimum sex-allocation ratio of workers (Trivers and Hare 1976). Furthermore, variation in paternity between colonies is expected to result in split sex ratios, with high paternity colonies rearing more queens and low paternity colonies more males (Boomsma and Grafen 1990; 1991; Ratnieks 1991). This pattern has been found in the ants *Formica truncorum* (Sundström 1994a; 1994b) and *Formica exsecta* (Sundström *et al.* 1996). Facultative sex allocation biasing also occurs in response to the number of queens in colonies of the ants *Myrmica tahoensis* (Evans 1995) and *Leptothorax acervorum* (Chan and Bourke 1994) and five epiponine wasp species (Queller *et al.* 1993b; Hastings *et al.* 1998), in line with kin selection predictions. Tests of these predictions are difficult in the vespine wasps owing to their long reproductive period, which would require continuous sampling over several weeks to get a precise estimate of colony sex ratio. Consequently, there are few studies. However, F. L. W. Ratnieks and J. J. Boomsma (unpublished data) did find evidence of split sex ratios in *Dolichovespula arenaria*. They included sexuals and sexual pupae in the analysis and were able to show that the proportional investment in gynes versus males was significantly negatively correlated with paternity. It appears, therefore, that *D. arenaria* workers facultatively bias sex allocation in the same way that *D. saxonica* workers facultatively police. Whether both traits co-occur in either species has yet to be investigated, although F. L. W. Ratnieks and J. J. Boomsma (unpublished) were unable to find a link between paternity and worker male production in their data. The sex ratio in five species of Japanese hornet (Matsuura 1984) varied from 0.65 to 0.42 (proportion of females) and was also variable among colonies (standard deviations 0.21 to 0.33) further consistent with the existence of split sex ratios. Archer's (1980) estimates of sex ratio in the British vespines suggest that near equal numbers of females and males are produced. With the dry mass of males lower than that of females (Edwards 1980 on *V. vulgaris*) this suggests a degree of female bias in sex allocation in line with kin selection predictions (Trivers and Hare

1976). However, dry mass estimates of sex allocation are likely to be inaccurate (Boomsma 1989) and better estimates of the cost of male versus female production in vespines colonies are required.

## 12.7 Conflict between founding queens

Several interspecific social parasites threaten vespine wasp colonies. *Vespula austriaca*, *Dolichovespula arctica*, *D. adulterina* and *D. omissa* are all obligate social parasites that have no worker caste, and queens of *Vespula squamosa*, *Vespula pensylvanica* and *Vespa dybowski* can facultatively parasitise nests of other species in addition to starting their own colony (reviewed by Greene 1991; Matsuura and Yamane 1990). There is also increasing evidence of the importance of *intraspecific* competition between founding wasp queens. Innumerable reports have been made of queen take-overs within species of *Vespa*, *Dolichovespula* and *Vespula* (reviewed by Greene 1991; Matsuura and Yamane 1990; Matsuura 1991). However, such usurpation has been associated with colony failure (Nixon 1985b, 1986) bringing into question whether usurpation is often a successful strategy (Greene 1991). Recent genetic data suggests that it is. Two worker matrilines in nests with one queen were found in 2/33 *Vespa crabro* (Foster *et al.* 1999, Chapter 4; Foster *et al.* 2000, Chapter 5) 1/10 *D. sylvestris*, 1/10 *D. norvegica*, 1/10 *D. media* (Foster *et al.* 2001, Chapter 7) and 1/17 *Vespula vulgaris* nests (Foster and Ratnieks 2001, Chapter 9). With workers taking from 3-5 weeks to develop from egg to adult (Edwards 1980; Matsuura 1984), this suggests that the new queens have been successful in establishing themselves following usurpation. The frequency of successful usurpation seems low but such genetic data are likely to significantly underestimate usurpation frequency because the worker offspring of a first queen will die mid season (adult workers live for around one month). This means that the founding queen of usurped nests will be undetectable in the latter part of the season when samples for analysing colony reproduction are taken. It is possible therefore that a high proportion of successful vespine queens do not found their own nest.

## 12.8 Conclusions

The vespine wasps provide strong support for kin selection predictions. There is comparative evidence at the genus, species and colony level indicating the importance of kin structure in the resolution of reproductive conflict (Table 12.3). As expected however, as kinship and relatedness do not explain all of the patterns found. The data suggest that the relative *power* of conflicting parties (the queen versus the workers) and the *cost* of conflict expression are also important (Ratnieks and Reeve 1992). For example, relatedness predicts the queen-worker conflict found in *Dolichovespula*, but why the queens retains the majority of the power over male production remains unexplained. In addition, worker policing was found at a lower paternity than predicted in *Vespula vulgaris*, *Dolichovespula saxonica* and most notably *Vespa crabro*. Worker policing may be favoured at paternity below two if worker reproduction reduces colony productivity (Ratnieks 1988), which could occur if reproducing workers work less or interfere with the queen's reproduction. Alternatively, selection for worker policing at paternity below two may result from an interaction between the conflicts over male production and sex allocation (Foster and Ratnieks submitted, Chapter 10). In ants, it has been shown that workers perform sex allocation biasing by killing male *larvae* (e.g. Sundström *et al.* 1996), presumably because they cannot determine the sex of the

Table 12.3. Summary of data on kin selection predictions in the vespine wasps.

Conflict	Prediction	Consistent	Inconsistent
Male production	Worker policing should occur in species where effective paternity > 2	Low paternity and male production by workers in <i>Dolichovespula</i>	Low paternity and worker policing in <i>Vespa crabro</i>
		High paternity and worker policing in <i>Vespula</i>	
	Facultative worker policing in species with variable paternity	Facultative worker policing in <i>D. saxonica</i>	
	Matricide most favoured at low paternity	Correlation of queen loss and paternity across 9 species	
Sex allocation	Female bias	Female bias in 7 British vespine species	
	Facultative sex allocation biasing in species with variable paternity	Facultative sex allocation biasing in <i>D. arenaria</i>	

queen's eggs (Nonacs 1993). This favours worker policing of eggs because policing reduces the cost of male larvae killing by removing some of the males at very low cost at the egg stage. At a general level, the widespread occurrence of worker policing in the Vespinae indicates a central role for worker policing in their social evolution. This supports the hypothesis that mutual policing is a key mechanism of conflict suppression in biological systems (Frank 1995; Maynard-Smith and Szathmáry 1995; Keller 1999; Foster and Ratnieks 2001, Chapter 9).

Despite the similar biology and small size of the group, the vespine wasps show significant diversity in both kinship and conflict resolution. While high paternity appears restricted to *Vespula*, the conflict over male production has had similarly peaceful resolutions in *Vespula* and *Vespa*. *Dolichovespula* is different, with significant worker reproduction and queen-worker conflict in queenright colonies. *Dolichovespula saxonica* is different again in its mix of single and double paternity colonies (Figure 12.3) and facultative worker policing. In comparison to general biology, therefore, paternity and conflict resolution appear highly labile traits in the Vespinae. This is well illustrated by the comparison of *D. norwegica* and *D. saxonica*. These species are close relatives and can be difficult to distinguish morphologically (Else 1994) but have very different patterns of paternity and male production (Figure 12.1).

One important character in which the vespine wasps do display variation is colony size. Colony size is interesting because it can be viewed as both a cause and effect of reproductive conflict (Bourke 1999). For example, all *Vespula* in which paternity and male production have been studied are large-colony species whose colonies commonly have several thousands of workers at the reproductive stage (*V. vulgaris* and *squamosa* species groups). This compares to *Dolichovespula* where tens to hundreds are the norm (Matsuura and Yamane 1990; *D. arenaria* is an exception reaching 1000 workers, F. L. W. Ratnieks and J. J. Boomsma, unpublished data). It is possible therefore, that by removing costly conflict the evolution of efficient worker policing in *Vespula* has caused the increase in colony size. However, it is equally possible that the benefits of

worker policing increase in large colonies and favour its subsequent evolution. Distinguishing between these hypotheses is extremely difficult and it is likely that there is coevolution between the two (Bourke 1999). However, the discovery of worker policing in *Vespa*, where colony size is generally also larger than *Dolichovespula* (Matsuura and Yamane 1990) further suggests an association.

There are many avenues for future work into reproductive conflict in the vespine wasps. There are three major groups where data on paternity and reproduction are needed: *Vespa*, *Provespa* and the *Vespula rufa* group. *Provespa* are enigmatic nocturnal wasps with cryptic nests few of which have ever been found (Matsuura and Yamane 1990). They appear to have royal courts around their single queen, as occurs in *Vespa*. In contrast to all other vespines it has been suggested that they are swarm founding due to the extremely low numbers of queen cells found in mature nests (7 to 29 out of 913 to 2276 cells in six nests of *P. anomala*, Matsuura and Yamane 1990). The *Vespula rufa* group have small colonies comparable to *Dolichovespula* and significant amounts of worker laying in some species (see above). In the groups where paternity is known, further tests for worker policing behaviour are desirable. In addition, accurate colony-level data on sex allocation and paternity have only been collected in one species (*D. arenaria*, F. L. W. Ratnieks and J. J. Boomsma, unpublished data). Finally, it will be interesting to study paternity in the workerless social parasites, such as *Vespula austriaca*. With no workers, paternity can have no repercussions on worker behaviour making it interesting to compare paternity in the parasite with its non-parasitic relatives.

Although their covered nests and hostile temperament have made them inherently secretive, the study of the vespine wasps has proved highly fruitful. Within their societies, we have found novel and varied resolutions to reproductive conflict that provide new insights into kin selection theory.

## 12.9 Acknowledgements

This paper is dedicated to William D. Hamilton (1936-2000). We thank Michael Goodisman, Joan Strassmann, Tom Wenseleers and John Gulliver. This work was funded by a BBSRC studentship to KRF.

---

## References

- Akre RD, Reed HC, Landolt PJ (1982) Nesting biology and behavior of the blackjacket *Vespula consobrina* (Hymenoptera: Vespidae). *J Kans Entomol Soc* 55: 373-405
- 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
- Archer ME (1980) Population dynamics. In: *Social Wasps. Their Biology and Control* (Edwards R), pp172-207. Rentokil Limited, East Grinstead, UK
- Archer ME (1993) The life history and colonial characteristics of the hornet, *Vespa crabro* L. (Hym., Vespinae). *Entomol Mon Mag* 129: 151-163
- Arevalo E, Strassmann JE, Queller DC (1998) Conflicts of interest in social insects: male production in two species of *Polistes*. *Evolution* 52: 797-805
- Aron S, Vargo EL, Passera L (1995) Primary and secondary sex ratios in monogyne colonies of the fire ant. *Anim Behav* 49: 749-757
- Balduf WV (1954) Observations on the white-faced wasp *Dolichovespula maculata* (Linn.) (Vespidae, Hymenoptera). *Ann Entomol Soc Amer* 47: 455-458
- Barron A, Oldroyd BP, Ratnieks FLW. Worker policing and anarchy in *Apis*. Submitted.
- Batra SWT (1980) Sexual behavior and pheromones of the European hornet *Vespa crabro germana* (Hymenoptera: Vespidae). *J Kans Entomol Soc* 53: 461-469
- Begon M, Harper JL, Townsend CR (1990) *Ecology: individuals, populations and communities*. Blackwell, Oxford
- Bekkevold D, Frydenberg J, Boomsma JJ (1999) Multiple mating and facultative polygyny in the Panamanian leafcutter ant *Acromyrmex echinator*. *Behav Ecol Sociobiol* 46: 103-109
- Benford FA (1978) Fisher's theory of the sex ratio applied to the social Hymenoptera. *J Theor Biol* 72:701-727
- Boomsma JJ (1989) Sex-investment ratios in ants: has female bias been systematically overestimated? *Am Nat* 133: 517-532
- Boomsma JJ, Grafen A (1990) Intraspecific variation and the Trivers-Hare hypothesis. *Evolution* 44: 1026-1034
- Boomsma JJ, Grafen A (1991) Colony-level sex ratio selection in the eusocial Hymenoptera. *J Evol Biol* 3: 383-407
- Boomsma JJ, Ratnieks FLW (1996) Paternity in the eusocial Hymenoptera. *Phil Trans Roy Soc Lond B* 351: 941-975
- Boomsma JJ, Fjerdingstad EJ, Frydenberg J (1999) Multiple paternity, relatedness and genetic diversity in *Acromyrmex* leaf-cutter ants. *Proc Roy Soc London B* 266: 249-254
- Bourke AFG (1988) Worker reproduction in the higher eusocial Hymenoptera. *Q Rev Biol* 63: 291-311
- Bourke AFG (1994) Worker matricide in social bees and wasps. *J Theor Biol* 167: 283-292
- Bourke AFG (1999) Colony size, social complexity and reproductive conflict in social insects. *J Evol Biol* 12: 245-257
- Bourke AFG, Franks NR (1995) *Social Evolution in Ants*. Princeton University Press, Princeton, NJ
- Brothers DJ, Carpenter JM (1993) Phylogeny of Aculeata: Chrysidoidea and Vespoidea (Hymenoptera). *J Hym Res* 2: 227-304
- Carpenter JM (1987) Phylogenetic relationships and classification of the Vespinae (Hymenoptera: Vespidae). *Syst Entomol* 12: 413-431
- Chan GL, Bourke AFG (1994) Split sex-ratios in a multiple-queen ant population. *Proc R Soc Lond B* 258: 261-266

- Chapuisat M, Sundström L, Keller L (1997) Sex ratio regulation: the economics of fratricide in ants. *Proc Roy Soc London B* 264: 1255-1260
- Crozier RH, Pamilo P (1996) *Evolution of Social Insect Colonies*. Oxford University Press, Oxford
- Don RH, Cox PT, Wainwright BJ, Baker K, Mattick J S (1991) "Touchdown" PCR to circumvent spurious priming during gene amplification. *Nuc Ac Res* 19: 4008
- Edwards R. (1980) *Social Wasps. Their Biology and Control*. Rentokil limited, East Grinstead, West Sussex, UK
- Else GR (1994) Identification: Social Wasps. *British Wildlife*. 5: 304-311
- Emlen ST, Wrege PH (1988) The role of kinship in helping decisions among white-fronted bee-eaters. *Behav Ecol Sociobiol* 23: 305-15
- Estoup A, Solignac M, Cornuet J (1994) Precise assessment of the number of patriline and of genetic relatedness in honeybee colonies. *Proc R Soc Lond B* 258: 1-7
- Estoup A, Scholl A, Pouvreau A, and Solignac M (1995) Monoandry and polyandry in bumblebees (Hymenoptera: Bombinae) as evidenced by highly variable microsatellites. *Mol Ecol* 4: 89-93
- Evans JD (1995) Relatedness threshold for the production of female sexuals in colonies of a polygynous ant, *Myrmica tahoensis*, as revealed by microsatellite DNA analysis. *Proc Natl Acad Sci USA* 92: 6514-6517
- Field J, Solis CR, Queller DC, Strassmann JE (1998) Social and genetic structure of paper wasp cofoundress associations: tests of reproductive skew. *Am Nat* 151: 545-563
- Fisher RA (1930) *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.
- Foster KR, Ratnieks FLW (2000) Facultative worker policing in a social wasp. *Nature* 407: 692-693
- Foster KR, Ratnieks FLW (2001) Convergent evolution of worker policing by egg eating in the honey bee and common wasp. *Proc Roy Soc London B*, in press
- Foster KR, Ratnieks FLW The effect of sex allocation biasing on the evolution of worker policing in hymenopteran societies. Submitted
- Foster KR, Ratnieks FLW, Raybould AF (2000) Do hornets have zombie workers? *Mol Ecol* 9: 735-742
- Foster KR, Gulliver J, Ratnieks FLW Why workers do not reproduce: worker policing in the European hornet *Vespa crabro*. Submitted
- Foster KR, Ratnieks FLW, Gyllenstrand N, Thorén PA (2001) Colony kin structure and male production in *Dolichovespula* wasps. *Mol Ecol*, in press
- Foster KR, Seppä P, Ratnieks FLW, Thorén PA (1999) Low paternity in the hornet *Vespa crabro* indicates that multiple mating by queens is derived in vespine wasps. *Behav Ecol Sociobiol* 46: 252-257
- Frank SA (1995) Mutual policing and the repression of competition in the evolution of cooperative groups. *Nature* 377: 520-522
- Gadagkar R (1993) Can animals be spiteful? *Trends Ecol Evol* 8: 232-234
- Getz WM (1981) Genetically based kin recognition systems. *J Theor Biol* 92:209-226
- Gobin B, Billen J, Peeters C (1999) Policing behaviour towards virgin egg layers in a polygynous ponerine ant. *Anim Behav* 58: 1117-1122
- Goodisman MAD, Matthews RW, Crozier RH. Multiple mating and worker reproduction in the European wasp, *Vespula germanica* in Australia. Submitted
- Goodnight KF, Queller DC (1994) Relatedness 4.2. Goodnight Software, Houston, Texas
- Goodnight KF, Strassmann JE, Klinger CJ, Queller DC (1996) Single mating and its implications for kinship structure in a multiple-queen wasp, *Parachartergus colobopterus*. *Ethol Ecol Evol* 8:191-198
- Grafen A (1985) A geometric view of relatedness. *Oxford Surv Evol Biol* 2: 28-89
- Greene A (1979) Behavioural characters as indicators of yellowjacket phylogeny (Hymenoptera: Vespidae). *Ann Entomol Soc Amer* 72: 614-619
- Greene A (1991) *Dolichovespula* and *Vespula*. In: *The Social Biology of Wasps* (eds Ross KG, Matthews RW), pp 263-304. Cornell University Press, Ithaca, NY
- Greene A, Akre RD, Landolt P (1976) The aerial yellowjacket *Dolichovespula arenaria* (Fab.): nesting biology, reproductive production, and behaviour (Hymenoptera: Vespidae). *Melandieria* 26: 1-34

- Grosberg RK, Levitan DR, Cameron BB (1996) Evolutionary genetics of allorecognition in the colonial hydroid *Hydractinia symbiolongicarpus*. *Evolution* 50: 2221-2240
- Haig D, Grafen A (1991) Genetic scrambling as a defence against meiotic drive. *J Theor Biol* 153: 531-558
- Hamilton, WD (1964) The genetical evolution of social behavior I and II. *J Theor Biol* 7: 1-52
- Hamilton WD (1970) Selfish and spiteful behaviour in an evolutionary model. *Nature* 228: 1218-1220
- Hamilton W D (1971) Selection of selfish and altruistic behaviour in some extreme models. In: Eisenberg JF, Dillon WS (eds) *Man and Beast: Comparative Social Behavior*, Smithsonian Press, Washington DC pp 57-91
- Hastings MD, Queller DC, Eischen F, Strassmann JE (1998) Kin selection, relatedness, and worker control of reproduction in a large-colony epiponine wasp, *Brachygastra mellifica*. *Behav Ecol* 9: 573-581
- Heinze J, Puchinger W, Hölldobler B (1997) Worker reproduction and social hierarchies in *Leptothorax* ants. *Anim Behav* 54: 849-864
- Hölldobler B, Bartz, SH (1985) Sociobiology of reproduction in ants. In: Hölldobler B, Lindauer M (eds) *Experimental Behavioural Ecology and Sociobiology*, Gustav Fischer Verlag, Stuttgart pp 237-257
- Honk CGJ van, Röseler PF, Velthuis HHW, Hoogeveen JC (1981) Factors influencing the egg laying of workers in a captive *Bombus terrestris* colony. *Behav Ecol Sociobiol* 9: 9-14
- Hurst GDD, McVean GAT (1998) Selfish genes in a social insect. *Trends Ecol Evol* 13: 434-435
- Hurst LD, Pomiankowski A (1991) Maintaining Mendelism: might prevention be better than cure? *BioEssays* 13: 489-490
- Hurst LD, Atlan A, Bengtsson BO (1996) Genetic conflicts. *Q Rev Biol* 71: 317-364
- Ikan R, Gottlieb R, Bergmann ED, Ishay J (1969) The pheromone of the queen of the oriental hornet, *Vespa orientalis*. *J Insect Physiol* 15: 1709-1712
- Ishay J (1964) Observations sur la biologie de la guêpe orientale *Vespa orientalis* F. *Insectes Soc* 3: 193-206
- Janet C (1895) Etudes sur les Fourmis, les Guêpes et les Abeilles. 9<sup>th</sup> note. Sur *Vespa crabro* L. Histoire d'un Nid depuis son origine. *Mém Soc Zool France* 8: 1-140
- Keller L (1997) Indiscriminate altruism: unduly nice parents and siblings. *Trends Ecol Evol* 12: 99-103
- Keller L (1999) *Levels of Selection in Evolution*. Princeton University Press, Princeton, NJ
- Keller L, Nonacs P (1993) The role of queen pheromones in social insects: queen control or queen signal? *Anim Behav*, 45: 787-794
- Keller L, Reeve HK (1999) Dynamics of conflicts within insect societies. In: Keller L (ed) *Levels of Selection in Evolution*. Princeton University Press, Princeton, NJ, pp 153-175
- Keller L, Ross KG (1998) Selfish genes: a green beard in the red fire ant. *Nature* 94: 573-575
- Keller L, Vargo EL (1993) Reproductive structure and reproductive roles in colonies of social insects. In: Keller L (ed) *Queen Number and Sociality in Insects*, Oxford University Press, Oxford pp 16-44
- Keller L, Milinski M, Frischknecht M, Perrin N, Richner H, Tripet F (1994) Spiteful animals still to be discovered. *Trends Ecol Evol* 9: 103
- Kerr WE (1969) Some aspects of the evolution of social bees (Apidae). *Evol Biol* 3: 119-175
- Kikuta N, Tsuji K (1999) Queen and worker policing in the monogynous and monandrous ant, *Diacamma* sp. *Behav Ecol Sociobiol* 46: 180-189
- Landolt PJ, Akre RD, Greene A (1977) Effects of colony division on *Vespula atropilosa* (Sladen) (Hymenoptera: Vespidae). *J Kans Entomol Soc* 50: 135-147
- Leigh EGJ (1977) How does selection reconcile individual advantage with the good of the group? *Proc Nat Acad Sci USA* 74: 4542-4546
- Leigh EGJ (1991) Genes, bees and ecosystems: The evolution of a common interest among individuals. *Trends Ecol Evol* 6: 257-262
- Leigh EGJ (1999) Levels of selection, potential conflicts, and their resolution: the role of the "common good". In: Keller L (ed) *Levels of Selection in Evolution*. Princeton University Press, Princeton, NJ, pp 15-30

- Liebig J, Peeters C, Hölldobler B (1999) Worker policing limits the number of reproductives in a ponerine ant. *Proc R Soc Lond B* 266: 1865-1870
- Lythgoe A, Read AF (1999) Catching the Red Queen? The advice of the Rose. *Trends Ecol Evol* 13: 473-474
- Martin SJ (1990) Queenless nests of *Vespa simillima* SMITH (Hymenoptera, Vespidae). *Jpn J Entomol* 58: 347-354
- Matsuura M (1984) Comparative biology of the five Japanese species of the genus *Vespa* (Hymenoptera, Vespidae). *Bull Fac Agric Mie Univ* 69: 1-131
- Matsuura M (1991) *Vespa* and *Provespa*. In: Ross KG, Matthews, RW (eds) *The Social Biology of Wasps*. Cornell University Press, Ithaca, NY, pp 232-262
- Matsuura M, Yamane S (1990) *Biology of the Vespine Wasps*. Springer-Verlag, Berlin
- Maynard Smith J (1964) Group selection and kin selection. *Nature* 201:1145-1147
- Maynard Smith J, Szathmáry E (1995) *The Major Transitions in Evolution*. Freeman, NY
- Michod RE (1997) Evolution of the individual. *Am Nat* 150: S5-S21
- Michod RE (1999) *Darwinian Dynamics. Evolutionary Transitions in Fitness and Individuality*. Princeton University Press, Princeton, NJ
- Miyano S, Hasegawa E (1998) Genetic structure of the first brood of workers and mating frequency of queens in a Japanese paper wasp, *Polistes chinensis antennalis*. *Ethol Ecol Evol* 10: 79-85
- Monnin, T. & Ratnieks, F. L. W. Policing in queenless ponerine ants. Submitted
- Nixon GEJ (1982) Colony failure in the hornet *Vespa crabro* L. (Hym.; Vespidae). *Entomol Mon Mag* 117: 241-244
- Nixon GEJ (1983) Notes on colony failure and the phenomenon of usurpation in the hornet, *Vespa crabro* L. (Hym., Vespidae). *Entomol Mon Mag* 119: 1-10
- Nixon GEJ (1985a) Secondary nests in the hornet, *Vespa crabro* L. (Hym., Vespidae). *Entomol Mon Mag* 121: 189-198
- Nixon GEJ (1985b) The phenomenon of usurpation in certain Vespidae, especially *Vespula vulgaris* L. (Hym.). *Entomol Mon Mag* 121: 145-148
- Nixon GEJ (1986) Piratical behavior in queens of the hornet *Vespa crabro* L. (Hym.) in England. *Entomol Mon Mag* 122: 233-238
- Nonacs P (1993) Male parentage and sexual deception in the social Hymenoptera. In Wrensch DL, Ebbert MA (eds) *Evolution and Diversity of Sex Ratio in Insects and Mites*. Chapman and Hall, NY pp 384-401
- Nonac P, Carlin NF (1990) When can ants discriminate the sex of brood? A new aspect of queen-worker conflict. *Proc Natl Acad Sci USA* 87, 9670-9673
- Owen RE, Plowright RC (1982) Worker-queen conflict and male parentage in bumble bees. *Beh Ecol Sociobiol* 11: 91-99
- Packer C, Gilbert DA, Pusey AE, O'Brien SJ (1991) A molecular genetic analysis of kinship and cooperation in African lions. *Nature* 351: 562-565
- Page RE (1980) The evolution of multiple mating behaviour by honey bee queens (*Apis mellifera* L.). *Genetics* 96: 263-273
- Page RE (1986) Sperm utilisation in social insects. *Annu Rev Entomol* 31: 297-320
- Pamilo P (1982a) Multiple mating in *Formica* ants. *Hereditas* 97: 37-45
- Pamilo P (1982b) Genetic population structure in polygynous *Formica* ants. *Heredity* 48: 95-106
- Pamilo P (1984) Genotypic correlation and regression in social groups: multiple alleles, multiple loci and subdivided populations. *Genetics* 107: 307-320
- Pamilo P (1985) Effect of inbreeding on genetic relatedness. *Hereditas* 103: 195-200
- Pamilo, P (1990) Sex allocation and queenworker conflict in polygynous ants. *Behav Ecol Sociobiol* 27: 31-36
- Pamilo P (1991a) Evolution of colony characters in social insects. I. Sex allocation. *Am Nat* 137: 83-107
- Pamilo P (1991b) Evolution in colony characteristics in social insects. II. Number of reproducing individuals. *Am Nat* 138: 412-433

- Pamilo P (1993) Polyandry and allele frequency differences between the sexes in the ant *Formica aquilonia*. *Heredity*, 70: 472-480
- Passera L, Aron S (1996) Early sex discrimination and male brood elimination by workers of the Argentine ant. *Proc R Soc Lond B* 263: 1041-1046
- Pedersen JS, Boomsma JJ (1999) Multiple paternity in social Hymenoptera: estimating the effective mate number in single-double mating populations. *Mol Ecol* 8: 577-587
- Peters JM, Queller DC, Strassmann JE, Solis CR (1995) Maternity assignment and queen replacement in a social wasp. *Proc R Soc Lond B* 260: 7-12
- Peters JM, Queller DC, Imperatriz-Fonseca VC, Roubik DW, Strassmann JE (1999) Mate number, kin selection and social conflicts in stingless bees and honeybees. *Proc R Soc Lond B* 266: 379-384
- Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* 43: 258-275
- Queller DC, Strassmann JE, Hughes CR (1993a) Microsatellites and kinship. *Trends Ecol Evol* 8: 285-288
- Queller DC, Strassmann JE, Solis CR, Hughes CR, Moralez DeLoach D (1993b) A selfish strategy of social insect workers that promotes social cohesion. *Nature* 365: 639-641
- Ratnieks FLW (1988) Reproductive harmony via mutual policing by workers in eusocial Hymenoptera. *Am Nat* 132: 217-236
- Ratnieks FLW (1990a) Worker policing in social insects. In: Veeresh GK, Mallik B, Viraktamath CA (eds) *Social insects and the environment*. (Proceedings of the 12th congress of IUSSI). Oxford, New Delhi pp 365-366
- Ratnieks FLW (1990b) The evolution of polyandry by queens in social Hymenoptera: the significance of the timing of the removal of diploid males. *Behav Ecol Sociobiol* 26: 343-348
- Ratnieks FLW (1991) Facultative sex allocation biasing by workers in social Hymenoptera. *Evolution* 24: 281-292
- Ratnieks FLW (1993) Egg-laying, egg-removal, and ovary development by workers in queenright honeybee colonies. *Behav Ecol Sociobiol* 32: 191-198
- Ratnieks FLW, Miller DG (1993) Two polygyne overwintered nests of *Vespula vulgaris* from California. *Psyche* 100: 43-50
- Ratnieks FLW, Reeve HK (1992) Conflict in single queen Hymenopteran societies: the structure of conflict and processes that reduce conflict in advanced eusocial species. *J Theor Biol* 158: 33-65
- Ratnieks FLW, Visscher PK (1989) Worker policing in the honeybee. *Nature* 342: 796-797
- Reed HC, Akre RD (1983) Comparative colony behavior of the forest yellowjacket, *Vespula acadica* (Sladen) (Hymenoptera: Vespidae). *J Kansas Entomol Soc* 56: 581-606
- Ridley M (1993) *The Red Queen. Sex and the Evolution of Human Nature*. Penguin Books, London
- Ross KG (1985) Aspects of worker reproduction in four social wasp species (Insecta: Hymenoptera: Vespidae). *J Zool Lond (A)* 205: 411-424
- Ross KG (1986) Kin selection and the problem of sperm utilisation in social insects. *Nature* 323: 798-800
- Ross KG, Matthews RW (1991) *The Social Biology of Wasps*. Cornell University Press, Ithaca, NY
- Ross KG, Vargo EL, Fletcher DJC (1988) Colony genetic structure and queen mating frequency in fire ants of the subgenus *Solenopsis* (Hymenoptera: Formicidae). *Biol J Linn Soc* 34: 105-117
- Sakagami SF (1954) Occurrence of an aggressive behaviour in queenless hives, with considerations on the social organisation of the honeybee. *Insectes Soc* 1: 331-343.
- Sakagami SF (1982) Stingless bees. In: Hermann HR (ed) *Social insects*, vol. 3. Academic Press, NY pp 361-423
- Schmid-Hempel R, Schmid-Hempel P (2000) Female mating frequencies in *Bombus spp.* from Central Europe. *Insectes Soc* 47: 36-41
- Seeley TD (1985) *Honeybee Ecology. A Study of Adaptation in Social Life*. Princeton University Press, Princeton, NJ
- Spradbery JP (1971) Seasonal changes in the population structure of wasp colonies (Hymenoptera: Vespidae). *J Anim Ecol* 40: 501-523

- Starr CK (1984) Sperm competition, kinship, and sociality in the Aculeate Hymenoptera. In: Smith RL (ed) Sperm Competition and the Evolution of Animal Mating Systems. Academic Press, Orlando, Florida pp 427-464
- Sundström L (1994a) Sex ratio bias, relatedness asymmetry and queen mating frequency in ants. *Nature* 367: 266-268
- Sundström L (1994b) Ant sex ratios. *Nature* 370: 257-258
- Sundström L, Chapuisat M, Keller L (1996) Conditional manipulation of sex ratios by ant workers: a test of kin selection theory. *Science* 274: 993-995
- Thorén PA (1998) Mating Structure and Nestmate Relatedness in Primitively Social Hymenoptera as Revealed by Microsatellites. Ph.D. Thesis, Uppsala University
- Thorén PA, Paxton RJ, Estoup A (1995) Unusually high frequency of (CT)<sub>n</sub> and (GT)<sub>n</sub> microsatellite loci in a yellowjacket wasp, *Vespula rufa* (L.) (Hymenoptera: Vespidae). *Ins Mol Biol* 4: 141-148
- Trivers RL, Hare H (1976) Haplodiploidy and the evolution of the social insects. *Science* 191: 249-263
- Tsuchida AK (1994) Genetic relatedness and the breeding structure of the Japanese paper wasp, *Polistes jadwigae*. *Ethol Ecol Evol* 6: 237-242
- Turillazzi S, West-Eberhard MJ (1996) Natural history and evolution of paper wasps. Oxford University Press, Oxford
- Villesen P, Gertsch PJ, Frydenberg J, Mueller UG, Boomsma JJ (1999) Evolutionary transition from single to multiple mating in fungus-growing ants. *Mol Ecol* 8: 1819-1825
- Visscher PK (1989) A quantitative study of worker reproduction in honeybee colonies. *Behav Ecol Sociobiol* 25: 247-254
- Visscher PK (1996) Reproductive conflict in honeybees: a stalemate of worker egg-laying and policing. *Behav Ecol Sociobiol* 39: 237-244
- Visscher PK, Dukas R (1995) Honeybees recognise development of nestmates' ovaries. *Anim Behav* 49: 542-544
- Walin L, Sundström L, Seppä P, Rosengren R (1998) Worker reproduction in ants - a genetic analysis. *Heredity* 81: 604-612
- West-Eberhard MJ (1981) Intragroup selection and the Evolution of insect societies. In: Alexander RD, Tinkle DW (eds) *Natural Selection and Social Behaviour*. Chiron Press, NY pp 3-17
- Wheeler WM (1911) The ant colony as an organism. *J Morphol* 22: 307-325
- Wilson DS, Sober E (1989) Reviving the superorganism. *J Theor Biol* 136: 337-356
- Wilson EO (1971) *The Insect Societies*. Harvard University Press, Cambridge, MA
- Wilson EO (1975) *Sociobiology: The New Synthesis*. Harvard University Press, Cambridge, MA
- Wilson EO (1985) The sociogenesis of insect colonies. *Science* 228: 1489-1495
- Woyciechowki M, Lomnicki A (1987) Multiple mating of queens and the sterility of workers among eusocial Hymenoptera. *J Theor Biol* 128: 317-327