

## Male Production: Predictions & Tests

Francis L. W. Ratnieks

Social Insects: C1139

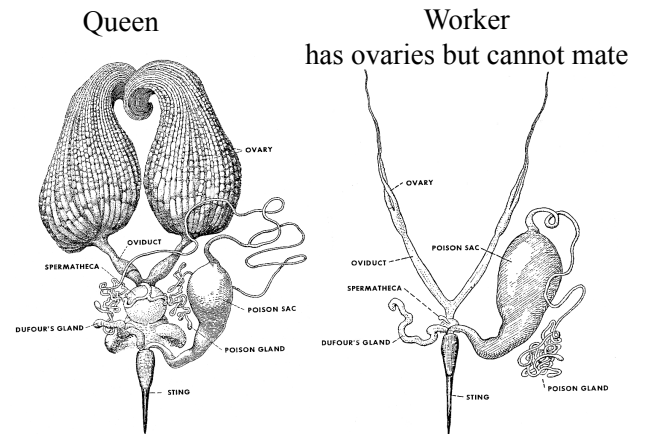
Laboratory of Apiculture & Social Insects  
Department of Biological & Environmental Science  
University of Sussex

## Whose Sons To Rear?

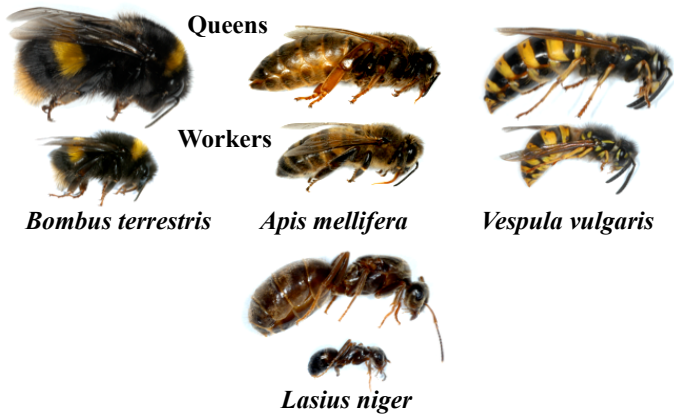
### Queen Honey Bee Laying an Egg



### Honey Bee: Female Reproductive Systems



### Workers With Ovaries but Cannot Mate



### Queenless Honey bee Colony with Worker-Laid Eggs



## Male Production by Workers is Rare

### Queenright honey bee colonies

Only 0.1% males are workers' sons

Visscher 1989 Behav Ecol Sociobiol

By using a body colour marker caused by the cordovan recessive gene, Visscher was able to visually screen thousands of males reared in queenright honey bee colonies. The results showed that approximately one male per thousand was a worker's son.

He set up colonies that were cc (queen) x C,C,C,C,C,C....C (males). The workers were all Cc, meaning that half the workers' sons were c (cordovan: pale colour) and half C (normal). Queens' sons were all c (cordovan: pale colour).

## Why Don't Workers Produce Males?

### More related to sons than brothers

1.0 v 0.5 regression relatedness

0.5 v 0.25 life for life relatedness

Ratnieks 1988 Am Nat

The fact that only 0.1% of the males are workers' sons seems to go against what we would expect from inclusive fitness theory, because (due to haplodiploidy) a worker bee is more related to sons than to brothers.

## Intracolony Conflict Over Male Rearing

### Regression Relatedness of

To sons of	Queen	Worker 1	Worker 2
Queen	1.0	0.5	0.5
Worker 1	0.5	1.0	0.25-0.75
Worker 2	0.5	0.25-0.75	1.0

Each female in the colony is more related to her own sons (1) than to the sons of other females. Workers are related to the sons of half sisters by 0.25 and sons of full sisters by 0.75. (Life for life relatedness values are just half the above values.)

## Why So Few Workers' Sons in Honey Bees?

## Worker Policing

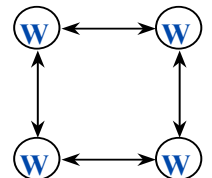
### Hypothesis

Workers prevent each other from reproducing

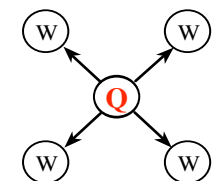
Ratnieks 1988 Am Nat

## Could Worker Policing be Effective?

Worker Policing  
*many against many*



Queen Policing  
*one against many*



Ratnieks 1988 Am Nat

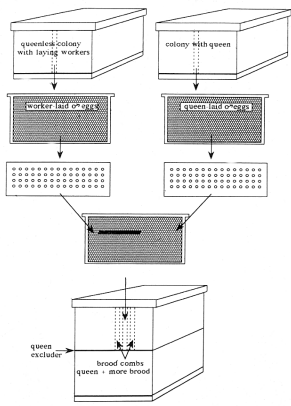


## Could Worker Policing be Effective?

In many species with small colonies, such as bumble bees or *Polistes* or wasps, the queen tries to stop workers from reproducing by killing worker-laid eggs or by aggression. A single queen can be quite effective if the nest is small and with few workers. But it would not be possible for such “queen policing” to be effective in large colonies, such as that of the honey bee, *Apis mellifera*, with up to 50,000 workers.

However, worker policing could be effective in a large colony. Worker policing is a mutual inhibition. The more workers there are, the more there are to stop each other reproducing.

## Policing Bioassay with Discriminator Colony



### 1. Obtain Eggs

Egg source colonies  
Queenright (queen-laid eggs)  
Queenless (worker-laid eggs)

### 2. Transfer Eggs

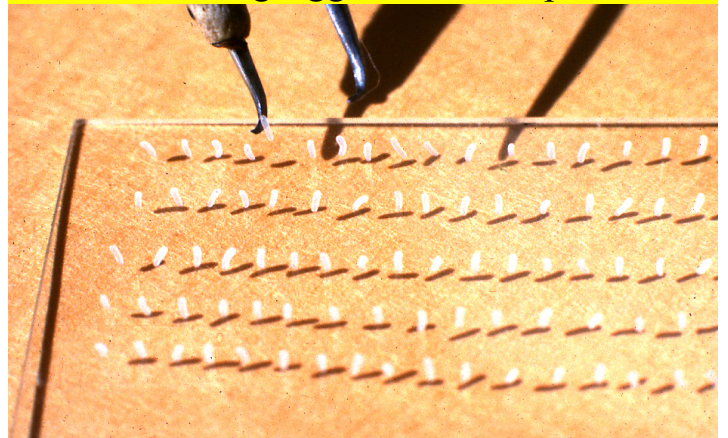
Harvest eggs and place on slide.  
Transfer eggs with forceps into adjacent drone cells.

### 3. Compare Egg Acceptance

Place comb into “discriminator”  
hive between two frames of brood  
above the queen excluder. Check at  
intervals.

## Discovery of Worker Policing in the Honey Bee

## Moving Eggs with Forceps

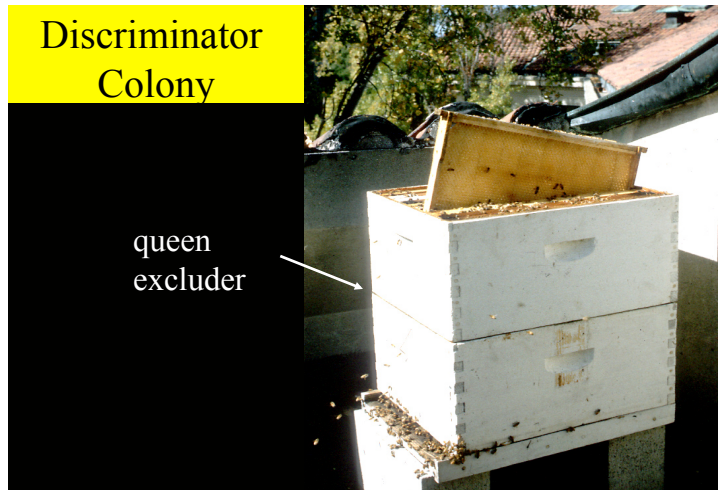


## Eggs Placed into Drone Cells by Hand



## Discriminator Colony

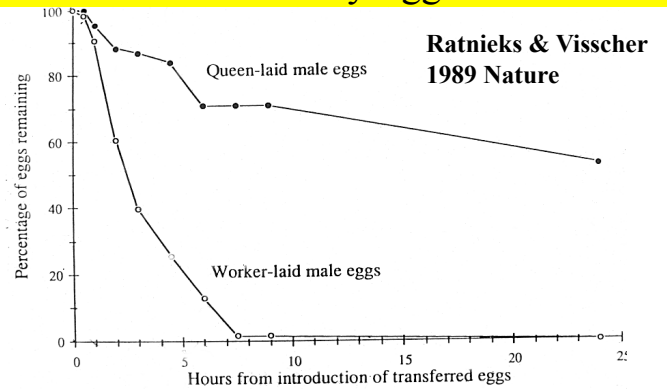
queen  
excluder



One Hour Later Many Eggs Have Gone



One Hour Later Many Eggs have Gone



All study colonies (n = 15) discriminated against worker-laid eggs

Worker Bee Inspecting Worker-Laid Egg



Worker Bee Killing Worker-Laid Egg



How Widespread in  
Worker Policing By Egg  
Eating?

How Widespread is Worker Policing?

Bees

*Apis mellifera*

Ratnieks & Visscher (1989, Nature)...and many others

*Apis cerana*

Oldroyd et al. (2001, Behav Ecol Sociobiol)

*Apis florea*

Halling et al. (2001, Behav Ecol Sociobiol)



## How Widespread is Worker Policing?

### Wasps

*Polistes chinensis*

Tsuchida (2005, Proc Roy Soc B)

*Vespa crabro*

Foster, Gulliver, Ratnieks (2002, Insectes Sociaux)

*Dolichovespula saxonica*

Foster & Ratnieks (2000, Nature)

*D. sylvestris*

Wenseleers, Tofilski, Ratnieks et al. (Behav Ecol 2005)

*D. norwegica*

Wenseleers, Tofilski, Ratnieks et al. (in preparation)

*Vespula vulgaris*

Foster & Ratnieks (2001, Proc Roy Soc B)

*V. rufa*

Wenseleers, Tofilski, Ratnieks et al. (Evolution, 2005)

## How Widespread is Worker Policing?

### Ants

*Diacamma sp.*

Kikuta & Tsuji (1999, Behav Ecol Sociobiol)

*Formica fusca*

Helantera & Sundström (2005, J Evol Biol in press)

*Pachycondyla inversa*

D'Ettore, Heinze & Ratnieks (2004, Proc Roy Soc B)

*Camponotus floridanus*

Endler et al. (2004, PNAS)

## Conditions that Favour Worker Policing of Worker-Laid Eggs

### Effect of Relatedness

- 1 sons (1) > workers' sons (0.75) > queen's sons (0.5)
- 2 sons (1) > workers' sons (0.5) = queen's sons (0.5)
- 10 sons (1) > queen's sons (0.5) > workers' sons (0.3)

The table above gives the relatedness of a worker to her sons, to the sons of other workers (nephews), and to the sons of the queen (brothers) when the queen is mated to 1, 2, or 10 males (assume equal sperm use).

Note that a worker is always most related to sons. But her number two choice changes. With single mating, nephews are more related than brothers. With multiple mating brothers are more related than nephews. With double mating they are equal.

Multiple mating has this effect because with multiple mating most workers are half sisters not full sisters.

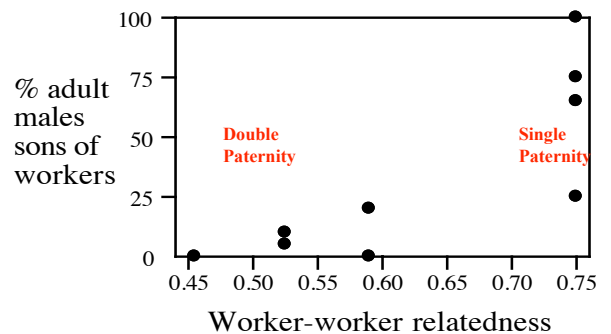
The implication is that worker policing will be favoured by multiple mating. Workers will have an incentive to lay eggs, but also to stop other workers from reproducing.

### Dolichovespula saxonica

Worker laying egg

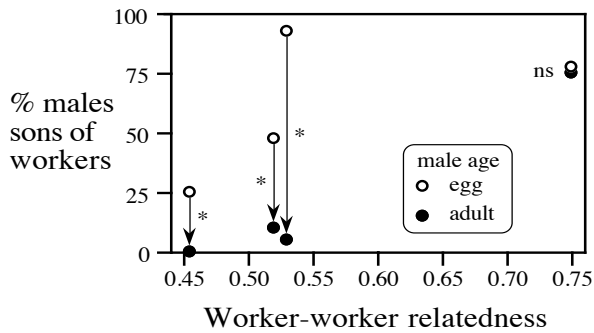


### Male Production in D. saxonica



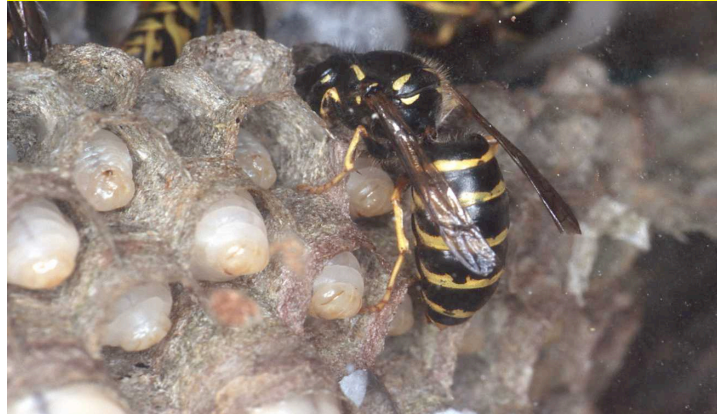
Foster & Ratnieks 2000 Nature

## Male Production in *D. saxonica*

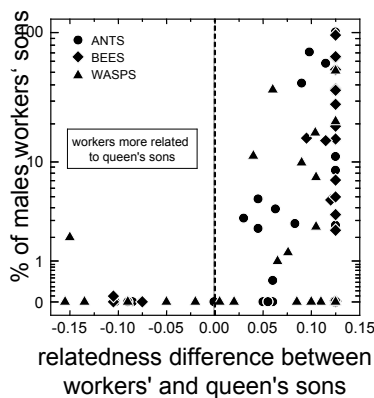


Foster & Ratnieks 2000 Nature

## Worker Policing in *D. saxonica*



## Comparative Study



## Comparative Study

The previous slide summarizes data on male production by workers in colonies with a queen and relatedness of workers to other workers' sons versus queen's (or in a few species queens') sons from nearly 90 species of bees, wasps and ants. To the left of the dotted line, workers are more related to queen's sons than other workers' sons. In these species very few of the males are workers' sons, supporting inclusive fitness predictions. To the right of the dotted line, workers are less related to queen's sons than other workers' sons. In these species a greater proportion of the males are workers' sons. This comparison is highly significant ( $p = 0.000000001$ ) and supports inclusive fitness predictions.

Wenseleers, T., Ratnieks, F. L. W. 2006. Comparative analysis of worker policing and reproduction in eusocial Hymenoptera supports relatedness theory. *American Naturalist* 168: E164-E179.

The abstract of this paper is on the next slide.

## Comparative Study

In many bees, wasps and ants, workers police each other in order to prevent individual workers from selfishly producing their own male offspring. Although several factors can selectively favour worker policing, genetic relatedness is considered to be of special importance. In particular, kin selection theory predicts that worker policing should be more common in species where the workers are more related to the queen's sons than other workers' sons. Here we provide strong novel support for this theory based on a comparative analysis of policing and male parentage in 109 species of ants, bees and wasps. First, an analysis of behavioural data confirms that worker policing occurs more frequently in species where workers are more related to the queen's sons (WP present in 7/7=100% of the species) than in species where they are more or equally related to other workers' offspring (WP present in 7/34=21% of the species). Second, an analysis of male parentage shows that, as predicted, a significantly higher percentage of the males are workers' sons in 75 species where the workers are more related to other workers' sons (avg. 14%) than in 15 species where they are more related to the queen's sons (avg. 0.14%). The correlations between relatedness and male parentage and the incidence of worker policing are also highly significant when phylogenetic non-independence is controlled for. Although our analysis provides strong overall support for the theory that the colony kin structure affects male parentage patterns, there is also significant unexplained variation. Several factors that may cause this residual variation are discussed.

## Comparative Study: Worker Policing

### Analysis of Behavioural Data

**In species in which workers are:**

**More related to queen's sons than workers' sons**  
 • worker policing occurs in 100% species (n = 7/7)

**Not more related to queen's than workers' sons**  
 • worker policing occurs in 21% species (n = 7/34)

p worker policing = 0.00015

p queen policing = 0.0009

p negative correlation queen and worker policing = 0.00009

## Comparative Study: Queen Policing

### Analysis of Behavioural Data

In species in which workers are:

**More related to queen's sons than workers' sons**

- worker policing occurs in 100% species (n = 7/7)
- queen policing occurs in 0% species (n = 0/4)

**Not more related to queen's than workers' sons**

- worker policing occurs in 21% species (n = 7/34)
- queen policing occurs in 91% species (n = 30/34)

p worker policing = 0.00015

p queen policing = 0.0009

p negative correlation queen and worker policing = 0.00009

## Consequences of Policing: Evasion

### Evasion: Non-Policed Worker Eggs



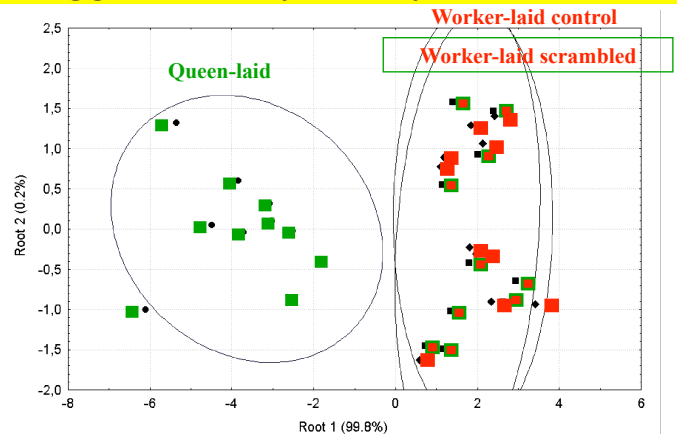
### Cue Scrambling: *Pachycondyla inversa*



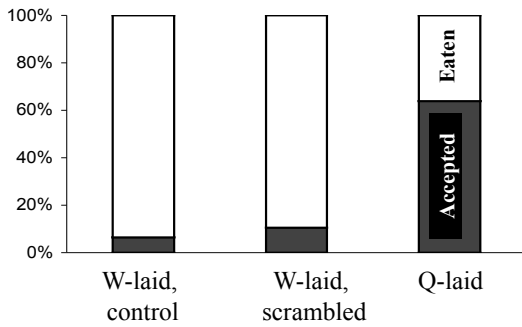
### Experimental Egg Pile



### Egg Chemistry: 30 Hydrocarbons



## Egg Acceptance: *Pachycondyla inversa*



## Consequences of Policing: Acquiescence



## Acquiescence

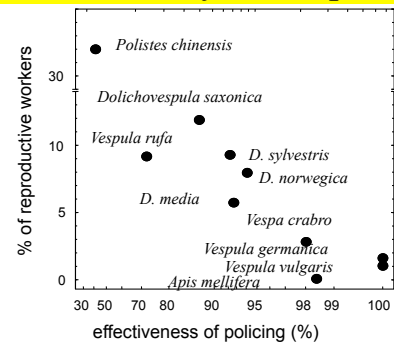
Policing can solve crimes (like Sherlock Holmes).  
But policing can also deter crime (which is what a patrolling Bobby is supposed to do).

So, if policing is effective, why try to reproduce?

- All honey bee workers have ovaries
- But only 0.1% - 0.01% lay eggs

Does better policing lead to greater altruism?

## Comparative Study of Acquiescence



This comparative study of 9 species of wasps and the honey bee shows that when policing is more effective (% of worker-laid eggs killed), fewer workers lay eggs. In other words, policing deters antisocial acts. Wenseleers, T., Ratnieks, F. L. W. 2006. Enforced altruism in insect societies. *Nature* 444: 50



## Comparative Study of Acquiescence

phylogeny	species	% of egg-laying workers in		relatedness among workers	effectiveness of policing (%)			type of policing	queen-worker dimorphism
		queenright colonies	queenless colonies		method 1	method 2	avg.		
	<i>Apis mellifera</i>	0.07%	36.4%	0.30	98.5%	98.4%	<b>98.4%</b>	W	1.38
	<i>Polistes chinensis</i>	32.0%	26.0%	0.75	41.4%	<b>41.4%</b>	Q+W	1.00	
	<i>Vespa crabro</i>	2.6%	11.6%	0.68	98.0%	<b>98.0%</b>	W	1.17	
	<i>Dolichovespula media</i>	5.7%	11.3%	0.71	92.6%	<b>92.6%</b>	Q	1.33	
	<i>Dolichovespula saxonica</i>	11.9%	12.1%	0.62	87.5%	<b>87.5%</b>	Q+W	1.32	
	<i>Dolichovespula sylvestris</i>	9.3%	14.3%	0.68	90.3%	94.1%	<b>92.2%</b>	Q+W	1.37
	<i>Dolichovespula norvegica</i>	8.0%	7.9%	0.71	94.1%	<b>94.1%</b>	Q+W	1.58	
	<i>Vespa vulgaris</i>	1.0%	29.7%	0.51	100.0%	100.0%	<b>100.0%</b>	W	1.38
	<i>Vespa germanica</i>	1.6%	31.7%	0.46	100.0%	<b>100.0%</b>	W	1.44	
	<i>Vespa rufa</i>	9.2%	20.0%	0.58	67.1%	77.5%	<b>72.3%</b>	Q+W	1.50

Supplementary Figure S1. Data on the phylogeny<sup>17</sup>, percentage of egg-laying workers (Table S1), relatedness among workers<sup>13</sup>, the effectiveness of policing (Tables S2, S3) and queen-worker dimorphism in 9 species of Vespidae wasps and the honeybee *Apis mellifera*. Worker-laid eggs can be policed by the queen (Q), by workers (W) or by both (Q+W)<sup>3,5-14,18-19</sup>.

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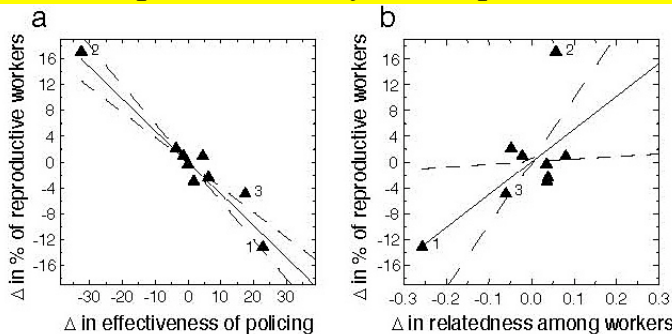
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## Comparative Study of Acquiescence



Supplementary Figure S2. Analysis of worker egg-laying in queenright colonies using phylogenetically independent contrasts ( $\Delta$ ). a. Contrasts in the percentage of egg-laying workers as a function of contrasts in the effectiveness of egg killing (policing). The relationship is significantly negative ( $F(1,8)=114.74, p=0.000006$ ). b. Contrasts in the percentage of egg-laying workers as a function of contrasts in the relatedness among workers. The relationship is significantly positive ( $F(1,8)=6.29, p=0.04$ ). Contrasts 1, 2 and 3 are *A. mellifera* vs. Vespidae, *P. chinensis* vs. Vespinae and (*V. vulgaris*, *V. germanica*) vs. *V. rufa*.