The phenomenon of *Apis mellifera capensis* laying workers in *Apis mellifera scutellata* colonies in the summer rainfall region of South Africa

by

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Submitted in fulfillment of the requirements for the degree

#### **Magister Scientiae**

In the

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Pretoria

October 2005

# **ABSTRACT**

African honeybee workers, *Apis mellifera scutellata* can activate their ovaries under queenless conditions to produce male (haploid) offspring. In contrast, laying workers of the Cape honeybee, *Apis mellifera capensis*, produce female (diploid) offspring via thelytokous parthenogenesis. In the early 1990's colonies of *A. m. capensis* were transported into the distribution area of *A. m. scutellata* (corresponding to the summer rainfall region of South Africa), leading to the "capensis calamity". Laying workers of *A. m. capensis* invaded and killed colonies of *A. m. scutellata* leading to losses of thousands of commercial colonies.

A survey of the apiaries in the *A. m. scutellata* region was conducted over 18 months from 1997 to 1998, to determine the extent of the problem. It was found that the parasites were established in many apiaries throughout the distribution range of *A. m. scutellata*. As the problem seemed to be more severe with commercial and migratory beekeepers, the apiaries surveyed were divided into risk groups related to beekeeping practices. The low risk group included apiaries of beekeepers in areas that are separated from commercial beekeepers and their high risk activities. These low risk colonies were sedentary vs the migration to high risk ares eg. Aloes, sunflower pollination areas, citrus and other fruit pollination areas of the high risk apairies.

The apiaries were monitored and records of the colonies' condition were taken. Samples of workers were collected for dissection. It was found that the low risk group had a lower rate of infection, a higher production of brood and honey and a higher rate of survival over a 12 month period.

The significant characteristics for identifying infection of a colony were determined as being the colour of the workers, the brood pattern, the presence of multiple eggs in cells and the presence of the queen. Indeed, the presence of dark workers with a black scutellum, an irregular brood pattern, the presence of

multiple eggs in cells and the absence of queen were all prevalent in infected colonies. As sample of workers from all inspected colonies were dissected and the average ovariole counts as well of the development stage of the ovaries proved to be significant variables in the diagnosis. Other variables eg. Ovariole counts, spermatheca size and aggression proved to be not significant, but in conjunction with other variables, could be used for diagnosis.

The genetic nature of the invasive parasitic population was determined using polymerase chain reaction (PCR) analysis. Nine loci were tested and the DNA fingerprints of all individuals sampled throughout the summer rainfall region were proved to be identical. This genetic identity led to the descripter of these individuals as a pseudoclone. In contrast, workers of *A. m. scutellata* were tested with the same loci and showed the normal distribution of an out-breeding population.

In order to investigate the spread of the parasite within an apiary, colonies were exposed to heavily infected hives and inspected regularly. Ninety five percent of the colonies had either died or absconded within 12 months.

It is concluded that this phenomenon of social parasitism is the consequence of apicaultural activities and that it can be managed by adopting low risk beekeeping practices.

# **FRONTICEPIECE**



Figure showing Apis mellifera scutellata workers with a queen.

# **CONTENTS**

| ABSTRAC*   | Гі   | i  |
|------------|--|----|
| FRONTICP   | IECEi  | V  |
| CONTENTS   | Sv   | ,  |
| LIST OF FI | GURESi   | X  |
| LIST OF TA | ABLESx   | ίi |
| PUBLICATI  | ONS ARISING FROM THIS STUDYx                                 | ίv |
| ACKNOWL    | EDGEMENTSx   | ۲V |
| CHAPTE     | ER 1   |    |
| INTRODUC   | TION 1   | ĺ  |
| 1.1        | SHORT NOTES ON BEEKEEPING HISTORY                            |    |
|            | IN SOUTH AFRICA1   | İ  |
| 1.2        | BACKGROUND TO THE CAPENSIS CALAMITY                          | 3  |
| 1.3        | HISTORY AND CONSEQUENCES OF THE                              |    |
|            | 'CAPENSIS CALAMITY'6   | 3  |
| 1.4        | ACTION AGAINST THE 'CAPENSIS CALAMITY'                       | 3  |
| 1.5        | RESEARCH PROJECTS ON THE CAPE PROBLEM BEE                    | )  |
| 1.5.1      | Information transfer   | )  |
| 1.5.2      | Survey of capensis laying workers and diseases               | )  |
| 1.5.3      | Penetration of the wild population1                          | 0  |
| 1.5.4      | Laying worker characteristic – inheritance1                  | 0  |
| 1.5.5      | Controlling element1   | 0  |
| 1.5.6      | Management practices   | 1  |
| 1.5.7      | A. m. scutellata reservoir1                                  | 1  |
| 1.5.8      | Modes of infestation/invasion of A. m. scutellata colonies 1 | 1  |
| 1.5.9      | Absconding swarms1   | 1  |
| 1.5.10     | Other projects resulting from original Working Group         |    |
|            | Projects1  | 1  |
| 1.5.10.1   | Hybridisation of Apis mellifera capensis and Apis mellifera  |    |
|            | scutellata:  |    |

#### University of Pretoria etd – Lubbe, A (2005)

|          | Does it occur and contribute to the capensis problem?    | 11 |
|----------|--|----|
| 1.5.10.2 | Dispersal of the pseudoclone in survey apiaries          | 12 |
| 1.6      | SCOPE OF THIS THESIS                                     | 12 |
| 1.7      | REFERENCES   | 13 |
| СНАРТ    | ER 2   |    |
| SURVEY   | FOR CAPENSIS LAYING WORKERS IN APIARIES                  | 15 |
| 2.1      | INTRODUCTION   | 15 |
| 2.2      | MATERIALS & METHODS                                      | 16 |
| 2.2.1    | Regions and background                                   | 16 |
| 2.2.2    | Observations   | 19 |
| 2.2.2.1  | External observations                                    | 19 |
| 2.2.2.2  | Internal observations                                    | 21 |
| 2.2.3    | Sampling bees for dissection                             | 25 |
| 2.2.4    | Dissection of bees                                       | 26 |
| 2.2.5    | Classification of apiaries by risk of capensis infection | 27 |
| 2.2.6    | Data Analysis  | 28 |
| 2.3      | RESULTS  | 28 |
| 2.3.1    | Colony and apiary classification                         | 28 |
| 2.3.2    | Observations   | 29 |
| 2.3.2.1  | Colour of worker bees                                    | 29 |
| 2.3.2.2  | Quality of brood pattern                                 | 30 |
| 2.3.2.3  | Colony defensive behaviour                               | 31 |
| 2.3.2.4  | Numbers of ovarioles/ovary                               | 31 |
| 2.3.2.5  | Ovariole development                                     | 32 |
| 2.3.2.6  | Queen survival   | 33 |
| 2.3.2.7  | Colony size  | 33 |
| 2.3.3    | Infection rates of colonies in three risk situations     | 34 |
| 2.3.4    | Productivity of colonies in three risk situations        | 36 |
| 2.3.5    | Survival of colonies in three risk situations            | 38 |
| 2.4      | DISCUSSION   | 40 |
| 2.4.1    | Characteristics used for diagnosis                       | 41 |
| 2.4.1.1  | Colour of worker bees                                    | 41 |

#### University of Pretoria etd – Lubbe, A (2005)

| 2.4.1.2  | Brood pattern                                | 41 |
|----------|--|----|
| 2.4.1.3  | Colony defensive behaviour                   | 41 |
| 2.4.1.4  | Number of ovarioles/ovary                    | 41 |
| 2.4.1.5  | Ovariole development                         | 42 |
| 2.4.1.6  | Queen survival                               | 43 |
| 2.4.1.7  | Colony size                                  | 43 |
| 2.4.2    | Infection rates                              | 43 |
| 2.4.3    | Productivity                                 | 44 |
| 2.4.4    | Survival                                     | 44 |
| 2.4.5    | Shortcomings of the survey                   | 44 |
| 2.5      | CONCLUSIONS                                  | 45 |
| 2.6      | REFERENCES                                   | 46 |
|          |  |    |
| CHAPT    | TER 3  |    |
| GENETIC  | BACKGROUND OF <i>CAPENSIS</i> LAYING WORKERS | 49 |
| 3.1      | INTRODUCTION                                 | 49 |
| 3.2      | MATERIALS & METHODS                          | 50 |
| 3.2.1    | DNA extraction procedure                     | 51 |
| 3.2.2    | Polymerase chain reaction (PCR) procedures   | 52 |
| 3.2.3    | DNA sequencing                               | 54 |
| 3.3      | RESULTS                                      | 55 |
| 3.4      | DICSUSSION                                   | 61 |
| 3.5      | REFERENCES                                   | 63 |
| <b></b>  |  |    |
| СНАРТ    | ER 4   |    |
| EFFECT ( | OF THE PSEUDOCLONE ON COLONIES IN AN APIARY  |    |
| 4.1      | INTRODUCTION                                 | 66 |
| 4.2      | MATERIALS & METHODS                          | 66 |
| 4.2.1    | Observations                                 | 67 |
| 4.2.1.1  | External observations                        | 68 |
| 4.2.1.2  | Internal observations                        | 68 |
| 4.2.2    | Sampling bees for dissection                 | 68 |
| 4.2.3    | Dissection of bees                           | 68 |

#### University of Pretoria etd – Lubbe, A (2005)

| 4.3    | RESULTS                       | 69 |
|--------|-------------------------------|----|
| 4.4    | DISCUSSION                    | 74 |
| 4.5    | REFERENCES                    | 75 |
|        |                               |    |
| CHAP   | PTER 5                        |    |
| SUMMA  | \RY                           | 77 |
| 5.1    | SUMMARY OF RESULTS OBTAINED   | 77 |
| 5.2    | POSSIBLE CONCLUSIONS          | 78 |
| 5.3    | RECOMMENDATIONS TO BEEKEEPERS | 79 |
| 5.4    | REFERENCES                    | 80 |
|        |                               |    |
| APPENI | DIX A                         | 82 |

### LIST OF FIGURES

- Figure 1.2 Geographical distribution of *A. m. capensis* (South of mountain ranges 1 4), *A. m. scutellata* (North of mountain ranges 5 10) and the hybrid zone between mountain ranges 1 4 and 5 10 as drawn from Hepburn & Crewe (1991). The numbers are described in the paragraph above.
- Figure 2.2.1 Map of South Africa showing the regions and sites where the surveys were conducted. The sites are indicated with letters close to where the apiaries were geographically located.
- Figure 2.2.2 The Inspector, on the left, inspects a brood frame while the recorder, (the author of this thesis) on the right, recorded the observations.
- Figure 2.2.3 Laying worker brood showing most of the signs of infection.

  The arrows point to the following characteristics: a. multiple eggs, b. raised cappings, c. & d. larvae of different ages in a group, e. uncapped prepupa.
- Figure 2.2.4 Yellow (level 3) honeybees, note the yellow scutellum indicated by the arrow marked a. A single black *capensis* bee (level 5) with a black abdomen and black scutellum, indicated by the arrow marked b.
- Figure 2.2.5 Equipment used to mark queens, TippEx, queen marker lid and queen cage.
- Figure 2.2.6 Sample bottles that were used to take samples of worker bees.

- Figure 2.2.7 Stages of ovariole development: a. Stage I ovarioles showing no development and no spermatheca. b. Stage IV ovarioles showing mature oocytes and the large spermatheca is indicated with the arrow.
- Figure 2.3.1 Colour ranking of worker bees in infected and non-infected colonies in apiaries classified into high, medium and low risk situations.
- Figure 2.3.2 Ranking of the quality of the brood pattern for infected and non-infected colonies in apiaries classified into high, medium and low risk situations.
- Figure 2.3.3 Level of colony defensiveness in the three risk groupings divided into infected and non infected colonies.
- Figure 2.3.4 Level of ovariole development in the workers of infected and non-infected colonies in apiaries classified into high, medium and low risk situations.
- Figure 2.3.5 Percentage infected colonies in three risk situations over time using the less conservative method of classifying colonies as infected.
- Figure 2.3.6 Brood production in colonies for three risk groups over time.

  Vertical lines denote 95% confidence intervals.
- Figure 2.3.7 Honey production in colonies of the three risk groups over time.

  Vertical lines denote 95% confidence intervals.
- Figure 2.3.8. Percentage survival of colonies in apiaries of the three risk groups during the three surveys.
- Figure 3.2.1 Two ml Eppendorf tubes that were used for DNA extraction.

- Figure 3.2.2 Ninety six well Eliza plates used for the PCR procedure.
- Figure 3.3.1 Electrophoresis gel showing the genetic identity of the clonal capensis laying workers. Each bee is represented in three adjacent vertical lanes with different loci (showing as green, blue and yellow bands) in each lane. The red bands are an internal standard. The purple arrows point to the control lanes.
- Figure 3.3.2 The alleles for the A113 and A14 loci as found in four *capensis* laying workers, showing the clonal nature of the individuals from colony N14.
- Figure 3.3.3 The alleles for the A113 and A14 loci as found in three *A. m.* scutellata bees from the same colony (N14) as the workers shown in Fig. 3.3.2.
- Figure 3.3.4 Electrophoresis gel showing on the left the genetic diversity in *A. m. scutellata* individuals and the lack of genetic diversity of the clonal *capensis* laying workers on the right. Each bee is represented in three adjacent vertical lanes with different loci (showing as green, blue and yellow bands) in each lane. The red bands are an internal standard.
- Figure 4.1 Decrease in the number of surviving colonies over time in weeks.

# LIST OF TABLES

- Table 2.3.1 Total number of apiaries and colonies inspected during the three surveys.
- Table 2.3.2 Number of colonies classified into each of the risk groups with number of apiaries in brackets.
- Table 2.3.3 Mean number of ovarioles/ovary found in workers sampled from the colonies in the three apiary risk groupings with the maximum count found in each case in brackets.
- Table 2.3.4 Percentage of colonies in Survey 1 in which queens were found and marked with light blue Tippex. The percentage of colonies in surveys 2 and 3 in which queens with a blue mark were identified. Colonies were classified into the three risk groups.
- Table 2.3.5 Mean number of frames filled with worker bees in the three categories of risk for apiaries during the three surveys.
- Table 2.3.6 Correlation of various variables with survival time (variables with p<0.05 are significantly correlated).
- Table 3.2.1 The micorsattelite loci with their associated primer sequences and PCR conditions for the amplification of the DNA extracted from the sampled worker bees.
- Table 3.2.2 Composition of the PCR reaction mixtures used for analysing the DNA of the workers sampled (All values are µl.).

- Table 3.3.1 Physical characteristics of a sample of bees from three colonies (N3, N14 & N18) with an assessment of whether they were *capensis* laying workers.
- Table 3.3.2 Field data of one colony (N03) at an early stage of infection by the clonal *capensis* laying workers.
- Table 3.3.3 Field data of one colony (N14) where *capensis* laying workers were active.
- Table 3.3.4 The ovariole counts and morphological characteristics of the bees used in the genetic analysis presented in Figures 3.3.2 and 3.3.3. Pseudoclone = clonal *capensis* laying workers
  - Table 4.1 Data collected from colony no 3 showing the criteria used to classify it as infected on 18/11/99. Shaded blocks indicate that infection has taken place.
- Table 4.2 Data showing the cumulative area of brood (dm²) present in different colonies, the highest number of ovarioles per ovary in dissected workers and the size of the spermatheca before a colony was infected, during the early stage of infection and when it was irreversibly infected.
- Table 4.3 The fate of the 20 colonies in the experimental apiary following the introduction of colonies infected with the pseudoclone workers. The experiment was initiated on 22 July 1999.

# PUBLICATIONS ARISING FROM THESE STUDIES

- Kryger, P. & Van der Schyf, A., 1999. The *capensis* bee problem: A genetic analysis using single locus DNA- fingerprinting. Final Report for the South African Bee Industry Executive. 6pp.
- Lubbe, A., 2001. Identifying the invader bee, *Apis mellifera capensis*, in colonies of *Apis mellifera scutellata* in the summer rainfall region of South Africa. *Abstracts of the 37th International Apicultural Congress*, Durban, South Africa. p. 30-31.
- Lubbe, A., 2003. The importance of the honeybee queen on the reproductive development of worker bees in an *Apis mellifera scutellata* colony. *Proceedings of the 14th Entomological Congress*. Pretoria, South Africa. p.51.
- Lubbe, A., 2003. Biodiversiteit in gevaar weens heuningby-pseudoklone. *SA Tydskrif vir Natuurwetenskap en Tegnologie* 22 (4): 115.
- Swart, D.J.\* & Lubbe, A., 2002. Is die Kaapse probleemby wat Afrika heuningbye (*Apis mellifera scutellata*) infesteer, 'n sosiale parasiet, of die gevolg van autoseleksie van lêende werkers van die Kaapse heuningby (*Apis mellifera capensis*)? *SA Tydskrif vir Natuurwetenskap en Tegnologie* 21 (1): 37.

<sup>\*</sup> Note that the author used the surname "Van der Schyf" up to 19 September 1999 and thereafter she uses the surname "Lubbe".

# **ACKNOWLEDGEMENTS**

Department of Agriculture: Directorate of Plant Health and Quality for funding.

Capensis Working Group for advice and co-ordination of programme.

The 44 Beekeepers for participating.

Co-workers at ARC-PPRI: Sidwell Banne, Riana Bezuidenhout, Petrus Dikgobe (late), Martin Johannsmeier, Sarah Kgapola, Elize Lundall-Magnuson, Andrew Masemola, Dawid Swart, Magda Swart, Sollie Tsede and Emil Von Maltitz for help collecting data, dissecting honeybees, typing data, advice and support.

Dr. Geoff Tribe, Martin Johannsmeier for valuable comments and suggestions on the first draft of the manuscript.

Prof. Robin Crewe, Dr. Vincent Dietemann and Dr. Per Kryger for guidance and comments on various stages of the manuscript.

Dr. Per Kryger and Dr. Vincent Dietemann for some of the photographs used.

Marie Smith and Liesl Morey for initial statistical analysis of the data.

My husband, Leon and my children, Yvonne and Marelize, for their understanding patience and moral support with the writing of this thesis.