Scientific Report

Full title:	Bee Research And Virology in Europe Identifying the research needs for protecting European Apiculture and ecosystems against viral diseases.
Acronym:	**BRAVE**
Number :	**513628**

Type of instrument: **Specific Support Action**

Duration : **the year 2005**

Consortium:

Michel AUBERT
Brenda BALL
Ingemar FRIES
Norberto MILANI
Robin MORITZ

Co-ordinator: **Michel AUBERT**
Agence française de sécurité sanitaire des aliments (Afssa)
m.aubert@afssa.fr
fax: 00 33 4 92 94 37 01

TABLE OF CONTENTS

1. Abstract.

The BRAVE project is the result of a call under the European Commission's 6th Framework Research and Development Programme in the area of policy-orientated research. The primary focus assigned to this Specific Support Action was "*to assess the level of risk and the likely consequences for bees and other closely related pollinators of the introduction of bee viruses into European bee colonies and ecosystems, and to provide advice to the EC on appropriate protective measures to prevent further incursions and spread.*" Building on initiatives arising from the European Association for Bee Research (EurBee), a response to this call was formulated by a Steering Committee comprising Dr Michel AUBERT, AFSSA, France (coordinator); Brenda BALL, Rothamsted Research, UK; Prof. Ingemar FRIES, Swedish University of Agricultural Sciences, Upsala; Prof. Norberto MILANI, University of Udine, Italy; and Prof. Robin MORITZ, University of Halle, Germany. The aims of BRAVE were firstly, to facilitate knowledge and skills transfer between researchers and advisors within the European Research Area of bee virus diseases through the establishment of closer contacts and collaborations, and secondly to identify significant gaps in the essential scientific knowledge required to support the formulation and integration of policy on the endemic and emergent diseases of bees. The project duration was of one year (2005) and the funding provided by the EC has been used for two meetings.

The first scientific meeting held at Sophia-Antipolis, Provence, France, from the 24th to 26th April 2005 was attended by 55 scientists, advisors and policy makers specifically invited because of their specialist knowledge and skills. Their expertise ranged from insect virology, virus taxonomy, immunology, epidemiology, disease risk assessment and international trade, to fundamental and applied research on pollinators and their pathogens. Delegates represented 15 European countries as well as Australia, Canada, Lebanon, Mexico, and the USA, together with representatives of the European Commission and the OIE (Office International des Epizooties). Young scientists and students had the opportunity to apply for additional places which allowed the consortium to go towards a more balanced age and gender participation.

The first sessions of the meeting explored the complex and evolving taxonomy of bee viruses, covered the range of diagnostic techniques now available for virus detection and discussion explored the appropriateness of the techniques for particular applications, for example, the lack of sensitivity of serological techniques may still make them relevant in the recognition of biologically significant overt virus infections against a background of inapparent and perhaps unimportant infections revealed by the now available molecular techniques.

Further sessions covered aspects of the genetics, physiology and behaviour of honey bees in relation to their resistance to virus infections. The genetic basis of disease resistance in bees was explored. Several papers considered the persistence of virus at sub-lethal levels in honey bees. The question of viruses being "triggered" by mite feeding, the association of virus infections with other parasites such as *Nosema apis*, and the possible depression of the honey bee immune response by exposure to sub-lethal doses of pesticides were also raised.
The evolutionary epidemiology of virus diseases and their virulence was considered in relation to the different routes of transmission available within social insect populations and the degree to which this picture has been changed in the honey bee system by *V. destructor*. Current information on the incidence, distribution and impact of honey bee viruses was reviewed, revealing large gaps in our knowledge. With increasing world trade and movement of stocks of social and solitary species of bees, this lack of knowledge of the pathogens present in populations is clearly a crucial deficiency. The management of bee diseases was

then considered, mainly in relation to *V. destructor*. The effect of acaricides on the behaviour and pollinating efficiency of bees, a consideration of economic treatment thresholds and the development of tolerance in bees to mites and resistance of mites to acaricides were all covered.

The final session concerned the current regulatory mechanisms governing the movement of honey bees into the EU and the assessment of the risk of pathogen introduction related to trade issues. The recent widespread commercial movement of various bumble bee species around the world, the virus pathogens of which are virtually unknown, was highlighted as a cause of concern. It became apparent that at present there is insufficient knowledge of the distribution of honey bee viruses and it is possible that the introduction of honey bee viruses to new locations has already occurred, although the implications of this are still largely unknown.

Every session was followed by a working session which produced short and comprehensive recommendations for the focus of future research efforts.

The proceedings of this meeting have been published. They include the scientific texts (more complete than the oral presentations) and the recommendations elaborated by the working groups and approved in plenary sessions.

A smaller **workshop meeting** took place later (2-7 September 2005) at which several experts including consortium of BRAVE, the rapporteurs of the first meeting and selected authors have prepared a synthesis of current knowledge in the different subject areas have also made recommendations about the means of achieving the identified research priorities at both the fundamental and applied levels. This task will be published in the form of **a book entitled "Virology and the Honey Bee"** and printed by the European Commission. The work started during the workshop and have been very well advanced during the following weeks: already six out of the nine main future chapters of "Virology and the Honey Bee" have been achieved. Serious personal problems of the co-author of all the 3 remaining chapters have caused some delay and of course, the whole still requires final editing input (general presentation, table of indexes, general introduction and conclusion). Anyhow, on the basis of which has already been achieved, we are now sure that the book will be published far before the end of this year.

The **dissemination of the work** has been accomplished via the BRAVE website : http://www.entom.slu.se/brave/ (and other linked websites), through scientific and professional media and using oral and poster presentations during scientific meetings. This dissemination will be pursued using the same means for publicising and distributing the proceedings of the first BRAVE meeting and the book "Virology and the Honey Bee".

The European BRAVE project will have produced tangible results : the proceedings of the BRAVE plenary scientific meeting and the book "Virology and the Honey Bee". Their aim was to synthesise the current knowledge required for protecting the honey bee and related pollinator insects from virus diseases and to propose a framework for future research programmes and integrating European research effort in support of Community policy.

2. Introduction : the objectives of BRAVE

The objective assigned by the call entitled *"Viral diseases of bees"* were : *"Assessment of the level of risk and the likely consequences for bees and other closely related pollinators of the introduction of bee viruses to European colonies of honey-bees and ecosystems and to provide advice to the EC on appropriate protective measures to prevent further incursions and spread."*

The overall aim of the proposed project (**B**ee **R**esearch **A**nd **V**irology in **E**urope - BRAVE) were a) to contribute actively to facilitate **knowledge and skills transfer** by establishing closer contact and collaboration **between researchers and European advisors** within the Research Area of bee virus diseases, and b) **to identify significant gaps in the scientific knowledge** required to support the formulation and integration of policy in the field of enzootic and emerging diseases.

Practically, BRAVE sought to achieve this objective by:

a) holding two meetings :

- a plenary scientific meeting : this first three day meeting gathered together two categories of experts:
  - experts with a broad base of skills in insect virology, diagnosis, immunology, disease epidemiology, international trade, policy formulation and disease risk assessment,
  - scientists involved in fundamental and applied research on bees and related pollinator species.

Within this meeting, we expected that exchanges between both categories of experts and between bee experts themselves favour novel approaches in apicultural research and take advantage of recent developments in the general fields of virology and insect immunology. For this purpose, following key lectures reviewed the more recent knowledge in the broad world of insects (or invertebrates) in designated subject areas, shorter talks on the same areas were more precisely restricted to the Honey Bee. After each session, future research priorities at the fundamental and applied levels had been proposed and discussed by all participants.

- an expert workshop : using the proceedings and conclusions of the first meeting, the second three day meeting was a workshop aimed at identifying future research priorities at the fundamental and applied levels for integrating an European research effort in bee virus diseases in support of Community policy.

b) creating a web site for communicating the BRAVE contents and the results of the scientific meeting to the public.

c) publishing the proceedings and recommendations of the two meetings :
- the proceedings of the plenary scientific meetings consisting in the scientific papers written by the speakers and the recommendations of the working groups,
- the task issued from the expert workshop under the form of a book which, in addition to being an overview of current virology status of the honey bee, also proposes a framework for future research programmes on virology and the honey bee.

e) disseminating the proceedings and the book with appropriate means.

3. A short history of the project

The BRAVE consortium.
Originally the answer to the call had been build on initiatives already established through EurBee, an European association of individuals and institutions for bee research whose object is to foster exchange of information between all European Bee researchers. Prof. Ingemar FRIES visited Brenda BALL at Rothamsted Research, UK (19 November 2003) and met Dr Michel AUBERT in Brussels, Belgium (4-5 December 2003). Contacts were pursued via emails and phone calls with Prof. Norberto MILANI (Udine University, Italy) and Prof. Robin MORITZ (Universität Halle-Wittenberg, Germany). They pooled their expertise and ideas to elaborate the BRAVE proposal which they submitted to the Commission in late December 2003.
For their meeting in Brussels (4-5 December 2003), Prof. Ingemar FRIES and Dr Michel AUBERT have been supported by funds from their own respective institutions.

Starting the project before its approval.
Expecting a positive answer to their submission, all the members of the consortium met in Callian (France) for two days (25-27 June 2004) for elaborating the scientific programme of the scientific meeting that was the first part of the submitted project: topics and titles of the sessions, the key lectures and shorter talks, choice of chairpersons, proposed speakers for key lectures and shorter talks.
For this first organisational meeting, every member of the consortium has been supported by funds from their own respective institutions.

To organise this meeting well in advance – even before knowing if the project would be accepted or not - brought two advantages :
- the earlier the proposed speakers were contacted, the better chance we had of obtaining their agreement to participate if the proposal were evaluated positively by the Commission,
- the invited speakers had more time to organise their travel: as soon the project was agreed, they were said to book their plane ticket. As early booking entails lower individual travel costs, with the same overall budget, we expected to be able to invite more scientists : a) several key European and non-European scientists (from North America, Australia, South Africa), and b) non-speaker scientists from new and future EU member states and young scientists that would not be able to attend otherwise.
Therefore, the following weeks, the consortium made contact with the proposed speakers. These contacts by emails and phone with all expected participants were intensified as soon as the official agreement of the project was received from the Commission.

Nevertheless, more direct contacts between the members of the consortium were still required for finalising the program and to up-date the list of participants of the plenary scientific meeting. Several members of the consortium took advantage of other planned meetings of bee researchers such as the EurBee meeting in Udine (19-23 sept 2004) and all the members met again on the 16 March 2005 in the Institut für Zoologie - Martin-Luther-Universität Halle-Wittenberg (Halle/Saale, Germany). This allowed the co-coordinator to present to the other members the current administration of the project regarding financial and other aspects and to obtain their common agreement.

The plenary scientific meeting and the workshop meeting.
The plenary scientific meeting was held at Sophia-Antipolis, Provence, France, from the 24th to 26th April 2005 and the **workshop meeting** took place later (2-7 September 2005) in Tourtour also in Provence. Both meetings are detailed in following chapters.

The management task.
As expected, the management task involved by the organisation of the two scientific meetings and the edition of their proceedings havebeen significant. Fortunately, all participants have expressed their satisfaction on this management. However, this task will not be described in the present report.

The proceedings of the scientific meeting.
The invited speakers had been asked well in advance to provide the text of their talk before or at least when arriving at the meeting in order to facilitate and accelerate the editing of the proceeding.
In fact, this was not so simple as several editing problems delayed the final edition of the proceedings: the last electronic version was sent to all participants by email and the printed version was received in November 2005.

Whereas the BRAVE project is officially closed, the BRAVE work is pursued.
As this will be explained in the chapter on the workshop meeting, today (13 February 2006), the work undertaken under the BRAVE project, whereas officially closed, is still and will be pursued until a) the complete achievement of the book and b) the dissemination of the results: i.e. a larger dissemination of the proceedings of the scientific meeting and of "Virology and the Honey Bee", when published.

4. The scientific meeting

4.1. The scientific meeting background.

Honey bee pathology is a large field of study, partly because of the importance of this insect to pollination of cultivated crops. One advantage of colleagues working on similar topics is the possibility of exchange of experiences in the field of interest. However, a disadvantage can also materialise under such circumstances: a large enough group of scientists working on similar topics may not extend the search for new information outside this group. This may seriously impair integration of new knowledge in related scientific fields into the field of honey bee pathology. We believe, that one reason for the slow progress made in honey bee pathology in general, and bee virology in particular in recent years, is a lack of integration of progress made in other scientific fields, such as general virology or epidemiology, into honey bee pathology. The present call was a good opportunity to create common access to the latest results obtained in insect virology, immunology and epidemiology for the European researchers specialised in bee diseases.
This inter-disciplinary approach had potential to lift European honey bee pathology research to new levels. This ambition was manifested through the composition of the scientific meeting where the first lecture of session α.1, α.2, β.1, β.2, β.3 and β.5 included overviews of the general topics of virology, immunology and epidemiology.

4.2. The programme.

A logical order have been followed, starting with the characterisation of bee viruses, ending with the regulatory issues that should be considered for prevention:

Additionally, to favour the emergence of novel approaches to address the problems of bee diseases - virus diseases in particular – we designated as Chairpersons several young but established researchers who held a real responsibility for organising their session.

Sunday 24 April

hour	duration of ind. talks	duration of sessions			
			α Bee Viruses - Chair : Max BERGOIN		
			α.1. Characterisation of viruses of honey bees and related species.- Co-Chair : Mike CARTER		
8:30	00:30		*Key-note*	1. Taxonomy of Picornaviruses.	**Peter CHRISTIAN**
9:00	00:30		*Review*	2. Taxonomy of Bee viruses.	**Mike CARTER**
9:30	00:30		*Review*	3. Phylogenetic of viruses isolated from bees.	**Tamas BAKONYI**
10:00	00:15		PAUSE		
10.15	00:20		*Review*	4. Genetic variability of bee virus isolates.	**Elke GENERSCH**
10:35	00:30		discussion		
		02:20			
			α.2. Diagnostic techniques for virus diseases in honey bees.- Co-Chair : Magali RIBIERE		
11:05	00:30		*Key-note*	1. Serological methods.	**Lesley TORRANCE**
11:35	00:30		*Key-note*	2.Molecular methods. Possibilities and limitation of molecular diagnostic techniques.	**Joachim DE MIRANDA**
12:05	01:30		LUNCH		
13:35	00:20		*Review*	3. Comparison of sensitivity and specificity between serological and molecular techniques.	**Mark STEVENS**
13:55	00:20		*Review*	4. Infectivity tests and their interpretation.	**Brenda BALL**
14:15	00:20		*Review*	5. Molecular detection of unapparent virus infections.	**Magali RIBIERE**
14:35	00:30		discussion		
		02:30			
15:05	00:15		PAUSE		
			β Bee Viruses and Infection.		
			β.1. Genetics, physiology and behaviour of virus infections in honey bees - Chair : Robin MORITZ		
15:20	00:30		*Key-note*	1. Resistance and virulence in insect-pathogen relationships: the baculovirus-lepidoptera case.	**Jenny CORY**

15:50	00:30	*Key-note*	2. Koch's postulates and RT-PCR as an new approach for proving viral disease causation in honey bees.	**Yanping CHEN**
16:20	00:20	*Review*	3. Application of the entire genome sequence for disease resistance.	**Jay EVANS**
16:40	00:15	PAUSE		
16:55	00:30	*Review*	4. RNAi, fat body genes and antibacterial peptide.	**Randi AAMODT**
17:25	00:15	*Review*	5. Mapping of genes controlling honeybee worker behaviour.	**Michel SOLIGNAC**
17:40	00:15	*Review*	6. Using drones in selection for brood disease resistance in honeybees.	**Robin MORITZ**
17:55	00:30	discussion		
	02:50			
	07:40	*<= total time (hours) except pauses and lunch*		

after lunch

1st step (1 hour) : the participants are split into working groups for elaborating "The recommendations of the day"

2nd step (until the work is acheived): participants meet in plenary session for discussing and adopting "The recommendations of the day".

Monday 25 April

hour	duration of ind. talks	sessions		
			β Bee Viruses and Infection. (cont.)	
			β.2. Insect immunity to infection - Chair : Peter ROSENKRANZ	
8:30	00:30	*Key-note*	1. Drosophila immune response to virus infection.	**Jean-Luc IMLER**
9:00	00:30	*Key-note*	2. Specific recognition of microbial substances and humoral reactions.	**Dan HULTMARK**
9:30	00:20	*Key-note*	3. Cellular immunity in insects : so far identified immuno-competent cell types.	**Tina TRENCZEK**
9:50	00:20	*Key-note*	4. Behaviour aspects of immunity or tolerance.	**Mark BROWN**
10:10	00:30	discussion		
	02:10			
10:40	00:15	PAUSE		
			β.3. Latency of virus infection in honey bees - Chair : Norberto MILANI	
10:55	00:30	*Key-note*	1. Overview on virus latency in insects.	**Robert POSSEE**
11:25	00:20	*Review*	2. Triggering of virus replication in honey bee.	**Denis ANDERSON**

11:45	00:30		discussion	
		01:20		
12:15			LUNCH	
		03:30	*<= total time (hours) except pauses and lunch*	

Afternoon : social programme = visit of the island of Sainte-Marguerite with its surviving relics of original Mediterranean flora and fauna – botanical guide provided + recital of baroque music

Tuesday 26 April

hour	duration of ind. talks	sessions			
			β.4. Epidemiology of virus infections in honey bees - Chair : Ingemar FRIES		
8:30	00:20		*Key-note*	1. Evolutionary epidemiology of infectious disease in social insects.	**Mark BROWN**
8:50	00:20		*Review*	2. Epidemiology of infectious disease in honey bees.	**Ingemar FRIES**
9:10	00:20		*Review*	3. Transmission routes of virus infections in honey bees.	**Brenda BALL**
9:30	00:20		*Review*	4. Some data on the impact of virus infections in honey bees.	**Michel AUBERT**
9:50	00:20		discussion		
		01:40			
10:10	00:15		PAUSE		
			β.5. Incidence and distribution of honey bee viruses.- Chair : Brenda BALL		
10:25	00:20		*Review*	1. Overview of the incidence and distribution of bee viruses in the EU.	**Laurent GAUTHIER**
10:45	00:20		*Review*	2. Honey bee movements in relation to the introductions of exotic pathogens.	**Denis ANDERSON**
11:05	00:20		*Review*	3. Bumble bee movements in relation to the introductions of exotic pathogens	**Niek STEEGHS**
11:25	00:30		discussion		
		01:30			
			γ Management of bee diseases : Economic Impact. Chair : Keith DELAPLANE		
11:55	00:30		*Key-note*	1. Importance of disease control in pollinating insects.	**Keith DELAPLANE**
12:25	00:20		*Review*	2. Limits of treatments : lack or limits of efficacy (resistance) of treatments applied.	**Marco LODESANI**

12:45	00:20		*Review* 3. Veterinary products for bees : a producer's point of view.	**Jérôme TROUILLER**
13:05	00:30		discussion	
		01:40		
13:35	01:30		LUNCH	
			δ Regulatory Issues for Preventing the Entry of Exotic Bee Viruses into the EU.	
			Chair : Mike BROWN	
15:05	00:20		*Review* 1. EU honey bee import regulations and protecting EU apiculture.	**Howard BATHO**
15:25	00:20		*Review* 2. Principles for risk analysis for support of bee health policies.	**Mike BROWN**
			Chair : Wolfgang RITTER	
15:45	00:20		*Review* 3. Trade issues and the WTO/OIE.	**Tomoko ISHIBASHI**
		01:00		
16:05	00:15		PAUSE	
16:20	00:20		*Review* 4. The significance of trade with honey bees and honey bee queens.	**Hermann PECHHACKER**
16:40	00:20		*Review* 5. The risks of trade with honey bees and honey bee queens.	**Wayne WEHLING**
17:00	00:20		*Review* 6. The OIE code and virus diseases of honeybees.	**Wolfgang RITTER**
17:20	00:30		discussion	
		01:30		

7:20 *<= total time (hours) except pauses and lunch*

after lunch

1st step (1 hour) : the participants are split into working groups for elaborating "The recommendations of the day"
2nd step (until the work is achieved): participants meet in plenary session for discussing and adopting "The recommendations of the day".

Wednesday 27 April

hour	
8:30	*Working groups - The organisation and tasks of these working groups will be defined during the meeting*
10:00	*and proposed to the participants by the organisers.*

4.3. The participants.

Scientists, advisors and policy makers were specifically invited because of their specialist knowledge and skills. Their expertise ranged from insect virology, virus taxonomy, immunology, epidemiology, disease risk assessment and international trade, to fundamental and applied research on pollinators and their pathogens.

Scientists not selected for delivering a talk, young scientists and students had the opportunity to apply for additional places. This opportunity had been publicised a) through an announcement in a plenary session and posters during the first EurBee meeting (Udine – Italy - 19-23 sept 2004) (see annex 1), b) through the EurBee Pathologists' Group. Newsletter 1, November 2004 (sent by email by Norman Carreck to a large audience of European specialists in the honey bee and related species), and b) through the BRAVE web site.

The candidates were advised to apply with a letter outlining their personal interest in attending the BRAVE scientific meeting, a Curriculum Vitae, and a list of recent publications. They were informed that the BRAVE consortium will then rank the applications according to several criteria: motivation, likelihood of the applicant to participate in future bee research programmes, with a special attention to balancing as much as possible, the number of representatives between the Member States and genders. Nineteen complete applications were received and 14 were selected according to the above mentioned criteria. Among the selected participants, six which were not financially supported by their laboratory (students and young scientists from new EU Member States or from developing countries) were granted full funding for travel and accommodation costs. Two additional students based in the AFSSA Sophia Antipolis laboratory participated in the meeting for helping the participants, a task that allowed them anyhow to attend most of the talks.

List of participants (grey colour indicates invited non-speaker following application):

First name	Family name	Organisation
Ales	GREGORC	Agricultural Inst. of Slovenia - SL
Antonio	LAVAZZA	IZSLER - Brescia - I
Brenda	BALL	Rothamsted Res - UK
Dan	HULTMARK	Umea Univ. - SW
Denis	ANDERSON	CSIRO - AustraliaUS
Dieter	BEHRENS	Univ. Halle - D
Elke	GENERSCH	Länderinst. Bienenkunde - D
Eva	FORSGREN	SLU - SW
Franco	MUTINELLI	IZS Venezie - I
Grazyna	TOPOLSKA	Warsaw Agricultural Univ. - PL
Hélène	BERTHOUD	ALP - CH
Hermann	PECHHACKER	Bienen Inst. - Lunz - Austria

Howard	NEEDHAM	European Commission
Ingemar	FRIES	SLU - SW
Jay	EVANS	USDA - Beltsville - USA
Jean-Luc	IMLER	CNRS Strasbourg - F
Jenny	CORY	Agloma Univ. - Sault Ste Marie, Ontario - CAN
Jérôme	TROUILLER	VITA – SWARM (Small or medium enterprise – UK)
Joachim	DE MIRANDA	Pen. State Univ. - USA
Keith	DELAPLANE	Univ. of Georgia - USA
Laurent	GAUTHIER	Montpellier Univ. - F
Lesley	TORRANCE	Scottish Crop. Research - UK
Magali	RIBIERE	AFSSA - F
Marco	LODESANI	INA Bologna - I
Mariano	HIGES	Consejeria de Agricultura de la Junta de Comunidades de Castilla-La Mancha - E
Marie-Pierre	CHAUZAT	AFSSA - F
Mark	BROWN	Univ. of Dublin - Irl
Mark	STEVENS	IARC Broom's Barn - UK
Max	BERGOIN	Montpellier Univ. - F
Mazen	HABAYEB	Lebanon - Student in UCMP - SW
Michel	SOLIGNAC	CNRS Gif - F
Michel	AUBERT	AFSSA - F
Mike	BROWN	CSL York - UK
Mike	CARTER	Univ. of Surrey - UK
Neil	BOONHAM	CSL - UK
Niek	STEEGHS	BBB – (Small or medium enterprise - NL
Norberto	MILANI	Udine Univ. - I
Norman	CARRECK	Rothamsted Res - UK
Olivier	CELLE	Student from Afssa-Sophia-Antipolis
Peter	CHRISTIAN	Nal Inst. for Biol. Stand. & Control - UK
Peter	ROSENKRANZ	Univ. Hohenheim - D
Pierangelo	BERNORIO	European Commission
Randi	AAMODT	Agric. Univ. Norway - NO
Reinhold	SIEDE	Bieneninstitut Kirchhain - D
Robert	POSSEE	NERC CEH Oxford - UK

Robert	PAXTON	Queen's Univ. Belfast - UK
Robin	MORITZ	Univ. Halle - D
Tamas	BAKONYI	Budapest Univ. - HU
Teresa	SANTILLAN-GALICIA	Mexico – Student in Rothamsted Res - UK
Tina	TRENCZEK	Giessen - D
Tomoko	ISHIBASHI	OIE
Violaine	Olivier	Student from Afssa-Sophia-Antipolis
Wayne	WEHLING	APHIS - USA
Wolfgang	RITTER	CVUA - D
Yanping	CHEN	USDA - Beltsville - USA

In total, the 56 delegates represented 15 European countries – including 13 Member states (Austria, Denmark, France, Germany, Hungary, Ireland, Italy, The Netherlands, Norway, Poland, Spain, Sweden, United Kingdom) and Switzerland, Slovenia – and Australia, Canada, Mexico, Lebanon and the USA, together with two representatives of the European Commission (Dr Howard NEEDHAM and Pierangelo BERNORIO) and one representative (Dr Tomoko ISHIBASHI) of the OIE (Office International des Epizooties). Two representatives of small or medium enterprises involved in veterinary products for the honey bee or in bumble bee commercial exchanges were also invited.

4.4. The proceedings.

The proceedings include :
a) the scientific texts at the basis of the oral presentations done during the meeting – most of these texts are of a high scientific level and, in any case, far more elaborated than the corresponding oral presentations,
b) the recommendations formulated during the discussion sessions of the working groups then approved in general sessions.

As explained earlier, the invited speakers had been asked well in advance to provide the text of their talk before or at least when arriving at the meeting in order to facilitate and accelerate the editing of the proceeding.
For producing the proceedings according to the standards of international scientific reviews, they received a list of recommendations they had to follow. Generally, as a counterpart of their invitation, almost all the invited scientists produced a paper but the recommendations were not always so strictly followed. This significantly delayed the edition of the proceedings. A first edition of the proceedings was sent by email on the 23 October 2005 to all participants. However, following the receiving of a late paper by a participant, a new edition of the proceedings had been produced and this new edition was re-sent by email to all participants on the 2nd of November 2005.

This last edition was sent to the printer, and received in late November 2005. The cover of this last edition is given in Annex 2 and a complete printed version has been sent together with the present report to the Commission.

4.5. The recommendations.

Whereas the recommendations have been included in the proceedings of the BRAVE plenary meeting, they are reproduced in this report as they constitute an important result of the project.

4.5.1. Recommendations of the working group
Characterisation of viruses of honey bees and related species and pathogenesis
Rapporteur : Mike CARTER

A Characterisation of viruses of honey bees and related species

1. State of the art.
Major advances have taken place in insect virology over recent years. Insect virology offers the most fruitful zone for the discovery of new viruses and replication strategies which in turn offer the potential for furthering our understanding of cell biology.
Study of bee viruses is advancing with this movement but more remains to be done. These opportunities for academic progress mean that there is an increasing and world-wide interest in the subject.

2. Challenges and problems :
- Many bee viruses remain poorly characterised.
- The study of bee viruses is impeded by lack of certified virus-free bees and *in-vitro* cultivation systems.
- There is a lack of characterised standard reagents and reference sera.
- Disease burden is underestimated; persistent infections are common but dismissed. Only gross disruption is detected, less obvious effects (decreased lifespan) must impact on productivity. Objective measures are required.
- Varroa has changed the rules, opening a new route for infection and possibly altering the behaviour of viruses by selecting mite-replicating variants. This has increased the significance of persistent infections.

3. Actions reagents and techniques required:
- Sequencing of bee viruses should be completed (for those whose sequencing has only being partly done), or performed and new agents sought,
- PCR procedures should be developed for newly sequenced agents,
- Recombinant antigens are needed to produce standardised antisera and antigens,
- An active (interventionist) approach is required to develop bee cell lines, whereas considering the numerous attempts with no avail made by numerous workers, this approach is now rarely encouraged.

B. Pathology and Pathogenesis :
High mutation rate and the organisation of viruses into quasi-species underlies potential for rapid RNA virus adaptive mutation have implications for host- and tissue tropism. However, these phenomenon are poorly characterised.
Among the most urgent needs in this area, the group identified :

- the need to locate virus-infected tissues following naturally acquired, acquired following parasitism by Varroa, and artificially acquired (following injection or other artificial infection procedure),
- the need to understand more of the dynamics of virus interaction with Varroa in *Apis mellifera* and particularly in *Apis cerana*, its original host.

4.5.2. Recommendations of the working group
Diagnostics techniques for virus diseases in honey bees
Rapporteur : Mark STEVENS

1. To develop an international database of appropriate diagnostic protocols, methods and tools such as antibodies and PCR primers.
Further sequencing of bee viruses will allow the opportunity to develop new PCR primer sets that can be exploited for diagnostics. It will also be necessary to develop standardized protocols for the collection, storage and initial processing of bee samples prior to any diagnostic tests, especially if samples being sent long distances.

2. To undertake a survey of the distribution and impact of the key viruses of bees within individual countries in order to determine their presence (for any quarantine lists that may be instigated) and to understand the epidemiology of these viruses.

3. To determine, via the diagnostic methods, the thresholds of detection for specified viruses and the thresholds at which these viruses cause 'damage' to bees.
Such methods could be exploited to standardize the terms used within bee virology, for example, quantification of terms such as 'unapparent' and 'latent'.

4. To undertake a series of ring tests among international laboratories to standardize methods for key viruses and to establish standard operating procedures for each system.

5. To establish a specific working group for KBP/ABPV.

4.5.3. Recommendations of the working group
Genetics, physiology, behaviour
Rapporteur : Robin Moritz

Researches to be developed must be carried out at the individual level or at the colony level.

A. Research need at the individual (bee) level.
Among the most urgent needs the group recommended :
1. screening for genetic variance of resistance to viral diseases in European honeybees,
2. to develop techniques for infecting drones with virus to take advantage of the haplo/diploid genetic structure of the bee species,
3. to identify disease resistance genes and their regulation (including the use of candidate genes from other model systems).
4. to develop molecular tools to assist in selection for virus disease resistance,
5. to study the effects of virus infection on the physiology of host organs (including gene expression in the host, structure, cell death, endocrine regulation).

B. Research need at the colony level.
1. Resistance to virus must be studied not only on groups of bee isolated from their hive but also at the colony level. Whereas simpler, the study at the level of groups of bee isolated from their hive, cannot be considered as a good model for entire bee colonies for two reasons : (i) isolated bees die rapidly (either they are infected with virus or not) and (ii) colonies may develop strategies to overcome virus infections.
2. Any behavioural changes in honeybees induced by virus infections that may affect colonial traits should be identified : e.g defence, hygienic behaviour, flight behaviour, intracolonial behaviour of infected and non infected workers.

4.5.4. Recommendations of the working group
Insect Immunity and virus latency
Rapporteur : Brenda BALL, Peter ROSENKRANZ and Jean-Luc IMLER

The insect response to infection is based on cellular and humoral defences but differs significantly from vertebrate systems in the degree to which recognition and memory contribute to immunity. The haemocytes of the honey bee have been characterised but in many instances their role and function are incompletely described or understood. Similarly, a range of antibacterial proteins produced by bees in response to various challenges have been recognised, but it is not known whether either of these defences systems are activated or effective against virus pathogens. Additionally to cellular and humoral defences, social insects can adapt behavioural responses to pathogen challenge at both the individual and colony level. A detailed investigation and understanding of all kinds of the innate immune and adaptive behavioural responses of populations, and the different life stages within them, would make a significant contribution to epidemiological studies.

Many of the viruses of bees persist within individuals at levels that are not readily detectable and that apparently cause no gross pathology. However, it has been demonstrated experimentally that these sequestered viruses can be activated and induced to multiply to lethal levels by a number of diverse triggers. Progress in the elucidation of some of the physiological and immunological pathways involved in these events has been made in other insects, facilitated by knowledge of the insect genome and functional approaches (genetics, transgenesis, RNAi). It is to be hoped that the honey bee genome project will ultimately make an important contribution in this area.

Insect immunity and virus latency are important fundamental areas that could provide valuable insights into infection processes and disease epidemiology, but of which at present we have only limited knowledge and understanding. Because of the close interdependence of these two areas research, progress would be greatly enhanced by bringing together specialists in each discipline for the design and undertaking of integrative studies. Some key questions and priority areas for further investigation were identified.

1. Investigation and characterisation of the response of honey bees to pathogen or other challenges could initially be based on currently known immune systems and pathways in other insects. Looking for homologies and differences to model systems would provide a simple first approach : in particular as infection markers are known for other insects, they should be looked for in infected honey bees.

2. The genetically regulated susceptibility to infection is well documented in Drosophila as proven by the lethal infection of mutant immuno-deficient individuals infected by opportunistic bacteria or fungi. The new information on the bee genome should be used in similar approaches.

3. Are the latent/occult honey bee viruses transcriptionally active and in what form do they persist; as intact particles or as RNA? What are the main tissues or cells in which these viruses persist and does this differ with different virus types and different life stages?

4.5.5. Recommendations of the working group
Evolutionary Epidemiology
Rapporteurs : Brenda Ball, Mark JF Brown and Ingemar Fries

1. Research shall be oriented to determine the basic biology of bee virus infections :
   - transmission routes and persistence of viruses,
   - transmission rates (vertically and horizontally between individuals, colonies, apiaries),
   - impact, especially with respect to environmental stress, eg. nutrition, environmental pollution by pesticides and other xenobiotics, etc.

Whereas some knowledge is available on transmission routes and persistence of some virus infections, virtually nothing is known about transmission rates. There is an urgent need for quantification of different transmission routes and for much better documentation of the persistence of virus infections both in the absence and presence of *Varroa destructor*.

2. Standardised epidemiological surveys should be conducted using appropriate direct sampling and detection techniques for measuring incidence, prevalence and distribution of bee virus infections across the EU.

3. Epidemiological and evolutionary models should be created, based on the knowledge acquired through the researches described in paragraphs 1 and 2, to understand bee/virus associations and predict their development.

4. Biogeographical studies of virus isolates should be developed to create a phylogeny of honey bee viruses.

Finally, the group proposed that studies initially may be concentrated on one particular honey bee virus to produce a detailed understanding of such a system.

4.5.6. Recommendations of the working group
Management of Bee Diseases: Economic Impact
Rapporteur : Keith S. DELAPLANE

1. Successful control of bee diseases and pests will depend on a sound understanding of the biology underlying the host/pest/parasite relationship.

2. Specific management programs should be built on the principles of Integrated Pest Management (IPM), stressing reduced reliance on the use of acutely toxic pesticides. Components of IPM include but are not limited to: development of economic (action)

thresholds, genetic host tolerance, cultural practices that interfere with parasite life history, social immune systems, beneficial disease-antagonistic organisms, and semiochemicals that can be used to trap pests or disrupt their mating. IPM can be expected to delay onset of economic thresholds and reduce overall use of acutely toxic pesticides.

3. Once economic thresholds are achieved, acutely toxic pesticides should only be used at lawfully-prescribed rates and in rotation, such that active ingredients are not used successively for avoiding or at least delaying the onset of resistance of the pest to the active ingredients.

4. The expected outcomes of IPM-based disease and pest control include, but are not limited to: reduced residues of acutely toxic pesticides in bee hive products and the environment, reduced occupational exposure of beekeepers to toxins, prolonged years of useful life for a limited pool of pesticides, improved reproductive performance of queens and drones, and increased colony population sizes and productivity.

5. Appropriate response to unknown causes of bee mortality requires establishment of standardized diagnostic avenues, establishment of regionalized baseline record keeping to discern historic trends in disease incidence, and beekeeper education programs to improve accurate diagnoses at the local level.

4.5.7. Recommendations of the working group

Regulatory issues
Rapporteur : Mike BROWN

For taking forward regulatory issues, the group recommended not to include honey bee viruses in the OIE Codes Terrestrial Animal health code at present, as there is not enough known about them or their distribution to legislate but it recommended :

1. to conduct delimiting surveys and monitoring programmes within the EU and elsewhere using recognised standard diagnostic methods and protocols to assist with: improving information on distribution and status for recognised bee viruses, assessing and identifying emerging or potential risks. In this way it will be possible to get a "handle" on what we may be dealing with, what their distribution is and what the economic impact might be.

2. to establish methods to measure pathological effects, impact in general including economic impacts, and set up studies to fill in identified gaps in knowledge.

3. to collect more detailed data on disease agents; epidemiology of honey bee virus infections to provide better information both to policy personnel at Commission and Member State level, for researchers and beekeepers. These information should include definitions and descriptions of the diseases, tests used, causative agents. {In order to consider legislation it is necessary to know what dealing with, what the distribution is, what are the disease signs, what is the impact, how to diagnose and so forth}.

4. to provide better training and education for beekeepers to identify disease problems, provide information on good husbandry, and information on international trade rules. This may help to reduce the risks of illegal or uncertified trade in honeybees and any introductions of exotic pests.

5. to rapidly investigate the causes of any new significant losses of honey bee colonies when they occur, and this could be through collaborative efforts across Europe.
Information obtained could be placed on a website, eg OIE but also EU wide website.

5. The expert workshop and the book "Virology and the Honey Bee".

5.1. The expert workshop background.

The aim of the workshop was to gather a smaller panel of experts to which would be assigned the task to use the scientific work presented during the scientific meeting to produce an overview of current virology status of the honey bee and to propose a framework for future research programmes on virology and the honey bee.

Of course, the BRAVE consortium had previously planned this task and already decided to give to it the form of a book that should ideally be a scientific reference for several years. But we had also foresaw to use the experience of the plenary scientific meeting to readjust our initial project. During the plenary scientific meeting, we (i.e. the consortium members) gathered several times, and before the end of the meeting, several possible authors were selected for the quality of their talk, their ability to encompass several scientific approaches which appeared during the working group discussions and their readiness to participate in our common objective.

5.2. The participants.

The list of the participants to this second part of the project was as follows :

First name	Family name	Organisation
Brenda	BALL	Rothamsted Res - UK
Elke	GENERSCH	Länderinst. Bienenkunde - D
Ingemar	FRIES	SLU - SW
Jean-Luc	IMLER	CNRS Strasbourg - F
Joachim	DE MIRANDA	Pen. State Univ. - USA
Magali	RIBIERE	AFSSA - F
Mark	BROWN	Univ. of Dublin - Irl
Michel	AUBERT	AFSSA - F
Mike	CARTER	Univ. of Surrey - UK
Norberto	MILANI	Udine Univ. - I
Rosie	HEILS	NERC CEH Oxford - UK
Robin	MORITZ	Univ. Halle - D

For personal reasons, it had been impossible for Dr Mike BROWN (CSL, York, UK) to attend the meeting. However, as shown in the following lines, he did participate efficiently in the book project.

It is worthwhile to note that this list gathers senior and well recognised scientists and younger scientists whose initial works and personal commitment are very promising. Additionally, even if the gender balance is not yet ideal, it is encouraging.

5.3. The programme and the task.

The participants arrived on the 2nd September and departed on the 7th. The work was intense during the four complete days. Following a one hour session where the advancement of the project was discussed, individual authors (or small groups of authors) worked separately on their respective chapters. At intervals, authors submitted their task to the other contributors for suggestions and editorial comments.

At the end of the meeting, the final plan of the book and the editor line were agreed, and all chapters had been at least partially written according to the following plan :

Title : Virology and the Honey Bee

Chapter 1. Introduction – by the editors.

Chapter 2. Natural history and distribution of honey bee viruses
– by Magali Ribière and Brenda Ball

Chapter 3. Molecular characterisation of honey bee viruses
– by Mike Carter and Elke Genersh

Chapter 4. Detection techniques for honey bee viruses
– by Joachim De Miranda and Brenda Ball

Chapter 5. Impact of virus infection in honey bees
– by Michel Aubert

Chapter 6. Covert infections in honey bees
– by Rosie Hails and Brenda Ball

Chapter 7. Evolutionary epidemiology of virus infections in honey bees – by Mark Brown and Ingemar Fries

Chapter 8. Innate immunity of insects to infection
- by Catherine Dostert and Jean-Luc Imler

Chapter 9. Honey bee Genomics and Breeding for Resistance to Virus Infections
– by Robin Moritz and Jay Evans

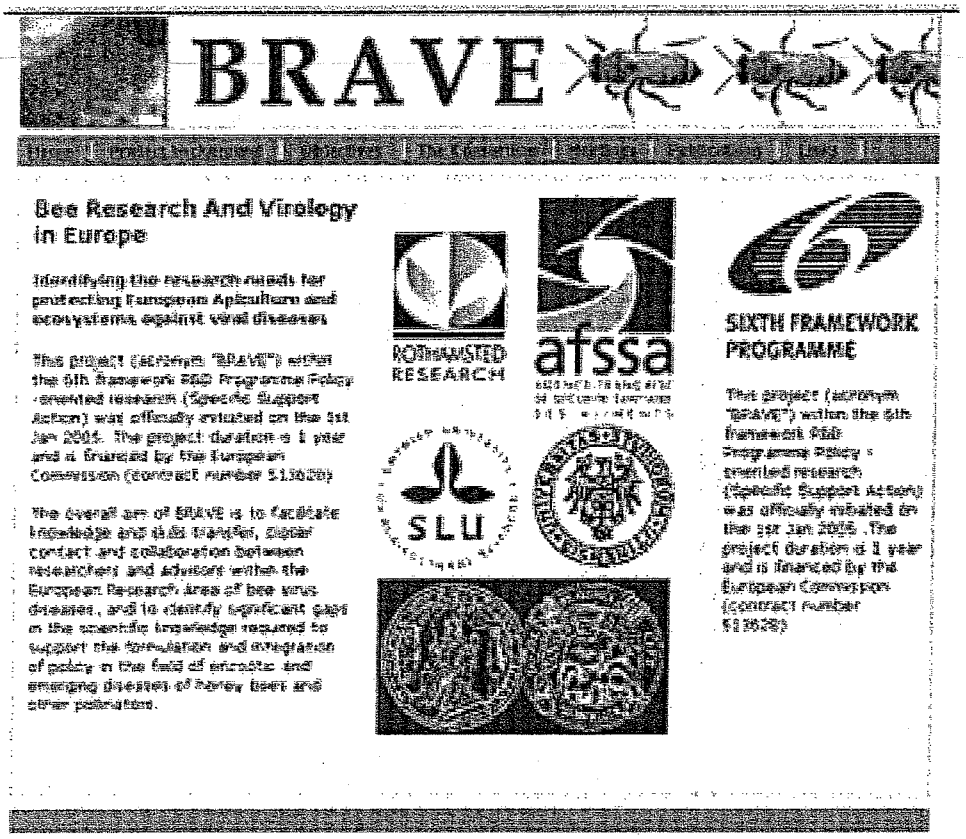
Chapter 10. Overview of the regulatory framework for apiculture
– by Michael Brown

Chapter 11. Conclusions – by the editors.

The task had been pursued during the following months. Already six out of the nine main future chapters of "Virology and the Honey Bee" have been achieved (the titles of those chapters are written in bold characters in the above box). Serious personal problems of the co-author of all the 3 remaining main chapters have caused some delay and of course, the whole book still requires final editing input (general presentation, table of indexes, general introduction and conclusion). Annex 4 gives an example of one chapter of the future book.
On the basis of which has already been achieved, we are now sure that the book will be published with the help of the European Commission far before the end of this year.

6. The web-site

The BRAVE website (http://www.entom.slu.se/brave/) has been accessible since the middle of February 2005 and is still active. Only the "home" page of the site is reproduced below. From this page, the reader can have access to the project background, its objectives, the consortium, the meetings, publications (from where the proceedings of the plenary scientific meeting can be downloaded), and links.



7. Impact and dissemination

The present call was an excellent opportunity to create common access to the latest results obtained in insect virology, immunology and epidemiology for the European researchers specialised in bee diseases. Without the support of the European Community it would not had been possible to organise such a meeting using the best experts in insect virology and immunology world-wide to interact with European (and non-European) experts in bee diseases.
The inter-disciplinary approach of the BRAVE initiative had the ambition to lift European honey bee pathology research, in particular the virus research, to new levels.

The scientific community, the first category of stakeholders, have been informed of the BRAVE project a) through an announcement in a plenary session and posters during the first EurBee meeting (Udine – Italy - 19-23 sept 2004) (see annex 1), b) through announcement in the scientific press specialised in bee diseases (see annex 3), and c) through the BRAVE web site.

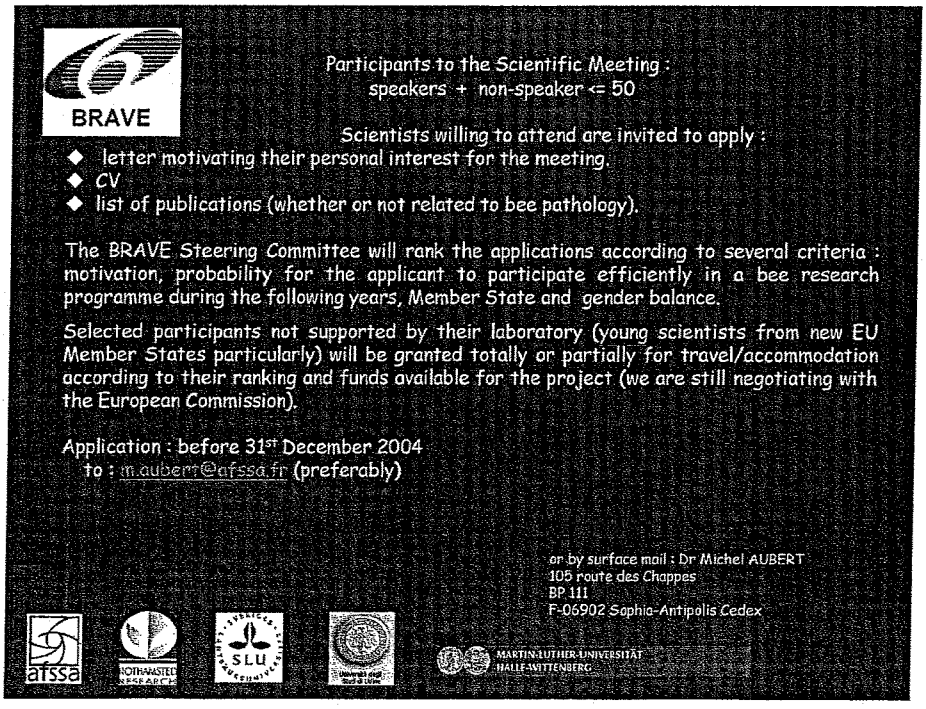
The scientific community will have access to the information provided during the scientific meeting through the proceedings of this meeting. The great many of the participants provided high quality papers that have been edited according to standards of international scientific meetings. An electronic edition (a "pdf" compressed file) of these proceedings have been disseminated thought email to each participants. They will additionally receive a bound paper edition by surface mail.
Moreover, the proceedings can be downloaded from the BRAVE website and more paper exemplars will be distributed during the next "EurBee" meeting that will be held in Prague (Czech Republic) 10-14 September 2006.

The second category of stakeholders were the policy makers. The BRAVE project had the ambition to shed light on the controversy where some veterinary officers and bee specialists suggest that viruses should not be included in trade regulations, while others claim that because of the possible economic impact of some bee viruses, inclusion should be considered. It was a great advantage to host during the scientific meeting two representatives of the EC and one of the OIE for the appropriateness of the discussion held on international trade measures for health protection of bees.

The third category of stakeholders were the bee keepers and more generally the whole apicultural industry. Viruses which were known for many years as inapparent infections only are now causing colony deaths due to the introduction of the *Varroa* parasite. This mite transmits viruses directly into the haemolymph of bees during its feeding. The feeding activities of the mite also triggers virus replication, where latent, non-lethal infections may develop into overt infections causing massive bee mortality. The increase in prevalence of some infections has introduced turmoil and large economic losses into the honey bee industry : unexplained bee colony mortality, and residues in hive products due to misuse of acaricides are two important consequences from the *Varroa* mite and associated virus infections. The dissemination of new understanding on the role of viruses in bee populations in Europe will be directly beneficial for the apicultural industry, whereas it may be difficult to measure this benefit.

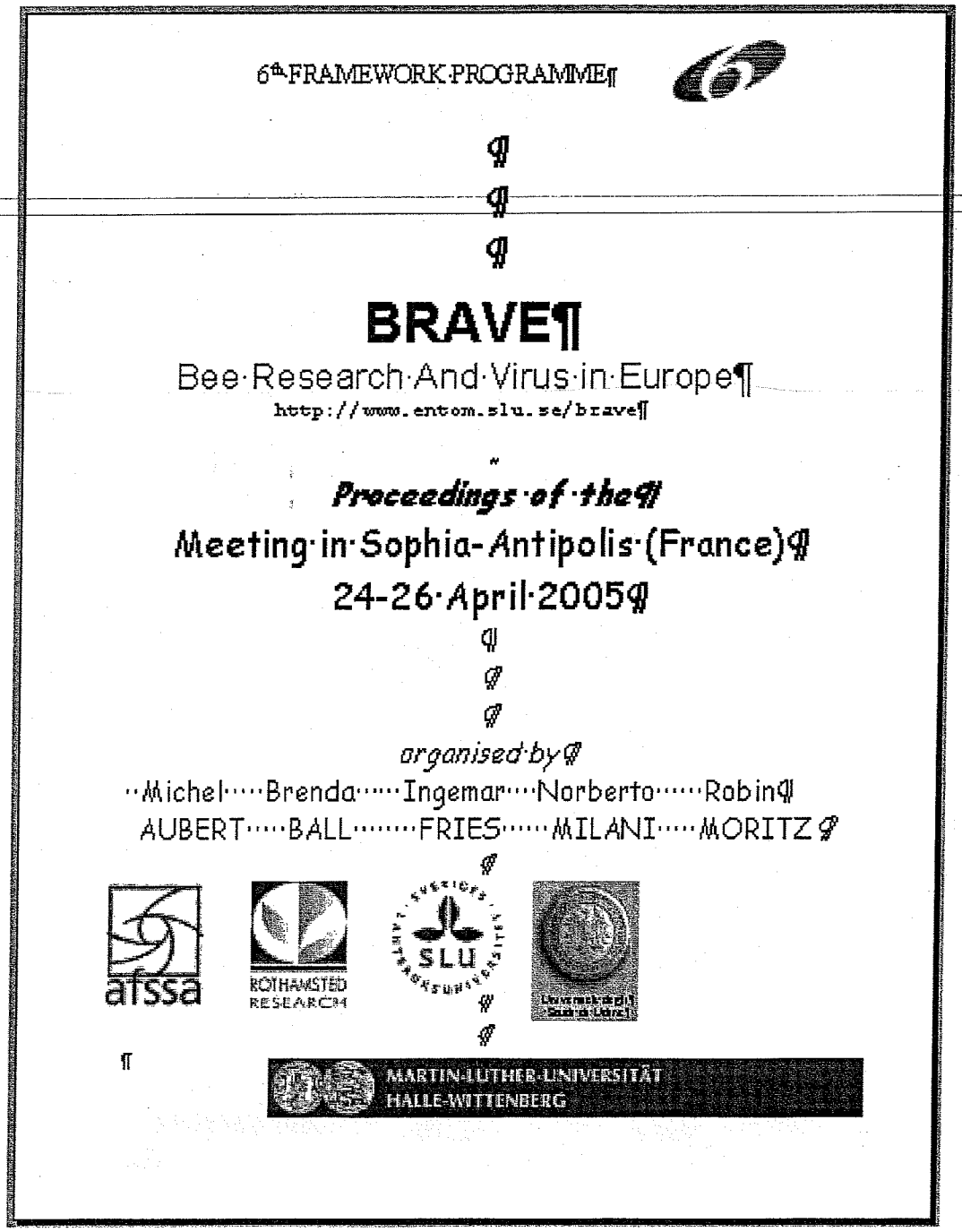
Annex 1

Poster on the BRAVE SSA during the first EurBee meeting (Udine – Italy - 19-23 sept 2004 calling for application to attend the BRAVE scientific meeting.



Annex 2

Front cover of the proceedings of the plenary scientific meeting.



Annex 3

Announcement of the BRAVE project
in the international scientific review APIDOLOGIE 36 (1) January-March 2005
(impact factor = 1.241)

SIXTH FRAMEWORK PROGRAMME
PRIORITY 6

BRAVE (**B**ee **R**esearch **A**nd **V**irology in **E**urope)

This project within the 6th framework R&D Programme Policy -oriented research (Specific Support Action) was officially initiated on the 1st Jan 2005. The overall aim of BRAVE is to identify the research needs for protecting European Apiculture and ecosystems against viral diseases

Approximately twenty different viruses have been described in bees so far but the pathogenicity has only been described partly for some of them. The consequences of bee virus infections have been underestimated and as in other food producing systems, apiculture is confronted to emerging and introduced pests and pathogens.. Due to international exchanges of bees, exotic viruses may be introduced into the EU.

The aim of BRAVE is to organise

1. A scientific meeting : organised in Sophia-Antipolis (24 to 26 April 2005), it will gather:
a) international experts with a broad base of skills in insect virology, diagnosis, immunology, disease epidemiology, international trade, policy formulation and disease risk assessment, and
b) internationally recognised scientists involved in fundamental and applied research on bees and related pollinator species.
Exchanges between both categories of experts should favour novel approaches in apicultural research and take advantage of recent developments in the general fields of virology and insect immunology.

2. A scientific workshop. Based on the proceedings of the meeting, several experts will subsequently meet and propose research priorities at the fundamental and applied levels. Their aim will be to put in place the framework for integrating an European research effort in bee virus diseases in support of the European Community policy.

Michel AUBERT (coordinator), **Brenda BALL, Ingemar FRIES, Norberto MILANI** and **Robin MORITZ** (members of the Steering Committee).

Annex 4

as an example of a chapter of the future book "Virology and the Honey Bee",
the chapter 7

Evolutionary epidemiology of virus infections in honey bees
– by Mark Brown and Ingemar Fries

---

is joined as a "pdf" file to the present report.

Chapter 7

EVOLUTIONARY EPIDEMIOLOGY OF VIRUS INFECTIONS IN HONEY BEES

Mark J.F. Brown and Ingemar Fries

Table of contents

TOC: to be updated at the end of the editing process

Initial page: arabitrary value

1. Introduction

The field of evolutionary epidemiology aims to understand how parasites and hosts interact over ecological and evolutionary time. One key aspect of this aim is to determine why different parasites damage their hosts to different degrees. In other words, why are some parasites apparently benign, whilst others cause rapid mortality? The traditional view of parasite virulence suggested that over evolutionary time parasites should decrease the amount of harm that they would do to their hosts (reviewed by Bull, 1994; Lenski and May, 1994). However, over the last 25-30 years, numerous theoretical and empirical investigations have demonstrated that this simplistic view is incorrect. This body of work relies upon the insight (or assumption) that parasite virulence is related to parasite fitness through its effects on increasing or decreasing the probability of transmission. Consequently, in contrast to the traditional view, we should expect parasites to exhibit a level of virulence that maximises their own reproductive success within a complex ecological, epidemiological and evolutionary world. A large number of factors related to the biology of parasites, hosts and their interaction have been suggested to play a role in the evolution of such parasite-optimal virulence. In Section 2 we provide a general review of investigations into these factors. In Section 3 we focus on the biology of honey bees in order to highlight host-specific traits that have implications for parasite virulence in this system. In Section 4, we bring these two themes together and ask what levels of virulence we might expect to find in viral parasites/diseases of bees, and whether our knowledge is sufficient to explain the levels of virulence that we observe, especially with respect to the recent involvement of the ectoparasitic mite, *Varroa destructor*. We end with a summary of our general points and provide perspectives on where future research into the evolutionary epidemiology of viruses in bees might best be directed.

2. Evolution of virulence – important parameters/models

2.1. Defining terms

The terms 'virulence' and 'parasite' mean many things to different people. In order to provide focus and avoid confusion, we start by defining these terms.

2.1.1. What is virulence?

Parasite virulence encompasses a wide variety of effects. A

broad definition of virulence would encompass any changes caused by a parasite in its host that reduce the evolutionary fitness of said host. This could include mortality, morbidity, and partial or complete castration. These terms themselves encompass a wide variety of parasite effects. For example, host mortality could increase if (i) parasites release toxins within their hosts that induce mortality, (ii) parasites successfully compete for internal (and essential) host resources, (iii) parasites manipulate host behaviour to enhance their own transmission. At the broader level, whether a parasite causes mortality, morbidity or castration (or some combination of these factors) has implications for the evolution of virulence (Day, 2002). Nevertheless, the majority of theoretical models treat virulence as being equivalent to additional host-mortality. This should be borne in mind when extrapolating the results of such models to biological systems.

While an understanding of virulence relies on treating it as an adaptation to maximise parasite transmission, it should be noted that virulence need not be an adaptive result of host-parasite evolutionary epidemiology. Virulence may be non-adaptive if it occurs after transmission has occurred (e.g., HIV), if it results from parasites invading atypical host tissues (e.g., poliomyelitis virus), or if a host-parasite relationship is novel (e.g., *Varroa destructor* in *Apis mellifera*, see Section 4).

2.1.2. What is a parasite?

Parasitism can be defined as an ecological relationship between individuals from two species, where one lives in or on and obtains resources from and consequently damages the other. Consequently, the term 'parasite' encompasses organisms as diverse as viruses and arthropods. From the perspective of theoretical models, parasites are often divided into micro- and macroparasites. Microparasites include viruses, bacteria, fungi and protozoa, and are assumed to have significantly faster generation times and significantly higher reproductive rates than their hosts. In contrast, macroparasites include the metazoa (animals) and are assumed to have much slower generation times and lower reproductive rates than microparasites. This division is theoretically important because micro- and macroparasites are modelled in different ways, and biologically important because it has implications for the evolution of virulence. It should be noted that the majority of the models discussed below are aimed at understanding the evolutionary epidemiology of microparasites. Finally, we note that we use the terms 'parasite', 'pathogen' and 'disease' interchangeably throughout this chapter.

2.2. Transmission routes and the evolution of virulence

Parasites vary in how they get from one host to another. Horizontal transmission, which involves the infection of one individual by another through direct or indirect transfer of the parasite, is probably the most common route of transmission. However, vertical transmission can play an important role in the propagation of parasites from one generation to the next. Unsurprisingly, the type of transmission used by a particular parasite – horizontal, vertical, direct, indirect, immediate or delayed – has consequences for the amount of damage done to the host.

2.2.1. Horizontal transmission

Imagine a host-parasite system where the parasite does little harm to its host and is horizontally transmitted. What should we expect to happen over ecological and evolutionary time? Parasite fitness relies on transmission to new hosts, and thus any parasite strain or mutation that transmits more rapidly to new hosts will be ecologically and epidemiologically more successful, and will eventually replace other strains in the population. However, enhanced transmission cannot come for free. The majority of theoretical models (see references throughout) assume that enhanced transmission is the result of a higher reproductive rate of the parasite – that is, the parasite is producing more propagules or infective stages more rapidly than its competitors. To do this, the parasite needs to take more resources from its host and, thus, should have a higher viru-

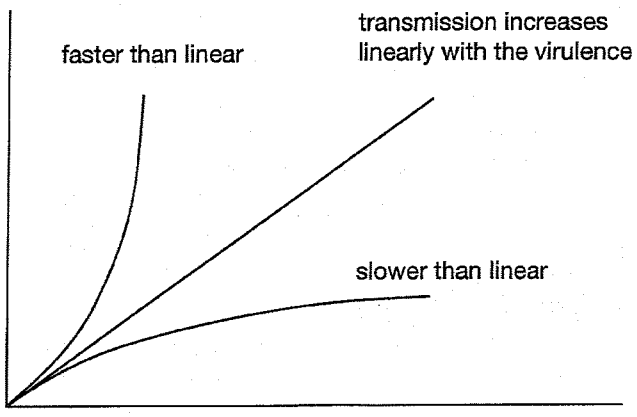


Figure 1. The graph shows three possible relationships between virulence (which is assumed to be due to parasite reproduction) and transmission. Natural selection to increase parasite transmission inexorably leads to the evolution of higher virulence.

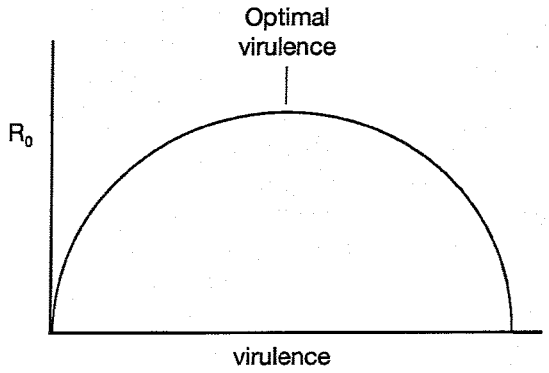


Figure 2. Host mortality constrains the evolution of virulence. The graph illustrates what happens when host mortality, either background mortality or due to parasite virulence, is incorporated into the model illustrated in Fig. 1. Because host mortality acts as a brake on parasite transmission, the relationship between virulence and transmission reaches an optimum where transmission is maximised, and thus an intermediate level of virulence is selected for.

lence, or impact on its host. This relationship can be modelled as a curve whereby parasite reproduction or transmission increases with increasing virulence (Fig. 1). However, an increase in parasite virulence may also result in increased host mortality, which in most cases truncates transmission. Thus, the relationship between parasite reproduction, transmission and virulence is constrained by host mortality (Fig. 2). This logic underpins the trade-off models for the evolution of virulence which assume that parasite virulence evolves as a result of selection for enhanced transmission (parasite fitness).

It should be noted that this result comes with a number of caveats. First, evidence for a positive relationship among parasite reproduction, virulence and increased transmission is rare, although it is beginning to accumulate (Ebert, 1994; Ebert and Mangin, 1997; Lipsitch and Moxon, 1997; Mackinnon and Read, 1999; Messenger et al., 1999). Second, it relies on the assumption that the host and parasite populations are at equilibrium (see Section 2.3.2). Nevertheless, evidence from serial passage experiments, where the trade-off between virulence and transmission is removed, provides strong evidence to support the idea that horizontal transmission selects for higher virulence (Ebert, 1998).

2.2.2. Vertical transmission

Vertically transmitted parasites rely on passing from one generation

to the next through successful reproduction of the host. In solitary organisms, this generally involves trans-ovarial transmission, while in social insects it generally implies transmission via new queens (but see Section 3 for the honey bee case). It is easily seen that, in strong contrast to horizontal transmission, vertical transmission should select for lower levels of virulence. Any vertically-transmitted parasite that decreases the fitness of its host will decrease its own reproductive success (as the number of offspring carrying it will be reduced). Consequently, natural selection will favour parasites which cause less harm to their hosts (have lower virulence), leading eventually to the evolution of completely benign parasites (or commensals). While there are exceptions to this general rule, e.g., *Wolbachia*-like organisms that manipulate host offspring sex-ratio, or even host functional gender, to increase their representation in the next generation, the prediction is a strong one.

2.2.3. Mixed horizontal and vertical transmission

If horizontal transmission selects for higher virulence and vertical transmission selects for lower virulence, what should we expect in parasites that rely on both modes of transmission? An initial verbal prediction was that there should be a continuum of virulence, ranging from low virulence in mainly vertically-transmitted parasites, to high virulence in mainly horizontally-transmitted parasites. A classic study of fig wasps and their nematode parasites provided apparently strong support for this prediction (Herre, 1993). However, more recent theoretical work has made the picture more complex. Lipsitch et al. (1996) modelled the interaction between horizontal and vertical transmission as it relates to virulence and parasite epidemiology. They examined two scenarios – one where higher rates of vertical transmission did not have to be correlated with increasing virulence, and one where higher vertical transmission came as a result of higher virulence. In the first case, they found that parasite strains showing vertical transmission coupled with low virulence dominated the parasite population (i.e., vertical transmission leads to lower virulence). In addition, they demonstrated that as rates of horizontal transmission increased (due to increased host density) horizontal transmission also selected for lower virulence. This surprising result emerges directly from the epidemiological parameters of their model. Host individuals could only contain single-strain infections and thus, as horizontal transmission increased in frequency the host population approached saturation by the parasite. At this point, vertical transmission becomes the dominant mode of transmission and lower virulence is selected. In the second case, the parasite population became dominated by strains of higher virulence

using horizontal transmission (horizontal transmission leads to higher virulence). However, as Lipsitch et al. (1996) noted, there are important caveats to their conclusions. First, they assumed an absence of multiple infections within hosts (see Section 2.4). Second, they assumed a fixed host population where prevalence levels for the parasite would increase and (potentially) saturate across time (see Section 2.3.2). Thus, it remains unclear exactly what levels of virulence we should predict for parasites which utilise both horizontal and vertical transmission.

2.2.4. Direct vs. indirect horizontal transmission

Horizontal transmission can be direct, requiring close physical proximity of the infected and uninfected individuals, or indirect, requiring a vector of some kind. Human malaria is a classic example of a parasite that uses indirect horizontal transmission, travelling between hosts via mosquitoes. Ewald (1983, 1994) was the first to propose that indirect transmission has implications for the evolution of virulence. He suggested that transmission of vectored parasites may be enhanced by the fact that virulence need not trade-off with parasite reproduction. The argument runs as follows: assume that a parasite causes morbidity (reduced locomotion) in its host. While this would reduce parasite transmission for directly horizontally transmitted parasites – by lowering the potential contact rate with susceptible individuals – for vectored parasites it might even increase transmission as transmission is determined by vectors rather than by contact rate. Consequently, the trade-off between virulence and transmission (caused by increased parasite reproduction within the host) may be lost, and thus we would expect higher virulence in vectored parasites. This verbal argument is logically appealing and some empirical evidence exists to support it. However, there are also vectored parasites that exhibit low virulence. Recent theoretical work by Day (2001, 2002) has suggested that Ewald's hypothesis only follows under very restricted conditions and, in fact, that there is no *a priori* reason to assume that vectored and directly transmitted parasites should differ in their level of virulence (but see Section 2.3.3.1).

An alternative approach to understanding virulence in vector-born parasites and diseases starts by noting that, because vectors actively search out new hosts, such parasites/diseases undergo frequency dependent transmission (O'Keefe, 2004). Despite fundamental biological and theoretical differences between mass action and frequency-dependent transmission, models of the latter still do not suggest that higher virulence should be expected in vector-born diseases.

2.2.5. Long-lived free-living propagules – the curse of the pharaoh?

The death of Lord Carnarvon from a mysterious disease after opening the tomb of Tutankhamen has inspired the suggestion that parasites with long-lived propagules will evolve high virulence. The verbal argument is based on the trade-off model, and runs that by avoiding the cost of virulence (that is, host death and the end of transmission), such parasites are free to evolve higher virulence. Bonhoeffer et al. (1996) explored this argument using a theoretical model and showed that, for most situations, parasite virulence should be independent of propagule longevity. However, if the host-parasite system is not at equilibrium and the parasite propagule dynamics are faster than those of the hosts, then increased longevity predicts higher virulence. More recent studies, incorporating mixed infections (see Section 2.4) and spatial structure in the host population (see Section 2.3.3) make the picture even more complicated.

2.3. Epidemiology and the evolution of virulence

The epidemiology of a parasite depends upon features of the host population and on the ability of the parasite to exist in single- and/or multiple-infections. These factors interact fundamentally with modes of transmission in determining parasite spread and prevalence within a host population, and the level of virulence to which a parasite might be expected to evolve.

2.3.1. Host demography

A key feature of host populations that interacts with both parasite epidemiology and the evolution of virulence is the background host mortality rate. The general expectation is that when hosts are long-lived, parasites should have low virulence (that is, parasite-related host mortality should be low or take a long time to occur). In contrast, when hosts are short-lived, parasites should have higher virulence (Lenski and May, 1994). This result comes directly out of the trade-off model (see Section 2.2.1). As host lifespan declines, parasites have decreased opportunities for transmission and thus the optimal virulence increases as a correlate of selection for increased transmission. This theoretical prediction has recently been supported experimentally in a study that manipulated transmission in the nuclear polyhedrosis virus of the gypsy moth (Cooper et al., 2002).

Unfortunately, and as with most other aspects of the evolution of virulence, the situation is not quite that simple. Recent work by Williams and Day (2001) has emphasised the fact that increased host mortality only automatically selects for higher virulence if background mortality and parasite-induced mortality are additive. If different causes of mortality

are not additive, which is the case when virulence is context-dependent (e.g., Brown et al., 2000, 2003), then reduced host longevity may or may not select for higher virulence, depending upon the exact relationship between background mortality rates and parasite-caused mortality. Nevertheless, in most cases it seems likely that this relationship is such that some increase in virulence will be selected for. Further complications result if multiple infections are taken into account (see Section 2.4).

2.3.2. Host population size

While the majority of the models discussed above assume that host populations are at a stable equilibrium, this is not necessarily true for many biological systems. Hosts may exhibit constant growth, or cyclical patterns of growth and decline. The latter pattern can be found in both annual, e.g., bumble bees, and perennial species, e.g., honey bees. This has a number of implications. First, predictions about the evolution of virulence that emerge from models relying upon equilibrium dynamics may not be applicable to more natural biological systems. Second, many parasites and diseases which appear at first sight to be endemic may more accurately be described as epidemic diseases, with patterns of rapid spread and then rapid decrease within the host population.

Of the models described above, only a few refer to systems which are not at equilibrium. Bonhoeffer et al. (1996) (see Section 2.2.5) compared the results of their analyses between host-parasite relationships in equilibrium and disequilibrium. They found that there was a significant effect of disequilibrium, with predictions for the relationship between propagule longevity and virulence depending upon whether the host or parasite was cycling faster in the system. Similarly, Lenski and May (1994) showed that in an epidemic (disequilibrium) system, where either the parasite is invading a new host population or the susceptible host population is constantly growing, higher levels of virulence evolve than in an endemic (equilibrium) system.

2.3.3. Host population structure - spatial

The epidemiological models that underlie most of our understanding of host-parasite dynamics, both in ecological and evolutionary time, depend upon the mass action principle with respect to transmission. Basically, they assume that transmission is a function of the numbers of infected and susceptible individuals in the host population. This ignores the fact that most host populations exhibit some form of spatial structure. This is especially true for social insects, where high levels of population structure (caused by division of labour) occur within colonies (Schmid-

Hempel, 1998). Only a few studies have examined the potential effect of host population structure on the epidemiology and evolution of parasites.

2.3.3.1. Structure and direct vs. indirect horizontal transmission

Boots and Sasaki (1999) used a lattice model to represent a spatially-structured host population. When parasite reproduction was local (that is, bound to the host) but transmission was global, they found that virulence evolved as expected under standard epidemiological models. However, as transmission became more locally restricted (modelling the situation where parasitised hosts are more likely to infect near- over far-neighbours) the predicted level of virulence similarly declined. This suggests that vectored diseases/parasites may indeed exhibit higher levels of virulence than directly transmitted parasites (see Section 2.2.4). However, and perhaps more importantly, it suggests that quantitative predictions about the level of parasite virulence derived from mass action or non-spatially explicit models are likely to be too high. Similar results were found by O'Keefe (2004) when spatial structure and frequency-dependent transmission were combined.

2.3.3.2. Structure and free-living propagules

Kamo and Boots (2004) examined the problem of the curse of the pharaoh (see Section 2.2.5) in spatially explicit populations (lattice models). While they found some situations in which propagule longevity correlated positively with virulence (see Section 2.5), in general they concluded that there was no evidence for the curse of the pharaoh and that in fact higher virulence may be selected for by shorter rather than longer-lived infective stages.

2.3.4. Host population structure – genetic

As well as being physically structured, host populations can exhibit genetic structure. This may be driven by low levels of offspring dispersal producing 'islands' of high genetic relatedness within the population (social insects provide an extreme example of this situation). The ability of parasites to infect and reproduce in hosts is assumed to be based on interactions between host and parasite genotypes (Schmid-Hempel, 1998). Consequently, host genetic population structure may influence both the spread and prevalence of parasites, as well as the evolution of virulence. Regoes et al. (2000) predicted that in a system with two host types, parasites should either evolve to be generalists, with correspondingly low levels of virulence, or to be specialists, exhibiting higher virulence on each host type. Gandon et al. (2002) examined the situation where host resistance varies (presumably due to genetic variation). In their model, if only sin-

gle infections occurred there was no effect on the evolution of parasite virulence (but see Section 2.4). While recent empirical work suggests a relationship (in ecological time) between the epidemiology and impact of parasites and genetic heterogeneity in social insects (bumble bees: Shykoff and Schmid-Hempel, 1991; Liersch and Schmid-Hempel, 1998; Baer and Schmid-Hempel, 1999, 2001; honey bees: Tarpy, 2003), the evolutionary implications for parasite virulence remain untested.

2.4. Single vs. multiple infections

As with all organisms, parasites compete with conspecifics for resources, i.e., hosts. If a host can only contain one infection at a time, then this competition occurs in the arena of transmission and underlies the evolutionary relationship between horizontal transmission and higher virulence (see Section 2.2.1). However, if multiple infections can infect a given host individual, competition will take place among parasite strains within a host. This has immediate implications for the epidemiology and evolution of the parasite.

Multiple infections are most likely when parasite prevalence is high, the probability of transmission is high, or when parasites mutate within their host (see Section 2.5). High host density, spatially structured populations and the coexistence of vertical and horizontal transmission may all contribute to increasing prevalence and probability of transmission. Consequently, the theoretical predictions described in Sections 2.2.1, 2.2.3, 2.2.4, 2.3.3 and 2.3.4 may all be modified by multiple infections.

Parasite competition via multiple infections can take two forms. Super-infection occurs when one parasite enters an already infected host and displaces its current parasite (superior competitiveness). Co-infection occurs when 2 or more parasites (or parasite strains) can occupy a single host at one time. In this case, competition for host resources will occur among those parasites. Both of these types of competition may lead to increases in the expected level of virulence. Parasites which utilise host resources most rapidly are likely to outcompete their conspecifics – this result is simply the extension of standard ecological competition theory. If virulence correlates with an increase in the rate of host resource use, then this competitive process should inexorably lead to an increase in parasite virulence.

Only a few theoretical studies have concentrated on the impact of single vs. multiple infections on the evolution of virulence (Claessen and de Roos, 1995; May and Nowak, 1995; van Baalen and Sabelis, 1995; Leung and Forbes, 1998; Gandon et al., 2001). In general, these studies predict that multiple infections should lead both to higher virulence (Claessen and de Roos, 1995; May and Nowak, 1995; van Baalen and Sabelis, 1995;

Leung and Forbes, 1998) and competitive exclusion among the parasite strains (Van Baalen and Sabelis, 1995; Leung and Forbes, 1998). In contrast to these theoretical predictions, Taylor et al. (1998) showed that mixed infections of rodent malaria exhibited higher virulence, but that this was not due to higher parasite reproduction. Even more surprisingly, Ebert and Mangin (1997) found results suggestive of the idea that mixed infections could lead to lower virulence in a microsporidian parasite of invertebrates. Gandon et al. (2001) used a theoretical model to show that this unexpected result could occur in a host-parasite system with superinfections. Because high rates of host mortality reduce the likelihood of multiple infections (by reducing contact rate and thus the opportunity for transmission), in a system with multiple infections virulence should be higher under low rather than high host background mortality rates. This prediction reverses the relationship among horizontal transmission, virulence and host mortality described in Section 2.3.1.

In other studies, Lipsitch et al. (1996) acknowledged that parasites with mixed horizontal and vertical transmission would evolve to higher virulence in the presence of multiple infections. Similarly, Gandon (1998) suggested that if mixed infections could occur then long-lived parasite propagules would be associated with higher virulence (see Section 2.2.5). Finally, one explanation for the empirical results of Herre (1993), previously taken to support the idea of a virulence continuum between horizontal and vertical transmission (see Section 2.2.3) is that higher virulence was driven by competition among nematodes in mixed or multiple infections.

This view of multiple infections is driven by the idea that within-host competition occurs through resource exploitation. However, parasites may compete more directly. Massey et al. (2004) showed that bacteria may compete directly via bacterial-specific toxins, making mixed infections less virulent than single infections. Obviously, to understand the impact of mixed or co-infections on the evolution of virulence we need a good biological understanding of how parasites interact within hosts.

2.5 Biology of the infection

So far in this review, the biology or epidemiology of parasite infections within hosts has been treated to a large degree as a black box. Most epidemiological models of parasite virulence assume that the production of transmission stages within infected hosts and the contact rates between infected and uninfected hosts are constant over the course of an infection (reviewed in Day, 2003).

The most obvious case where such an assumption is invalid is when parasites are semelparous, that is, host death is required for parasite trans-

mission. Ebert and Weisser (1997) showed that in such cases parasite virulence (the timing of host death) should be higher (earlier host death) when carrying capacity within the host is low (i.e., when parasite growth and reproduction within the host is limited) or when host mortality rates are high (mirroring the general relationship between background host mortality and virulence, see Section 2.3.1). Kamo and Boots (2004) applied the idea of semelparous parasite reproduction to the relationship between longevity of free-living propagules and virulence, and found that in a spatially explicit model (see Section 2.3.3.2) this was the only scenario under which both high virulence and high propagule longevity would co-evolve.

Day (2001, 2003) has examined within-host parasite biology in more detail. He has shown that when transmission occurs at an early stage within the lifespan of an infection (Day 2001) or if there is a timelag between the onset of transmission and the onset of the effects of parasite virulence (Day 2003) we should predict higher virulence.

An additional aspect which was examined by Bonhoeffer and Nowak (1994) was the effect of parasite mutation within individual hosts. They developed a model to look at the role of intra-host mutation and competition among parasite strains in the evolution of virulence. They found that intra-host competition generated a virulence polymorphism in the parasite population, shifted mean virulence beyond the optimal level for parasite transmission, and also that the parasite could evolve to intermediate levels of virulence even in the absence of a trade-off between transmission rate and virulence. Their model is particularly appropriate for viral diseases with high mutation rates and long infection periods.

From this work, it seems clear that incorporating details of the within-host biology of infections into epidemiological models will be an important step in understanding the evolutionary epidemiology of parasites.

2.5.1 Behaviour and transmission

Our focus in this chapter precludes an in-depth coverage of the impacts of parasites on host behaviour, and the obvious implications of such changes for either enhancing or controlling the spread of parasites (Schmid-Hempel, 1998). Nevertheless, such changes (due to host-manipulation by the parasite or adaptations of the host) are an integral part of the biology of host-parasite interactions. For example, sacbrood virus changes the behaviour of infected bees in a way that reduces further viral transmission (Chapter 2; Bailey and Ball, 1991). While such changes in behaviour may be implicit within transmission parameters in theoretical models, their inclusion as explicit factors may well lead to different dynamics and predictions for the evolution of virulence.

2.5. Mixed species infections

The vast majority of empirical and theoretical work has examined the epidemiological and evolutionary properties of host-parasite systems through single-species interactions. However, hosts can support many different parasite species at the same time. How these parasites interact, directly and indirectly, and impinge on each other's epidemiology and evolution, is an area that remains to be explored. Interactions among mixed infections within hosts may increase the population of both parasites, decrease one and increase the other, or decrease the population growth of both (Cox, 2001). If virulence and transmission are the result of increased parasite reproduction then such interactions have obvious implications for parasite population dynamics and virulence in both ecological and evolutionary time. Recent work on insect pathogens has suggested that even otherwise avirulent parasites can play an important role when in mixed infections (Thomas et al., 2003). Similar results have been found for viruses and mite interactions in honey bees (reviewed in Schmid-Hempel, 1998, pp. 27-31; see Section 4 below).

2.6. Discussion

It should now be clear that a large number of factors play a role in the evolutionary epidemiology of host-parasite relationships. Virulence is a key feature of such relationships, and its management is a key aim of evolutionary parasitologists. Despite this array of potential causal factors in the evolution of virulence, it may still be possible to make some preliminary generalisations. Firstly, from the parasite perspective, horizontal transmission and mixed (co-)infections are likely to result in the evolution of higher virulence. Secondly, from the host perspective, a high background host mortality rate and rapidly growing populations will also select for higher virulence in parasites. In contrast, spatially and genetically structured host populations appear, in general, to select for lower levels of virulence. Finally, vectored diseases may evolve to higher levels of virulence, but there is probably no reason to expect diseases that rely on free-living propagules for transmission to have higher levels of virulence than directly transmitted parasites and diseases. All of these factors turn out to be relevant in understanding honey bee viruses.

3. The honey bee as a host

Diseases in honey bees have been studied intensively within colonies and much is known about the intra-colonial spread and virulence of

a variety of parasites. However, only a few attempts have been made to discuss honey bee epidemiology from the colony level perspective (Royce and Rossignal, 1990; Schmid-Hempel, 1998; Fries and Camazine, 2001). Here we outline why epidemiological studies in honey bees must consider both individual-level and colony-level parasite reproduction and transmission if we are to understand and manage levels of virulence in parasites of honey bees.

The field of evolutionary epidemiology is rapidly expanding (see Section 2 above) as it has become apparent that an understanding of how parasite virulence evolves may also enable the management of disease processes to decrease virulence. However, in spite of the importance of this emerging field, the perspectives provided by evolutionary epidemiology have only recently been applied to social insects. The works of Paul Schmid-Hempel (e.g. Schmid-Hempel, 1995, 1998, 2001) have developed the framework for understanding the evolution of social insect hosts and their parasites. In this section we apply these ideas to honey bees to determine how the biology of *Apis mellifera* relates to their interaction with parasites. A search in the BIOSIS data base for the years 1945-2005 revealed only one relevant hit when searching for the combination "epidemiology" and "honey bees" (using the term 'epizootiology' produced no relevant hits). The application of evolutionary epidemiological considerations to honey bees and their parasites may shed new light on our understanding of virulence in this system. While we concentrate below on *A. mellifera*, and later on their viral infections, most of our discussion is equally relevant for other species of honey bee as well as for parasites other than viruses.

In this section we discuss the biology and reproductive system of the honey bee, delineating its reproduction at both the individual and colony levels. We then discuss the consequences of this reproductive system for horizontal and vertical transmission of parasites within as well as between colonies. We also comment on the consequences of the spatial structure of the host, at both levels, for parasite transmission.

3.1. Within colony reproduction

During the foraging period in temperate climates, a colony of honey bees normally consists of one queen bee, 20-50 thousand worker bees and a few hundred drones. The queen bee is the only reproducing female within the colony and has the capacity to produce over 2000 eggs per day under optimal conditions. The determination of offspring caste and sex is a result of two different mechanisms:

(i) worker bees are normally the product of fertilised eggs where the hatched larvae receive brood food (glandular secretions from young

bees) diluted after about three days post-hatching with pollen and nectar. If these larvae were to receive brood food only, the ovaries would develop fully and the hatching bee would develop into a queen bee. Thus, caste determination is based on nutrition during larval development.

(ii) drones are normally the result of unfertilized eggs (arrhenotoky). However, it is not non-fertilization *per se* that produces drones. Honey bees (and probably most haplodiploid hymenopteran insects) have a sex-determining system with are multiple alleles at the sex locus. The gene responsible for sex determination (the complementary sex determiner, *csd*), has recently been identified in the honey bee (Beye *et al.*, 2003). Heterozygosity at this locus results in females. When there is only one functional allele present at this locus as a result of homozygosity (fertilized eggs) or, as in the case of honey bees, hemizygosity (unfertilized eggs), the resulting individual is a male. Adult diploid males are never found in colonies, however, since they are detected as young larvae and eaten by the nurse bees. It has been estimated that a large bee population may hold around 15-20 sex alleles.

Thus, within a colony there is both sexual and parthenogenetic reproduction. Because the queen will be mated (mostly on a single occasion) with some 15 or so different drones, the genetic composition of the colony becomes complex. As meiosis does not occur during spermatogenesis in haploid drones, each drone produces genetically identical sperm. This produces groups of super-sisters within the colony with a relationship coefficient of 0.75 when they have the same "father". These groups of super-sisters are then related to one another by 0.25, as normal half-siblings.

While within colony reproduction is essential for colony growth and survival, the colony as a 'super-organism' (Moritz and Southwick, 1992) also has to reproduce.

3.2. Colony-level reproduction

The Darwinian fitness of a honey bee colony requires reproduction not only at the individual level, but also at the colony level. Honey bees are super-organisms that consist of individual units (bees) that have no function or survival capacity when removed from their colony context. Conceptually, this can be compared to taking simple neurons in the brain out from their brain context. Individual (dumb) bees become a functioning (smart) colony when integrated. The simple (dumb) neurons become a thinking (at best) brain when integrated into the whole. In many social Hymenoptera, colony-level reproduction occurs with the release of new queens that found new colonies from scratch; the system in honey bees is quite different (and also seen in a few swarm-founding wasps and ants).

Colony level (super-organism) reproduction in honey bees occurs when the colony swarms, that is, reproduction occurs by colony fission. When honey bees divide by swarming, the first swarm issued normally contains the old laying queen and subsequent swarms, if any, contain unmated queens. Although there is great variation in swarm sizes, each swarm issued can be expected to depart with 50-60% of the adult bee population (Winston, 1987). There is an age-related tendency to depart with the swarm, with younger workers dominating the swarm, although all age categories of bees will be represented (Muszynska, 1976; Winston, 1987). This mode of reproduction by colony fission has obvious implications for parasite transmission and is likely to be important for the evolution of parasite virulence (see Section 3.4 below).

3.3. Potential routes for parasite transmission

It is obvious from the description above that the transmission of parasites in honey bees must occur both within and between colonies. Unsurprisingly, given the complexity of honey bee biology, a variety of routes are potentially available, each of which may have implications for parasite virulence. There is the potential for vertical and horizontal transmission both among individuals and among colonies. Below we discuss these different modes of transmission within the sections for intra- colony and inter-colony transmission respectively.

3.3.1. Intra-colony transmission

Inside the colony, transmission can be either horizontal or vertical. Although recent evidence suggests a potential role for vertical transmission of deformed wing virus via eggs (transovarial transmission; Chen et al., 2005), the vast majority of intra-colony transmission appears to be horizontal in nature. In general, queens are rarely carriers of parasites and when they do become infected they are replaced by bees through supersedure (where a young queen is raised and starts laying eggs before the old queen disappears from the colony). As discussed in Section 2, horizontal transmission can take a variety of routes, each of which has implications both for the epidemiology of the parasite within the honey bee colony and the evolution of virulence. There is evidence for both direct (adult-to-adult, adult-to-larvae) and indirect transmission of parasites in honey colonies, with indirect transmission occurring both via vectors and relatively long-lived propagules (Bailey and Ball, 1991).

The spatial structure of the host may largely determine transmission. The honey bee has within colony spatial structure both at the level of brood – where the comb imposes structure – and at the level of the

adult population – where division of labour imposes structure on interaction patterns and the location of individuals. Theoretical work has identified the potential importance of such structure for parasite transmission (Schmid-Hempel, 1998; Pie et al., 2004). Currently, there are no specific data on whether colony comb structure and position of the brood influence transmission in honey bee colonies. However, there is some evidence to suggest that the age-dependent division of labour in honey bees may have an impact on transmission. Sacbrood virus (SBV) propagates in the hypopharyngal glands of adult bees without causing disease symptoms, and the larvae become infected as they feed on the gland secretions (Bailey, 1969). Cleaning activities (cleaning out of diseased brood) and feeding activities of young bees overlap in the age dependent sequence of tasks performed by bees (Winston, 1987). Thus, division of labour is likely to increase virus transmission rates in the case of SBV as nurse bees also become contaminated with virus particles as they clean out diseased larvae. In addition, there may even be a host response to reduce transmission, since infected adult bees are less likely to engage in feeding activities than non-infected bees (Bailey & Fernando, 1972).

3.3.2. Inter-colony parasite transmission

Mirroring the situation of intra-colony transmission, parasite transmission between colonies can be both horizontal and vertical in nature. Horizontal transmission may occur in a variety of ways, including:

1. *Drifting.* Although the colony entrance is effectively protected by guards against intruders in search of food stores, bees often enter the wrong colony within apiaries by accident. Drifting of bees may even occur over large distances between apiaries, in particular with drones that are readily accepted by any colony during some part of the season (Pfeiffer and Crailsheim, 1998). Any bee that is infected by parasites or carries infective propagules may then transfer disease to new colonies.
2. *Robbing.* When nectar sources become scarce while flying conditions prevail, honey bees will attempt to rob the stores of other colonies. If colonies cannot effectively defend the hive entrance they will soon succumb to intruders. Colonies may be weak for a variety of reasons, and disease may be a key reason for such weakness. Robbing out of infected colonies is an effective mechanism of parasite transmission, with even mite infestations being effectively transferred in this manner (Sakofski, 1990).
3. *Contact with infectious material from the environment.* This transmission route is limited to parasites that can survive outside of the

host environment and may occur perhaps via flowers during foraging (as is the case for the trypanosomatid *Crithidia bombi* in bumble bees; Durrer and Schmid-Hempel, 1994) or via abandoned nest sites. This survival capacity is extreme for the brood disease American foulbrood (AFB) caused by the spore forming bacterium *Paenibacil-*

Table I. List of common honeybee pathogens, trivial names, mode of intercolony transmission and virulence. + or +++ under transmission indicates which mode of transmission is estimated to be most important for moulding the host-parasite relationship under natural conditions; signs in parentheses indicate the impact of apiculture on transmission. Virulence is estimated from apicultural data (adapted and reworked from Fries and Camazine, 2001).

Pathogen			Transmission		Virulence	
Group	Latin name	Trivial name	Horizontal	Vertical	Individual level	Colony level
Protozoa	*Nosema apis*	Nosema disease	+ (++)	+++ (+)	Benign	Benign
	Malpighamoeba mellificae	Amoeba disease	+ (++)	+++ (+)	Benign	Benign
Fungi	*Ascosphaera apis*	Chalkbrood	+ (++)	+++ (+)	Lethal	Benign
	Aspergillus flavus	Stonebrood	+ (++)	+++ (+)	Lethal	Benign
Bacteria	*Paenibacillus larvae*	American foulbrood[1]	+ (+++)	+++ (+)	Lethal	Lethal[1]
	Melissococcus plutonius	European foulbrood	+ (++)	+++ (+)	Lethal	Benign
Virus	ABPV	Acute bee paralysis virus	+ (++)	+++ (+)	Lethal	Benign to Lethal[2]
	DWV	Deformed wing virus	+ (++)	+++ (+)	Lethal	Benign to Lethal[2]
Mites	*Acarapis woodi*	Tracheal mite	+ (++)	+++ (+)	Benign	Benign to Lethal[3]
	Varroa destructor[4]	Varroa mite	+ (++)	+++ (+)	Benign	Benign to Lethal[4]

[1] Colony level virulence may be an apicultural phenomenon – this needs to be investigated

[2] Only severe effects when vectored by varroa mites

[3] Only severe where the mite has been recently introduced or after owerwintering in cool temperate regions (McMullan and Brown, 2005)

[4] Only severe where the mite has been recently introduced and/or where mite control removes selective pressure from being virulent

lus larvae. Nest sites where the bees have succumbed to AFB may be infectious for decades and infected nest sites are not avoided by swarming bees (Ratnieks and Nowakowski, 1989).

Other routes of inter-colony horizontal transmission, such as contact between infected and uninfected individuals from different colonies during foraging, are probably of minor importance in general, although they may be involved in the transfer of spiroplasma (Clark, 1977).

While spatial structure of the host may be likely to influence within colony transmission (see Section 3.3.1), it must certainly have an effect on colony level transmission. In a natural system, honey bees are likely to appear as scattered units with suitable nest sites limiting colony density, as compared to the apicultural context where beekeepers crowd colonies together in apiaries. At least some evidence suggests the existence of different levels of parasite transmission depending upon the density and distribution of honey bee colonies. Adult bees from wild colonies in areas without beekeeping rarely carry detectable levels of AFB spores, whereas bees from wild colonies in areas with beekeeping are often contaminated by AFB spores (Hornitzky et al, 1996), indicating an effect of colony density on transmission. Furthermore, a clear correlation has been demonstrated between colony density and the incidence of chronic paralysis, suggesting an influence of colony density on virus transmission (Bailey et al., 1983). Intuitively, inter-colony transmission of parasites in the honey bee system must be dependent on the spatial structure of the host but published data demonstrating this causal effect are, again, lacking. Unpublished data on AFB strongly support the hypothetical influence of colony density on transmission; colonies within apiaries containing clinically diseased neighbour colonies contract detectable spore levels in their honey and on adult bees faster than colonies at different distances from this apiary, with the distance from the diseased apiary determining the horizontal transmission rate (Fries & Körpela, unpublished data).

While vertical transmission may be relatively unimportant within colonies, it is another matter entirely for inter-colony transmission. As stated earlier, colony reproduction in honey bees is by fission, and thus vertical transmission may occur when a swarm leaves an infected colony. Transmission may be via either infected workers, or the transfer of infected material (e.g., honey) from the maternal hive to the new colony's nest site. Consequently, vertical transmission has the potential to play a major role in the spread and maintenance of parasites within honey bee populations.

In Table I we list some common parasites of honey bees. The table also includes assumed main routes for disease transmission between colo-

nies, i.e., how new colonies are most likely to contract the respective disease agents. Very little data exists on this topic for honey bee diseases in general but it is obvious from the reproductive biology of honey bees that swarming must be an important route for pathogens to become transmitted to new host colonies (Fries and Camazine 2001). All the parasites mentioned in Table I are either carried inside or outside of bees, or can be isolated from adult bees from infected colonies.

3.4. Implications of transmission for parasite evolutionary epidemiology in honey bees

From the parasite's viewpoint, it must overcome three distinct fitness hurdles in order to reproduce and disperse to new honey bee hosts:

1. The parasite must infect an individual (and usually must be able to multiply within this new host).
2. The parasite must be able to infect additional individuals within the colony to maintain itself either endemically or epidemically.
3. The parasite must successfully gain access to new colonies.

In terms of fitness, the successful transfer of a parasite's offspring to a new colony is a critical step in its life history. If a parasite fails to achieve a foothold in another host colony, it will not increase its reproductive fitness, regardless of how prolific it has been within the original host colony. Thus, the first two hurdles (intra-individual and intra-colony transmission) are important aspects of parasite fitness only to the extent that they contribute to more efficient inter-colony transmission (hurdle 3). Thus, for the parasite there are two trade-offs: (i) between growth/reproduction within and the consequent impact on individual bees vs. transmission among bees, and (ii) spread and impact within colonies vs. transmission among colonies.

4. Understanding virulence levels of honey bee viruses

In section 2.1.1 we listed various ways of defining virulence. Here we begin by explaining how we define virulence in viral diseases of honey bees. Our benchmark for virulence is host mortality, of either individual infected bees or colonies. We use mortality for the sake of simplicity, but note that many parasites cause morbidity (that is, they lower host fitness without causing mortality), and thus the absence of mortality in any given honey bee/virus system should not be taken to mean the absence of an impact of the virus on honey bee colonies. While using mortality as a metric may seem to be straightforward, the presence of covert infections

(see Chapter 6) puts a serious wrinkle into the picture. Let us take three hypothetical situations: (i) the virus is present in 80 out of 100 colonies at any point in time and always leads to colony death, (ii) the virus is present in the same number of colonies but only 1 of the 80 colonies dies due to the virus, (iii) the virus is present in the same number of colonies but in 79/80 colonies as a covert infection and the single colony with an overt infection dies. [Given our current level of knowledge, each of these situations is equally likely to be true for any given virus] In the first case the virus clearly has high virulence, while in the second case, even though it causes colony mortality (and thus, in that colony, has high virulence), at the population level the virus exhibits low virulence. What about the third case? If covert and overt infections are all part of one viral population, then our conclusion from the second case applies. However, if overt and covert infections are effectively independent, perhaps due to quasispecies and mutation (see Chapter 3), then the virus must be redefined as having high virulence. We can make the same argument at the level of individual bees. Clearly, to define viral virulence accurately we need to know the level of prevalence, the level of mortality associated with it, and the relationship between covert and overt infections. At present, we do not know this for any honey bee virus. Consequently, our definitions of parasite virulence at this point in time are best guesses based on incomplete data.

4.1. In the absence of *Varroa destructor*

As detailed in section 3, honey bee colonies represent multi-level systems as far as the evolution of viral virulence is concerned. At each level, different factors may drive the evolution of virulence, and between levels these factors may act either in concert or in opposition.

At the lowest level, we have viral infections within individual bees. Assuming an overt infection, selection should act to favour those viral strains that replicate most rapidly. Given the presence of mutation and quasispecies, the theoretical prediction is clear (Bonhoeffer and Nowak, 1994) and we should expect the evolution of highly virulent viral strains at the level of the individual bee.

The next level up is the evolution of viral infections within individual colonies. At this point, trade-offs between within-host growth and between-host transmission appear. If transmission within colonies is vertical, this should select strongly for low levels of viral virulence and act in opposition to selective forces at the intra-individual level. However, we believe that despite recent evidence (Chen et al., 2005) the vast majority of intra-colony transmission, especially during overt infections, must be horizontal in nature. Studies have demonstrated horizontal transmission

of viral infections from workers to brood and workers to workers, and at least in the case of sacbrood virus this seems to be sufficient to explain the maintenance of infections within colonies (Bailey and Ball, 1991). The simple expectation from section 2 is that horizontal transmission should select for higher virulence leading to some optimum level of virulence that maximises the trade-off between within host reproduction and between host transmission. However, spatiogenetic structure within honey bee colonies (section 3.3.1) should lead to a reduction in this optimum (section 2.3.3, 2.3.4). Even if covert infections act as the equivalent of long-lived propagules, this should make no difference to patterns of virulence (section 2.7). What about host-biology? As detailed in section 3.1, honey bee colonies go through an annual cycle of growth and decline. During the growth phase, a rapidly increasing host population should lift the upper constraint on virulence (section 2.3.2). Interestingly, an increase in background host mortality levels is also expected to lift this upper constraint (section 2.3.1), and so when colonies are growing and actively foraging, viruses should be selected for higher virulence. In contrast, as colony birth rate and foraging decrease in the autumn, selection should act to drive down the level of virulence as the opportunities for transmission decrease. So, when looking at the evolution of virulence within colonies, we should expect relatively high levels of virulence – based on the predominance of horizontal transmission among bees and viral competition within bees – to be selected. An additional, but important factor, is the presence of multiple viral species, or virus' and other parasites, within individual bees and colonies (see Chapter 2). For example, the chronic bee paralysis-virus associate reduces the virulence of CBPV, and the impact of black queen cell virus is intimately associated with co-infection by the microsporidian, *Nosema apis* (Bailey & Ball, 1991). How such interactions may modify the evolution of viral virulence will probably turn out to be system-specific, but at present we lack sufficient empirical data about the biology of these interactions to make any specific predictions.

The final level may be termed colony-level selection, and refers to our expectations for the evolution of viral virulence in a population of colonies. Theory suggests that the key point at this level is the relative importance of horizontal versus vertical transmission in maintaining the virus within the honey bee population. While opportunities for horizontal transmission among colonies are likely to be high in managed apiaries (e.g., Hornitzky, 1998), where high colony densities result in a large amount of drifting among hives (section 3.3.2), nothing is known about the potential for horizontal transmission among colonies in natural populations. However, given the likely distribution and density of wild colonies,

Table II. Transmission and virulence in viruses of honey bees (Apis mellifera). Virulence is as measured in apicultural systems and may not reflect the impact of these viruses in unmanaged populations. For more detailed information about mechanisms of transmission and impact, see Chapters 2 and 5.

Virus	Natural transmission[1]		Transmitted by Varroa?[2]	Virulence without Varroa		Varroa-related virulence	
	intra-colony	inter-colony		individual	colony	individual	colony
Chronic paralysis	H	H? V?	I	Severe	Severe	–	–
Cloudy wing	H	H? V?	I	Severe	Severe	–	–
Filamentous	?	H? V?	I	Benign	Benign	–	–
X	H	H? V?	No	Severe	Severe	–	–
Y	H	H? V?	No	Benign	Benign	–	–
Kashmir	H	H? V?	Yes	Severe	Benign	Severe	Severe
Deformed wing	H; V?	H? V?	Yes	Benign?	Benign?	Severe	Severe
Sacbrood	H	H? V?	Yes	Moderate	Benign	–	–
Arkansas	H?	H? V?	?	?	?	Severe?	–
Slow paralysis	H?	H? V?	Yes	Benign	Benign	Severe	–
Black queen cell	H	H? V?	?	Severe	Benign	–	–
Acute paralysis	H	H? V?	Yes	Benign	Benign	Severe	Severe
Egypt	H?	H? V?	?	?	?	Severe?	–

[1] H: horizontal transmission; V: vertical transmission; H? and V? indicate possible but untested routes

[2] Yes: Varroa destructor shown to transmit; I: injection shown to transmit, but unknown for mite; No: no transmission via mite

[3] – : no relevant data are available

vertical transmission during fission events to offspring swarms should be the dominant mode of transmission. If this is true then there should be strong selection at the colony-level for low virulence in viral pathogens, as highly virulent pathogens will either kill off their host colony prior to fission (and thus have no opportunity for transmission) or will reduce their growth to the point that they are unable to swarm (with similar impacts on transmission). Given that maintenance of the virus in the honey bee population ultimately depends upon inter-colony transmission, vertical transmission as the dominant route should result in viruses that have little or no effect at the colony level. In fact, unless there is some horizontal transmission among colonies, theory suggests that viruses should ultimately be expected to evolve to be commensals. Both acute paralysis virus and slow paralysis virus, which prior to the advent of *Varroa* mites were never associated with disease, provide evidence for such a scenario. However, the existence of generally low, but still noticeable in some cases (e.g. CPV), colony level impact from virus infections (in the absence of *Varroa destructor*) suggests that some horizontal transmission among natural colonies must take place.

What about the presence of covert infections? If covert infections represent the equivalent of a reservoir for the virus, i.e., they act to maintain the virus in the same way that a seedbank maintains plant species in the absence of adult plants, then the dynamics may be substantially different. In this case, overt infections may well represent epidemic rather than endemic diseases, perhaps triggered by particular environmental conditions (e.g., bad weather or low food availability) or host contexts (e.g., overcrowding within a hive). CPV represents a potential example of such a system (Allen and Ball, 1996). In natural populations, where opportunities for horizontal transmission are likely to be low, such an epidemic might rage through a colony, resulting in colony death, but would be unlikely to spread through the honey bee population (that is, it would be an intra- rather than inter-colony epidemic). However, in the managed system where opportunities for horizontal transmission are high, such an epidemic might well run through the larger honey bee population, causing mass colony mortality before dying down. Thus, at the population-level, viral impact may simply be a phenomenon of honey bee management. For evidence of such dynamics related to *V. destructor* infestation, see Chapter 2.

Table II summarises what is known about transmission and virulence in viral pathogens of bees. In contrast to Table I, here we report only known modes of transmission, or the absence of such data, rather than estimations based on host biology. We do this deliberately, in the hope that it will inspire research into viral transmission routes. We have catego-

rised virulence based on the assumption that if a virus has been known to cause mortality it has high virulence – thus, we ignore the complications of covert maintenance and parasite prevalence in the host population. It can be seen that, under natural conditions, viruses can vary in their level of virulence. However, it should be noted that even for those viruses that exhibit severe virulence (e.g., CPV), killing individual host bees as well as colonies, for much of the time the virus is maintained within the host population in an inactive state. Thus, high prevalence may not translate into a major impact on honey bee populations even for these viruses. The main feature that can be taken home from Table II is that we know remarkably little about inter-colony transmission of viruses. Consequently, it is unclear how selective forces acting at inter- and intra-colony levels interact to determine virulence in these viral pathogens. However, the presence of variation in virulence (at the individual and colony levels) across honey bee viruses indicates that variation in modes of transmission within and among colonies is likely to be shaping the evolution of virulence in these host-parasite systems.

4.2. In the presence of *Varroa destructor*

The current increasing virus problems facing apiculture are caused by the introduction of an exotic parasitic mite, *Varroa destructor*, into European honey bee, *Apis mellifera*, populations. The mite probably feeds on adult bees by piercing through the ventral membranous inter-segmental connections on the bee abdomen and sucking up haemolymph (Bowen-Walker & Gunn, 2001). During the reproductive phase of the mite it feeds on bee pupae by piercing through the pupal skin (Bailey & Ball, 1991). The feeding activity of the mite probably triggers replication of certain virus infections in individual bees and bee larvae. The mite then acts as a vector for virus transmission between adult bees and brood, thereby fundamentally changing the routes of within colony virus transmission of all virus types that replicate upon injection into bee haemolymph (see Table II and Chapter 2 for more details). As overt infections develop within colonies, inter-colony virus transmission rates probably also increase. Indeed, high rates of between colony horizontal transmission of deformed wing virus (DWV) have been demonstrated when mite free colonies with undetectable levels of DWV infections (using serology) develop overt virus infections within a few months after being introduced into a heavily mite infested and DWV infected apiary (Nordström et al., 1999). To complicate the matter further, this virus may even replicate in the mite (Yue and Genersch, 2005). Vector borne pathogens in general are often severe to the host, but benign to the vector (e.g.malaria) (Ewald, 1993). Clearly, the introduction

of *V. destructor* has fundamentally changed the transmission routes for some honey bee viruses. Because of the novelty of this change it is not yet clear what the outcome will be of these changes in modes of transmission of certain viral infections. Predictions based on evolutionary epidemiology theories suggest that more virulent variants may evolve where the virus is vectored by the mite – vectoring removes the contraint of host mortality and dramatically increases rates of horizontal transmission, both of which enhance the fitness of virulent strains that emerge within individual host bees. Given the potential for rapid mutation and mutant swarms in RNA viruses, it seems likely that these viruses have already evolved away from their pre-mite state. Because beekeepers remove the selective disadvantage of being virulent at colony level, by removing the vector through mite control measures, the current problems with virus induced colony mortality are likely to continue, or even increase, unless mite tolerant stock and/or virus resistant stock is developed. Experiments using natural selection demonstrate that honey bee populations infested by *V. destructor* and infected by DWV can not only survive , but increase in fitness, after initial high levels of mortality (Fries et al., 2005). Actually, honey bee populations that do survive mite infestations – and associated virus infections - exist in several locations where man has not interfered (Rosenkranz, 1999). As a caveat, we note that mite-related virulence may be analogous to the case of Polio in humans, and thus a non-adaptive expression of virulence, but even if this was initially true, the evolutionary potential of RNA viruses suggests that virulence should rapidly evolve in this system in the ways outlined above.

The honey bee – mite – virus association probably offers an opportunity to study evolutionary epidemiology in the field. As demonstrated in natural systems, although mite infested colonies are likely to succumb to virus infections if left untreated, the species *A. mellifera* is unlikely to perish without the involvement of apiculturists. Hypothetically, by studying transmission routes and transmission rates of virus infections within and between colonies we should be able, not only to understand the epidemiology involved, but also to follow the evolutionary process as the host-parasite system co-evolves. Without data on basic epidemiological parameters such as transmission routes and transmission rates (at the individual bee level as well as at the colony level), however, it will remain impossible to model and understand the system. With at least some data it would be possible to adapt current evolutionary epidemiology models to the complex two-level transmission system of honey bees. It is increasingly clear that theoretical models have helped in understanding epidemiology in other systems and may suggest interesting possibilities for the management of

disease development and the evolution of virulence in pathogens (see Section 2). For the honey bee system, this remains to be done.

5. Summary

Evolutionary epidemiology of parasites and diseases in social insects in general, and honey bees specifically, is in an early stage of development. While theoretical models about the evolution of virulence abound we have two major hurdles to jump before we can understand the evolutionary interaction between viruses and honey bees. Firstly, we are severely lacking in good data on the true impact of viruses on their honey bee hosts and on how viruses are transmitted within colonies and between colonies, in both natural and managed systems. These data need to be collected and we indicate below in more detail what we believe the important factors are. Secondly, we need models which represent the hierarchical host system that honey bees present to viral parasites.

Important empirical questions that must be addressed include:

(i) the mode of transmission within colonies (horizontal vs. vertical)
(ii) the mode of transmission between colonies (in both managed and natural systems)
(iii) the relative rates of transmission by different modes within and between colonies
(iv) the frequency of multiple-infections (both of viral strains and viral species) within individual bees and individual colonies
(v) evidence for the presence or absence of competition/enhancement among viral strains and species, and between viruses and other co-existing pathogens (e.g., BQCV and *Nosema apis*)
(vi) the relationship between parasite reproduction within a host and its potential transmission
(vii) the relationship between covert and overt infections
(viii) the impact of the virus on individual bees and individual colonies

Once we understand the biology of the bee-virus systems at this level we should be able to parametrise a model to determine which of these factors control the levels of virulence in bee viruses. And this, in turn, will help us to know which factors to manipulate if we hope to manage their virulence.

6. References

Allen M., Ball B. (1996) The incidence and world distribution of honey bee viruses, Bee World, 77, 141-162.

Anderson R.M., May R.M. (1982) Co-evolution of hosts and parasites, Parasitology, 85, 411-426.

Baer B., Schmid-Hempel P. (1999) Experimental variation in polyandry affects parasite loads and fitness in a bumble-bee, Nature 397, 151-154.

Baer B., Schmid-Hempel P. (2001) Unexpected consequences of polyandry for parasitism and fitness in the bumblebee, *Bombus terrestris*, Evolution 55, 1639-1643.

Bailey L. (1969) The multiplication and spread of sacbrood virus of bees, Ann. Appl. Biol. 63, 482-491.

Bailey L., Ball B.V. (1991) Honey bee pathology 2nd Edition, Academic Press, London.

Bailey L., Ball B.V., Perry J.N. (1983) Honeybee paralysis: its natural spread and its diminished incidence in England and Wales, J. Apic. Res. 22, 191-195.

Bailey L., Fernando E.F.W. (1972) Effects of sacbrood viruse on adult honeybees, Ann. Appl. Biol., 72, 27-35.

Bailey L., Gibbs A.J. (1964) Acute infection of bees with paralysis virus, J. Insect Pathol., 6, 395-407.

Beye M., Hasselmann M., Fondrk M. K., Page R.E. Jr., Omholt S. W. (2003) The gene *csd* is the primary signal for sexual development in the honeybee and encodes an SR-type protein, Cell 14, 419-429.

Bonhoeffer S., Lenski R.E., Ebert D. (1996) The curse of the pharaoh: the evolution of virulence in pathogens with long living propagules, Proc. R. Soc. Lond. B 263, 715-721.

Bonhoeffer S., Nowak M.A. (1994) Mutation and the evolution of virulence, Proc. R. Soc. Lond. B 258, 133-140.

Boots M., Sasaki A. (1999) 'Small worlds' and the evolution of virulence: infection occurs locally and at a distance, Proc. R. Soc. Lond. B 266, 1933-1938.

Bowen-Walker P.L., Gunn, A. (2001) The effect of the ectoparasitic mite, *Varroa destructor* on adult work honeybee (*Apis mellifera*) emergence weights, water, protein, carbohydrate, and lipid levels, Entom. Exp. Appl. 101, 207-212.

Brown M.J.F., Loosli R., Schmid-Hempel P. (2000) Condition-dependent expression of virulence in a trypanosome infecting bumblebees, Oikos 91, 421-427.

Brown M.J.F., Schmid-Hempel R., Schmid-Hempel P. (2003) Strong context-dependent virulence in a host-parasite system: reconciling genetic evidence with theory, J. Anim. Ecol. 72, 994-1002.

Bull J.J. (1994) Virulence, Evolution 48, 1423-1437.

Chen Y.P., Higgins J.A., Feldlaufer M.F. (2005) Quantitative real-time reverse transcription-PCR analysis of deformed wing virus infection in the honeybee (*Apis mellifera* L.), Appl. Env. Microbiol., 71, 436-441.

Claessen D., de Roos A.M. (1995) Evolution of virulence in a host-pathogen system with local pathogen transmission, Oikos 74, 401-413.

Clark T.B. (1977) *Spiroplasma* as a new pathogen in the honeybees, J. Invertebr. Pathol. 29, 112-113.

Cooper V.S., Reiskind M.H., Miller J.A., Shelton K. A., Walther B.A., Elkinton J.S.,

Ewald P.W. (2002) Timing of transmission and the evolution of virulence of an insect virus, Proc. R. Soc. Lond. B 269, 1161-1165.

Cox F.E.G. (2001) Concomitant infections, parasites and immune responses, Parasitol. 122, S23-S38.

Day T. (2001) Parasite transmission modes and the evolution of virulence, Evolution 55, 2389-2400.

Day T. (2002) The evolution of virulence in vector-borne and directly transmitted parasites, Theor. Pop. Biol. 62, 199-213.

Day T. (2003) Virulence evolution and the timing of disease life-history events, Trends Ecol. Evol. 18, 113-118.

Durrer S., Schmid-Hempel P. (1994) Shared use of flowers leads to horizontal pathogen transmission. Proc. R. Soc. Lond. B, 258, 299-302.

Ebert D. (1994) Virulence and local adaptation of a horizontally transmitted parasite, Science 265, 1084-1086.

Ebert D. (1998) Experimental evolution of parasites, Science 282, 1432-1435.

Ebert D., Mangin K.L. (1997) The influence of host demography on the evolution of virulence of a microsporidian gut parasite, Evolution 51, 1828-1837.

Ebert D., Weisser W.W. (1997) Optimal killing for obligate killers: the evolution of life histories and virulence of semelparous parasites, Proc. R. Soc. Lond. B 264, 985-991.

Ewald P.W. (1983) Host-parasite relations, vectors and the evolution of disease severity, Ann. Rev. Ecol. Syst. 14, 465-485.

Ewald, P.W. (1993) The evolution of virulence. Scientific American, 268, 56-62.

Ewald P.W. (1994) Evolution of infectious diseases, Oxford University Press, Oxford UK.

Fries I., Camazine S. (2001). Implications of horizontal and vertical pathogen transmission for honey bee epidemiology, Apidologie, 32, 199-214.

Fries I., Rosenkranz P., Imdorf A. (2005) Survival of mite infested (*Varroa destructor*) honey bee (*Apis mellifera*) colonies in a Nordic climate. In manuscript (for Apidologie)

Gandon S. (1998) The curse of the pharaoh hypothesis, Proc. R. Soc. Lond. B. 265, 1545-1552.

Gandon S., Jansen V.A.A., van Baalen M. (2001) Host life history and the evolution of parasite virulence, Evolution 55, 1056-1062.

Gandon S., van Baalen M., Jansen V.A.A. (2002) The evolution of parasite virulence, superinfection, and host resistance, Amer. Nat. 159, 658-669.

Ganusov V.V., Antia R. (2003) Trade-offs and the evolution of virulence of microparasites: do details matter?, Theor. Pop. Biol. 64, 211-220.

Herre E.A. (1993) Population structure and the evolution of virulence in nematode parasites of fig wasps, Science 259, 1442-1445.

Hornitzky M.A.Z. (1998) The spread of *Paenibacillus larvae* subsp. *larvae* infections in an apiary, J. Apic. Res. 37, 261-265.

Hornitzky M.A.Z., Oldroyd B.P., Sommerville D. (1996) *Bacillus larvae* carrier status of swarms and feral colonies of honeybees (*Apis mellifera*) in Australia, Aus. Vet. J. 73, 116-117.

Kamo M., Boots M. (2004) The curse of the pharaoh in space: free-living infectious stages and the evolution of virulence in spatially explicit populations, J. Theor. Biol. 231, 435-441.

Koella, J.C., Restif, O. (2001) Coevolution of parasite virulence and host life history. Ecology Letters, 4, 207-214.

Lenski R.E., May R.M. (1994) The evolution of virulence in parasites and pathogens – reconciliation between 2 competing hypotheses, J. Theor. Biol. 169, 253-265.

Leung B., Forbes M.R. (1998) The evolution of virulence: a stochastic simulation model examining parasitism at individual and population levels, Evol. Ecol. 12, 165-177.

Liersch S., Schmid-Hempel P. (1998) Genetic variation within social insect colonies reduces parasite load, Proc. R. Soc. Lond. B 265, 221-225.

Lipsitch M., Moxon E.R. (1997) Virulence and transmissibility of pathogens: what is the relationship? Trends Microbiol. 5, 31-37.

Lipsitch M., Siller S., Nowak M.A. (1996) The evolution of virulence in pathogens with vertical and horizontal transmission, Evolution 50, 1729-1741.

Mackinnon M.J., Read A.F. (1999) Genetic relationships between parasite virulence and transmission in the rodent malaria *Plasmodium chabaudi*, Evolution 53, 689-703.

Massey R.C., Buckling A., Ffrench-Constant R. (2004) Interference competition and parasite virulence, Proc. R. Soc. Lond. B 271, 785-788.

May R.M., Anderson R.M. (1990) Parasite-host coevolution, Parasitology 100, 89-101

May R.M., Nowak M.A. (1995) Coinfections and the evolution of parasite virulence, Proc. R. Soc. Lond. B 261, 209-215.

McMullan J., Brown M.J.F. (2005) Brood pupation temperature affects the susceptibility of honeybees (Apis mellifera L.) to infestation by tracheal mites (*Acarapis woodi*), Apidologie 36, 97-105.

Messenger S.L., Molineux I.J., Bull J.J. (1999) Virulence evolution in a virus obeys a trade-off, Proc. R. Soc. Lond. B 266, 397-404.

Moritz R.F.A., Southwick E.E. (1992) Bees as superorganisms, Springer Verlag, Berlin.

Muszynska J.(1976) Porównanie pszczól z roju i macierzaka, Psczel. Zesz. Nauk. 20, 191-201.

Nordström S., Fries I., Aarhus A., Hansen H., Korpela S. (1999). Virus infections in Nordic honey bee colonies infested with *Varroa jacobsoni*, Apidologie 30, 475-484.

O'Keefe K.J. (2004) The evolution of virulence in pathogens with frequency-dependent transmission, J. Theor. Biol. 233, 55-64.

Pfeiffer K.J., Crailsheim K. (1998) Drifting of honeybees, Insect. Sociaux 45, 151-167.

Pfennig K.S. (2001) Evolution of pathogen virulence: The role of variation in host phenotype, Proc. Roy. Soc. Biol. Sci. Series B, 268, 755-760.

Pie M.R., Rosengaus R.B., Traniello J.F.A. (2004) Nest architecture, activity pattern, worker density and the dynamics of disease transmission in social insects, J. Theor. Biol. 226, 45-51.

Ratnieks F.L.W., Nowakowski J. (1989) Honeybee swarms accept bait hives contaminated with American foulbrood disease, Ecol. Entomol., 14, 475-478.

Regoes R.R., Nowak M.A., Bonhoeffer S. (2000) Evolution of virulence in a heterogeneous host population, Evolution 54, 64-71.

Rosenkranz P. (1999) Honey bee (*Apis mellifera* L.) tolerance to *Varroa jacobsoni* Oud. in South America, Apidologie, 30, 159-172.

Royce L.A., Rossignol P.A. (1990) Epidemiology of honey bee parasites, Parasitol. Today 6, 348-353.

Sakofski F. (1990) Transfer of *Varroa jacobsoni* between honey bee colonies, In Proc. Int. Symp. Recent Res. Bee Pathol. (ed W. Ritter), pp. 70-72. Janssen Pharmaceutica, Beerse, Belgium, 5 – 7 Sept. 1990, Gent, Belgium.

Schmid-Hempel P. (1995) Parasites and social insects, Apidologie 26, 255-271.

Schmid-Hempel P. (1998) Parasites in social insects, Princeton University Press, Princeton N.J.

Schmid-Hempel P. (2001) On the evolutionary ecology of host-parasite interactions: addressng the question with regard to bumblebees and their parasites, Naturwiss., 88, 147-158.

Shykoff J.A., Schmid-Hempel P. (1991) Parasites and the advantage of genetic variability within social insect colonies, Proc. R. Soc. Lond. B 243, 55-58.

Tarpy D.R. (2003) Genetic diversity within honey bee colonies prevents severe infections and promotes colony growth, Proc. R. Soc. Lond. B 270, 99-103.

Taylor L.H., Mackinnon M.J., Read A.F. (1998) Virulence of mixed-clone and single-clone infections of the rodent malaria *Plasmodium chabaudi*, Evolution 52, 583-591.

Thomas M.B., Watson E.L., Valverde-Garcia P. (2003) Mixed infections and insect-pathogen interactions, Ecol. Lett. 6, 183-188.

van Baalen M., Sabelis M.W. (1995) The dynamics of multiple infection and the evolution of virulence, Amer. Nat. 146, 881-910.

Williams P.D., Day T. (2001) Interactions between sources of mortality and the evolution of parasite virulence, Proc. R. Soc. Lond. B 268, 2331-2337.

Winston M.L. (1987) The biology of the honey bee, Harvard University Press, London, UK.

Yue C., Genersch E. (2005) RT-PCR analysis of *Deformed wing virus* (DWV) in honey bees (*Apis mellifera*) and mites (*Varroa destructor*), J. Gen. Virol., *in press*.