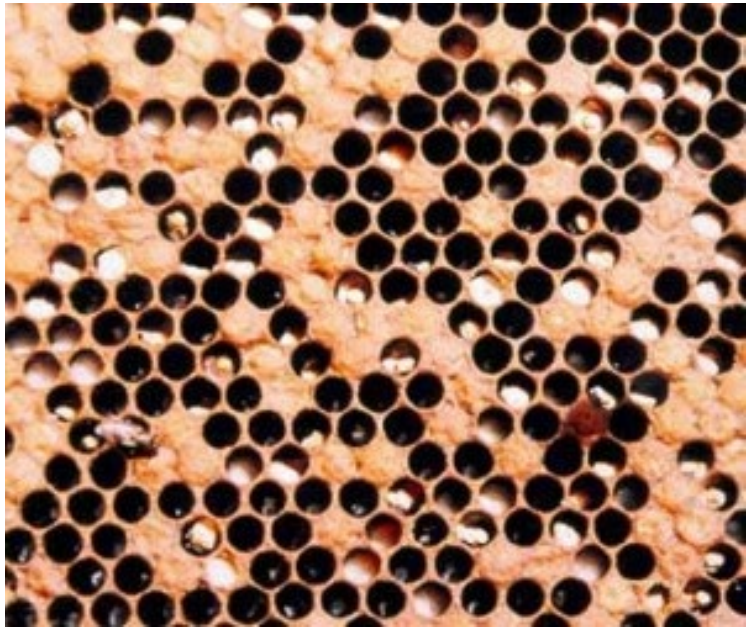
Chalkbrood and stresses





- Not so much UK research now on chalkbrood – but US, Spain, Denmark, Australia
- Biggest commercial breeding effort in Scotland/UK places a high emphasis on brood pattern in hybrid bees
- Still a serious disease in many countries and with some types of beekeeper

Chalkbrood – nutrition – stress?

- Environmental effects vs ‘genetic’ effects in the broad sense
- Often said that ‘stress’ induces chalkbrood outbreaks
- Stress claimed to be:
  - Low temperature
  - Moving hives
  - Poor nutrition
  - Damp, shady environments
- Anything else?
  - Some infection ‘just happens’ if there are spores in the food and the right genetics

Low temperature stress

- Early season weather and colonies over-extending themselves
- Beekeeper interventions: too many, too long
- Scientific demonstration of low temp sensitivity in presence of ingested spores *at a certain growth stage*

- Most research on the pathogen now uses a deliberate cold period to induce high levels of infection

Chalkbrood development in honeybee brood under controlled conditions

F Puerta, JM Flores, M Bustos, F Padilla, F Campano

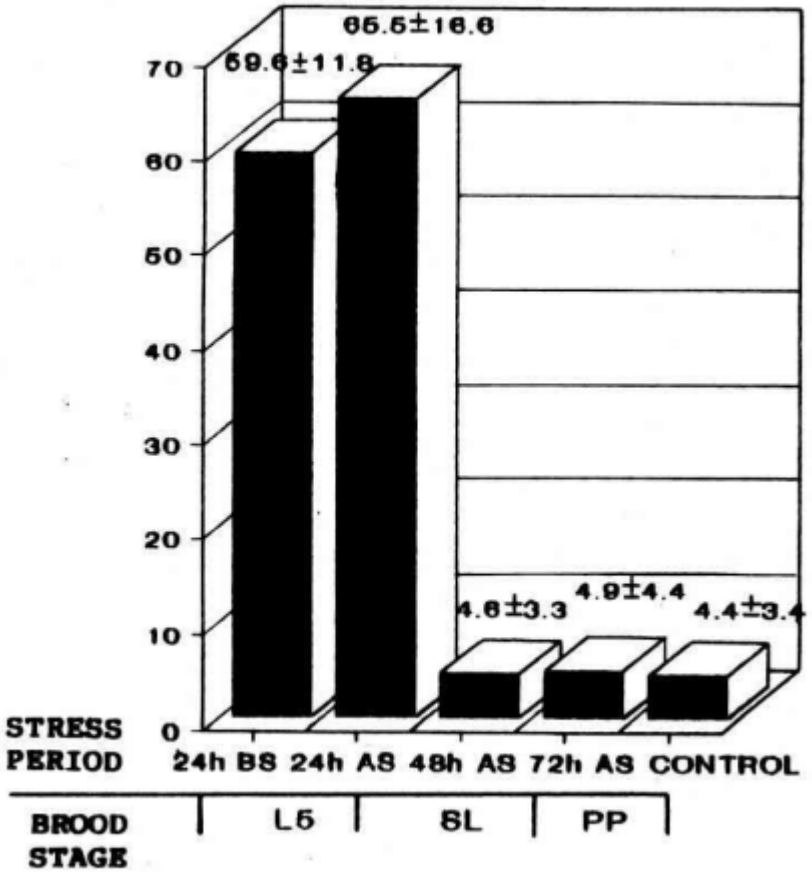
Departamento de Biología Animal, Cátedra de Biología Aplicada, Sección de Apicultura, Facultad de Veterinaria, Universidad de Córdoba, 14005 Córdoba, Spain

(Received 17 February 1994; accepted 10 June 1994)

Summary — Third instar larvae from a honeybee colony were fed with high doses of spores of *Ascosphaera apis*, the causative agent of chalkbrood disease. Optimal survival of spores was detected during a short period after sealing the cell and before larval spinning by culture of the gut contents removed from 4 stages of brood development. The inocula (5 x 10^5 spores/larva) did not induce the disease and were not present in the digestive tract before pupation. In a second experiment, third instar larvae, fed with the same amounts of spores as before, received a cooling stress (22 ± 2°C, for 24 h). When chilling was applied 24 h before or after operculation, mummification occurred in the majority of larvae (59.6 and 65.5%, respectively). Chilling of older brood (spinning larvae or pupa) produced a much lower incidence of chalkbrood. This confirms the need for predisposing conditions over a short period of brood development for the development of this disease.

- 3rd instar larvae fed 5x10^5 spores
- Normally cleared from gut before pupation with few mummies
- Chilling stress applied 24 hr before to 24 hr after sealing induces high level of disease (~60%)

ing stress (fig 2) statistical analysis shows 2 clearly differentiated blocks: those larvae



Nutrition?

Genetics

- Susceptibility of Amm – is it real?
- Larval-mediated resistance/susceptibility
- What about intra-colony diversity? (ie no of drones the queen mated with, number of patrilines)

- Hygienic bees *not* thought to confer significant resistance

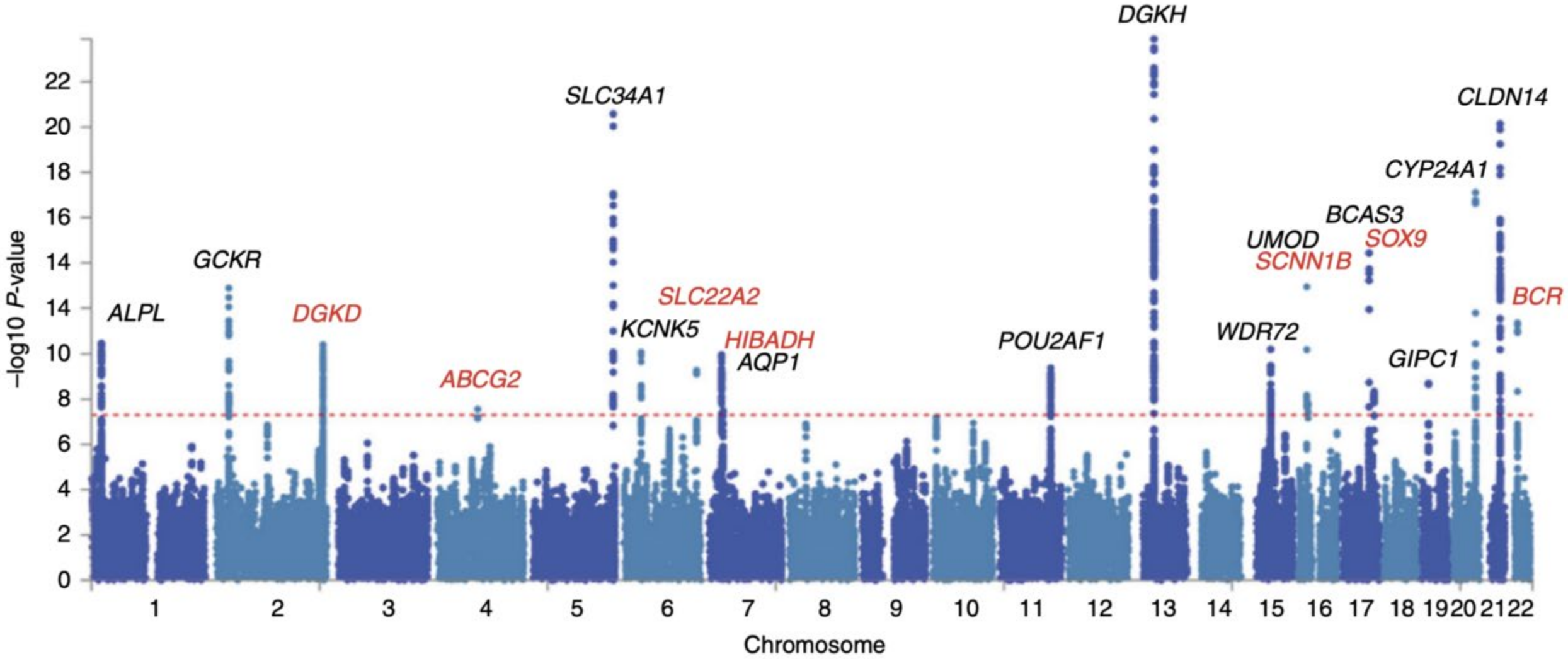
How to find genetic control, the modern way

- Genome-
- Wide
- Association
- Study

GWAS

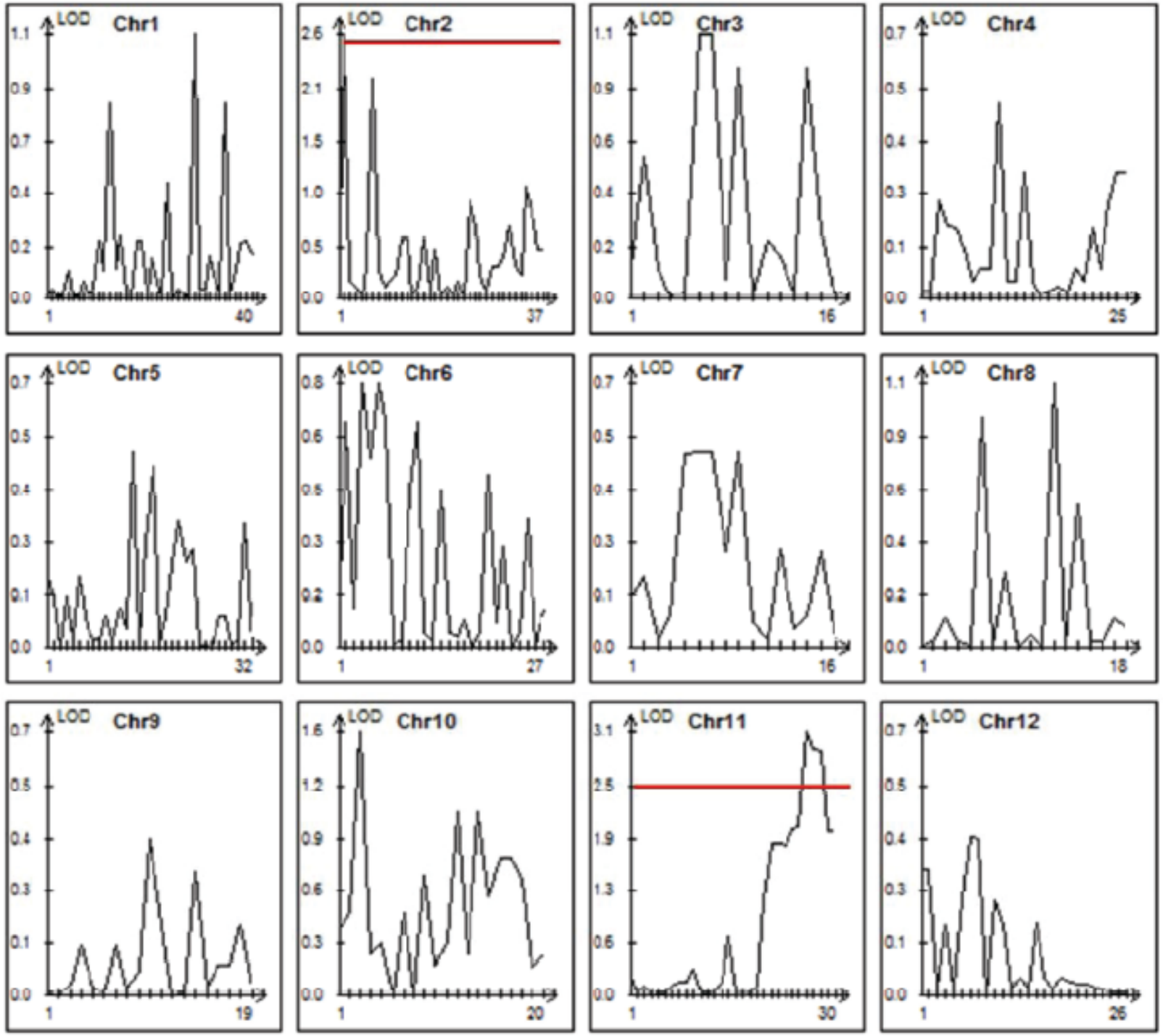
GWAS – human kidney stone disease

‘Manhattan Plot’



Holloway, Sylvester, Bourgeois and Rinderer 2012

- SNP- based ‘GWARS’
- Two genes, chm 2 and chm 11
- About 200 genes are in the vicinity of the SNPs
- Research suggests *JHBP-like* gene might be one candidate on chm 11



Li et al (2016) Larva-mediated chalkbrood resistance-associated single nucleotide polymorphism markers in the honey bee *Apis mellifera*. Insect Molecular Biology (2016) 25(3), 239–250

Single larvae were harvested and separated into resistant and susceptible categories after *in vitro* feeding with spores. Study confirms genes conferring resistance on chromosomes 2 and 11. SNPs in a gene coding for a Royal jelly protein seemed closest to the resistance effects:

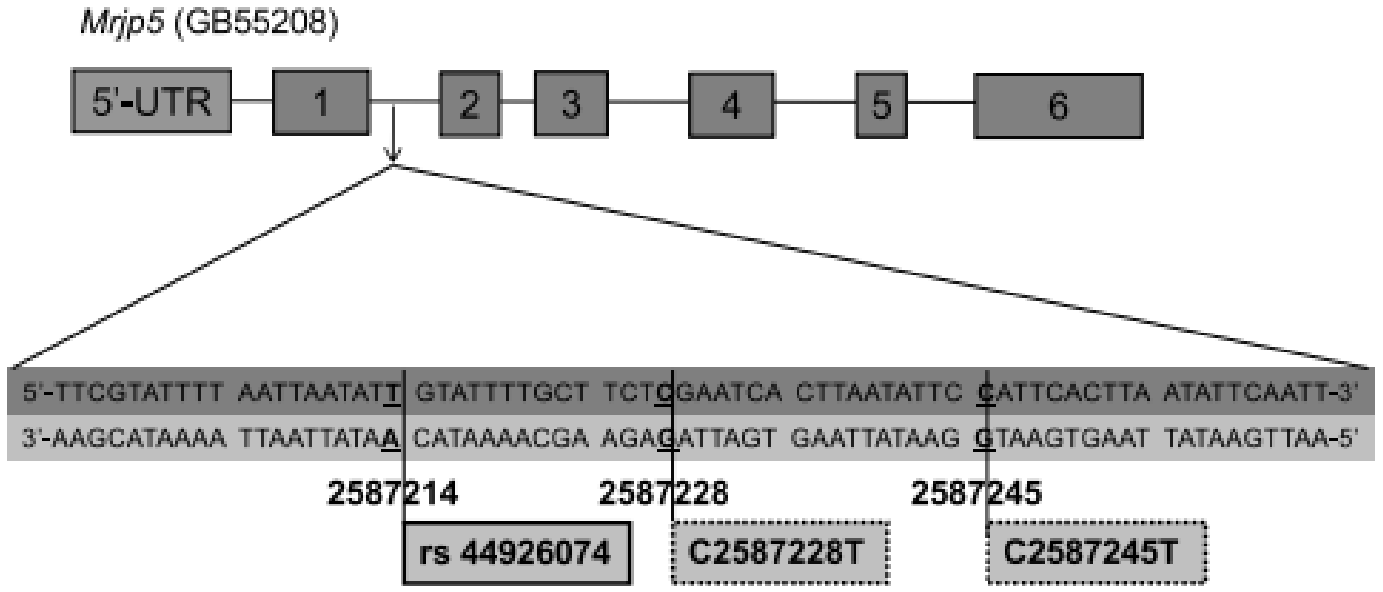


Figure 3. Diagram of single nucleotide polymorphism (SNP) distribution in the second intron of the gene for major royal jelly protein 5 (*Mrjp5*) with 5'-untranslated region (UTR). The positions of the three SNPs were derived from their position on the assembled genome sequence; the solid box represents a SNP submitted previously to the National Center for Biotechnology Information database, whereas the two dotted boxes represent the SNPs identified in this study. SNPs were named after their positions and alleles on the genome sequence of chromosome 11.

What did he just say?!

- Two main genes confer resistance to chalkbrood infection
- They aren’t really sure which genes have the effect
- At least one of them has the resistant form as dominant

Genetic Variation in Virulence among Chalkbrood Strains Infecting Honeybees

Svjetlana Vojvodic ,Annette B. Jensen,Bo Markussen,Jørgen Eilenberg,Jacobus J. Boomsma

https://doi.org/10.1371/journal.pone.0025035

- The strains from one clade caused 12–14% mortality while those from the other clade induced 71–92% mortality.
- Larvae from one colony showed significantly higher susceptibility to chalkbrood infection than larvae from the other two colonies, confirming the existence of genetic variation in susceptibility across colonies.
- Our results are consistent with antagonistic coevolution between a specialized fungal pathogen and its host, and suggest that beekeeping industries would benefit from more systematic monitoring of this chronic stress factor of their colonies.

Intracolonial genetic variability in honeybee larval resistance to the chalkbrood and American foulbrood parasites

Insectes Sociaux volume 56, pages233–240 (2009)

C. Invernizzi, F. Peñagaricano & I. H. Tomasco

- .. the resistance of different patrilines within a colony to parasites of different species should be independent, as a result of independent gene-for-gene interactions with each parasite. To test this aspect of the hypothesis, two honeybee colonies (*Apis mellifera*) were infected with the fungus *Ascosphaera apis* and two colonies with both A. apis and the American foulbrood bacterium *Paenibacillus* larvae.
- Patrilines were found to vary in resistance of larvae to A. apis in all four colonies, but similar variation in resistance was not found to P. larvae. Common resistance to both pathogens was not detected.
- This study supports the hypothesis that polyandry in social insects could have originated as an adaptation to decrease the impact of diseases.

Lessons

- If you are collecting pollen to feed colonies, avoid chalkbrood+ colonies assiduously (risk of high level of spores from mummies)
- Be hygienic with all equipment and especially comb
- Don’t breed from high chalkbrood stocks
- Try to maximise numbers of fathers
- Avoid any kind of chilling, keep inspections brief, keep colonies strong
- Consider feeding high quality protein in seasons with pollen deficits
- Avoid transporting chalkbrood to different countries/regions