

How to invade a country and have fun with the locals

ROS GLOAG, BEN OLDROYD, EMILY REMNANT
Behaviour and Genetics of Social Insects Lab
University of Sydney

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Honey bees of the world

- A. florea* (Dwarf bees)
- A. andreniformis* (Dwarf bees)
- A. dorsata* (Giant bees)
- A. laboriosa* (Giant bees)
- A. mellifera* (Giant bees)
- A. koschevnikovi* (Cavity nesting bees)
- A. nuluensis* (Cavity nesting bees)
- A. nigrocincta* (Cavity nesting bees)
- A. cerana* (Cavity nesting bees)

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The Asian honey bee

- Closest relative of 'our' honey bees
- Original host of *Varroa* mite
- Potential competitor for 'European' honey bees




AHB EHB

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Apis cerana colonizes Australia

1970s
West Papua


1993
Torres Strait

2003
Solomon Is.

2007
Queensland

■ Native-range

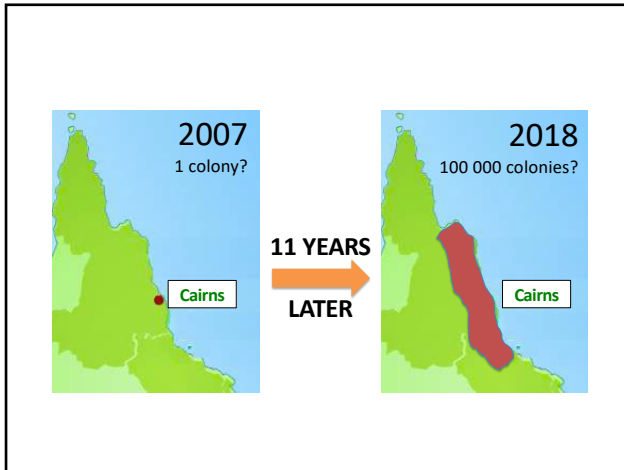
■ Invasive range



Cavity nesters with small colony sizes = good stowaways!

Koetz 2013, Department of Ag, Fish and Forestry QLD

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How come this huge population grew so fast despite brother-sister matings?

Trevor Weatherhead

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Inbreeding is a *particular* problem for small populations of honey bee

Because sex (male or female) is determined by a single gene, *csd*

 Female	 Normal Male	 INVIABLE Diploid Male
 Two distinct <i>csd</i> alleles	 One <i>csd</i> allele (haploid)	 Two identical <i>csd</i> alleles

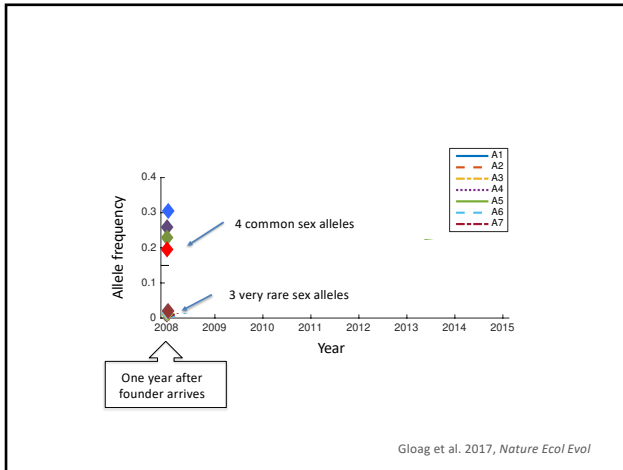
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Plenty of samples so let's study it!

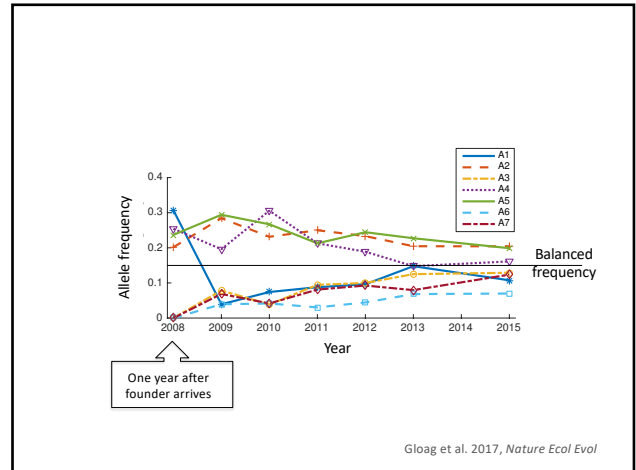
Queensland Biosecurity
 Eradication 2007-2011
 Management 2011-2013

Our own collections 2014-2018

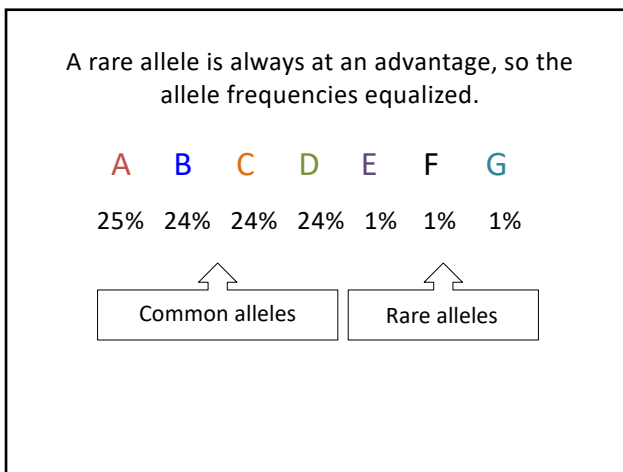
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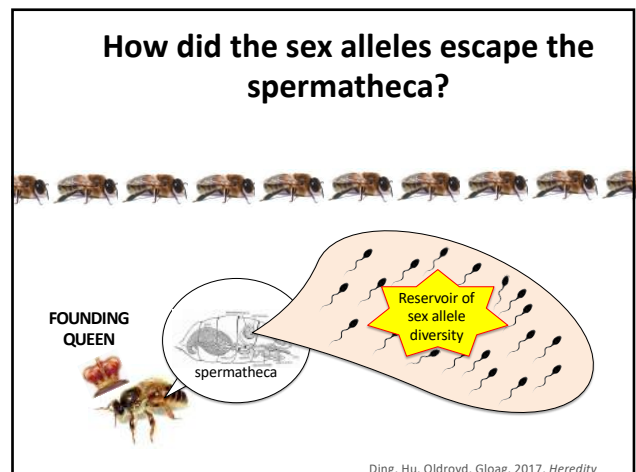
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11% of nests we collected were queenless and workers were rearing drones




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Trapped drones are of two sizes




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- 31% males at mating congregation are small worker-laid males ($N=2947$)

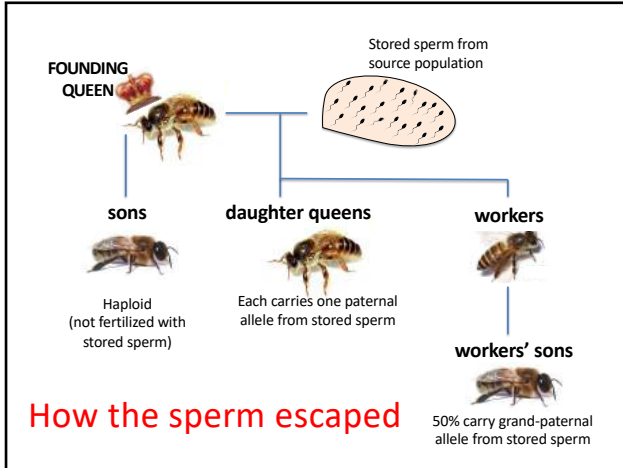
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SMALL drone = son of a worker 18%

LARGE drone = son of a queen 82%

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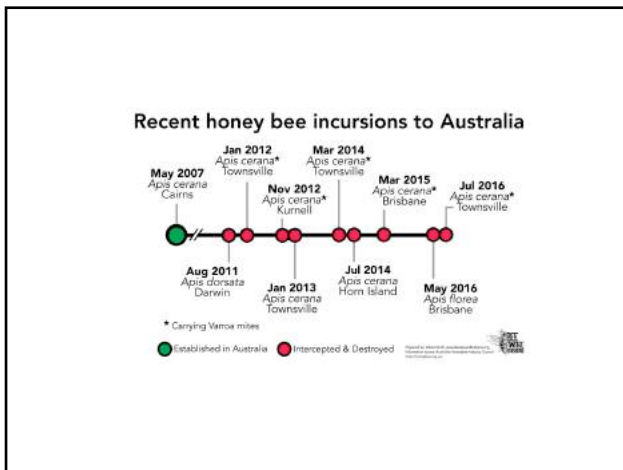
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Conclusions

Genetic evidence is consistent with a single mated queen.

Worker-laid males were critical for releasing genetic diversity

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And they keep on coming....

AUSTRALIAN HONEY BEE INDUSTRY COUNCIL INC
 ABN: 62 559 624 424
 Address: P.O. Box 4213, Roseville Q. 4305 Phone: 07 5467 2205
 Email Address: ahbic@honeybees.org.au Web Site: www.honeybees.org.au

INDUSTRY NOTICE NO. 1

AHIBC has been advised by the Department of Agriculture and Water Resources that a swarm of Asian bees (*Apis cerana*) has been found in Darwin in the suburb of Karrara. A local beekeeper was called, on 13 May 2018, to collect a swarm of bees and noticed that they were most likely Asian bees and reported this to the local Apisary Officer. All bees have been destroyed including the queen.

Examination has shown no presence of external mites i.e. Varroa or Tropilaelaps. Examination for internal mites is also being conducted. DNA identification will also be carried out.

Northern Territory Departmental Officers are starting a surveillance program initially concentrating on a one (1) kilometre radius for the presence of other Asian bees.

The AHIBC Executive is considering a response to this incursion.

Further information will be sent out as it is received.

Trevor Weatherhead AM
 Executive Director
 16 May, 2018

16 May 2018: *A. cerana* found in Darwin


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How has our research helped us prepare for a response to the Darwin incursion?

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1. The *csd* gene (= sex locus) is a powerful tool for estimating how many generations have passed since the founder colony, and the origin of the founder

Better than the conventional genetic tools available to Queensland Biosecurity



We showed:

- Townsville invasion was from Papua New Guinea
- Arrived 2-3 years ago

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2. Method for trapping drones at mating congregations can be used to identify infested areas




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What effect will *A. cerana* have on our industry and the environment?

- Competition for food
- Competition for nest sites
- Disease reservoir
- **Interspecific matings**



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Matings happen in an open space surrounded by trees



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Can *A. mellifera* and *A. cerana* mate?

- Germany: *A. cerana* queen released on mating flight. Returned with trauma to sting chamber (did not survive).
- Japan: *A. mellifera* queens released on an island where there are only *A. cerana* colonies. Several queens mated (sperm in spermatheca).

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What happens after interspecific matings of *A. mellifera* queens by *A. cerana* males?

- Most eggs (62%) were unfertilized
- 36% were interspecific hybrids, but these failed to develop beyond early embryonic stages.



Tsushima Island

Nakamura, Takahasi. IUSSI conference, Adelaide 1998

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So....

- We can expect:
 - A high frequency of eggs that don't hatch
 - Lots of drones in worker cells



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What we did as part of the Transition to Management effort

- Study the sperm in the spermatheca of queens from China and Cairns
- Interspecific crosses using AI
- Study eggs from Cairns queens



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Why China?

- China has a big *A. mellifera* industry, and a big native *A. cerana* population.



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Spermatheca dissections

The slide titled "Spermatheca dissections" contains three images. The top-left image shows a dissection of a spermatheca, a small, pale, oval-shaped structure. The top-right image is a diagram of several sperm cells, each with a red head and a long, thin tail. The bottom image shows a dissection of a spermatheca with a label "Tracheal Not Covering Spermatheca" pointing to a specific area.

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- *A. cerana* (22 from Cairns, 30 from China)
- *A. mellifera* (12 from Cairns, 42 from China)

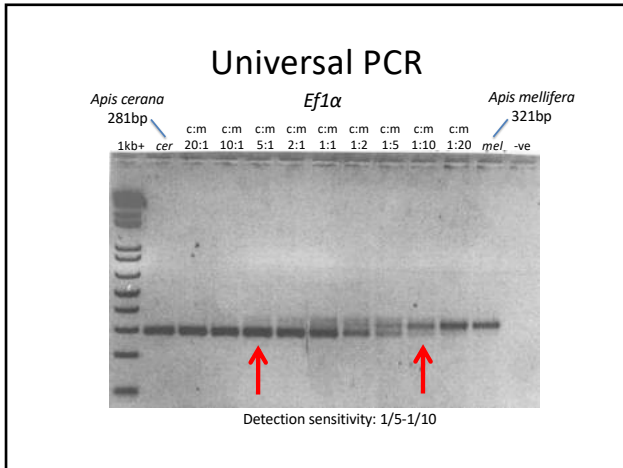
The slide contains two photographs of honeycomb frames. The top photograph shows a close-up of a honeycomb frame with many bees and brood cells. The bottom photograph shows a close-up of a honeycomb frame with many bees and brood cells.

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Agarose gel electrophoresis

The slide titled "Agarose gel electrophoresis" contains two photographs of agarose gel electrophoresis setups. The left photograph shows a gel electrophoresis tank with a red gel and a power supply unit. The right photograph shows a similar setup. A small inset photo in the bottom-left corner shows a person, identified as "Emily Remnant", working in a laboratory.

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Interspecific matings

Site	Queen species	n	Queens that mated with at least one interspecific male	% interspecific mating
Caoba Basin, Yunnan, China	<i>A. mellifera</i>	42	6	14.0
	<i>A. cerana</i>	30	0	0
Cairns, Queensland, Australia	<i>A. mellifera</i>	12	4	33.3
	<i>A. cerana</i>	22	0	0

1/3 of Cairns queens mated with at least one cerana male

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Location	Queen	Number of eggs sampled	Number of successful PCR amplifications (%) ¹	Number of heterospecific embryos (%) ²
Caoba Basin, Yunnan, China	1	190	67.4	0
	2	95	92.6	0
	3	47	87.2	0
	Total	328	77.4	0
Cairns, Queensland, Australia	1	66	43.9	0
	2	67	71.6	0
	3	80	67.5	0
	Total	213	61.5	0

No evidence of hybrid eggs

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So.... Do *A. mellifera* mate with *A. cerana* males?

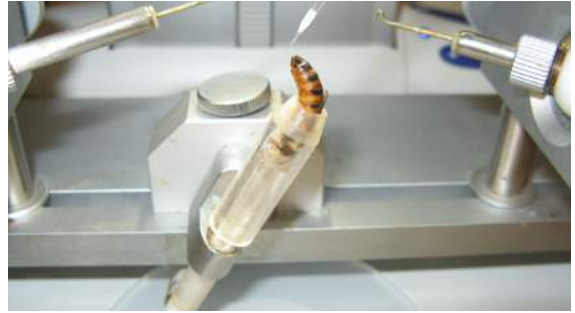
- Yes! Definitely happens in China and Cairns

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Artificial inseminations



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Artificial insemination in Cairns

	AI Treatment	Offspring
Hive 1	<i>cerana</i> sperm	drones + 1 worker*
Hive 2	<i>cerana</i> sperm	drones
Hive 3	saline	½ drone
Hive 4	saline	
Hive 5	<i>cerana</i> sperm	eggs only*
Hive 6		drones
Hive 7		drones
Hive 8		drones

All bees killed at pupal stage

Genotyping:
Hive 3 and Hive 1:
 Workers were
 thelytokously produced

Hive 5:
 Eggs had been fertilised
 with *cerana* sperm

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Conclude

- Matings between *A. cerana* and *A. mellifera* are very likely to become frequent in Cairns
- Minimum consequence is reduced brood viability
- Worst case is initiation of thelytoky

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What does it mean for the industry?

- Reduced brood viability
- Small colonies
- No queen breeding in affected areas



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More info

- <http://sydney.edu.au/science/biology/socialinsects/index.shtml>
- Remnant, et. al. 2014. Reproductive interference between honey bee species in Australia and China *Molecular Ecology* 23:1096-1107.
- Glog, R., et al., *An invasive social insect overcomes genetic load at the sex locus*. *Nature Ecology & Evolution*, 2016. **1**: p. 11.



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