How to invade a country and have fun with the locals

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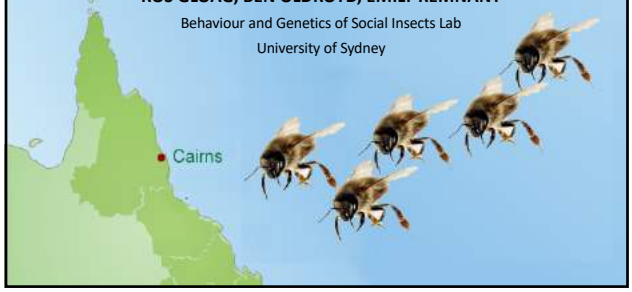

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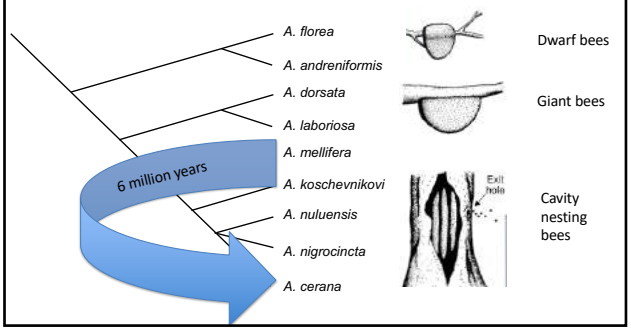
Honey bees of the world



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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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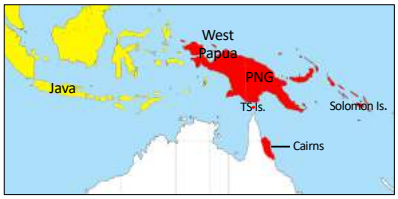
Apis cerana colonizes Australia

1970s West Papua → 1993 Torres Strait → 2003 Solomon Is. → 2007 Queensland



Cavity nesters with small colony sizes = good stowaways!

Native-range Invasive range

Koetz 2013, Department of Ag, Fish and Forestry QLD

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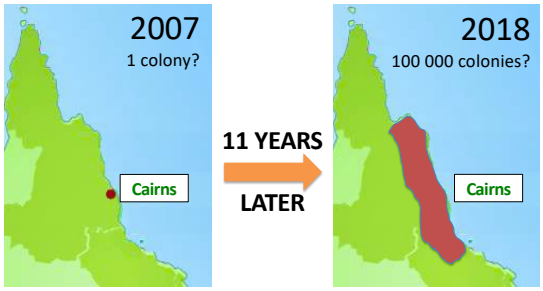

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How come this huge population grew so fast despite brother-sister matings?

Trevor Weatherhead

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

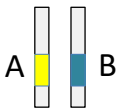
Because sex (male or female) is determined by a single gene, *csd*

Female

Normal Male

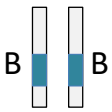
INVIABLE

Diploid Male



Two **distinct** *csd* alleles

One *csd* allele (haploid)



Two **identical** *csd* 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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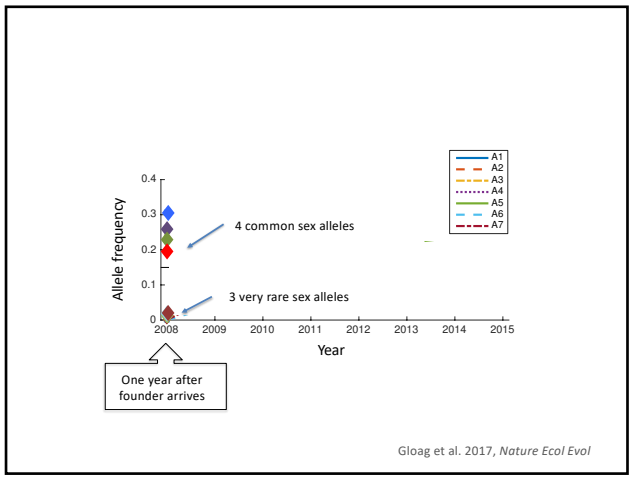

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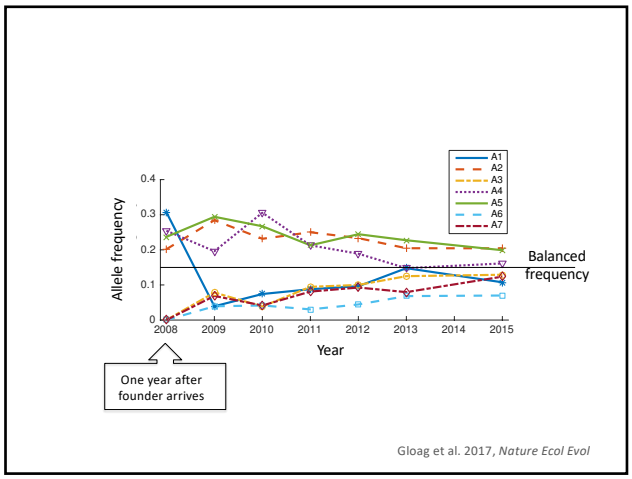

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A rare allele is always at an advantage, so the allele frequencies equalized.

A	B	C	D	E	F	G
25%	24%	24%	24%	1%	1%	1%

Common alleles

Rare alleles

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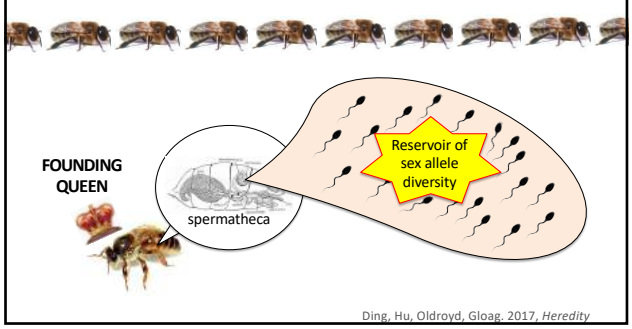
How did the sex alleles escape the spermatheca?



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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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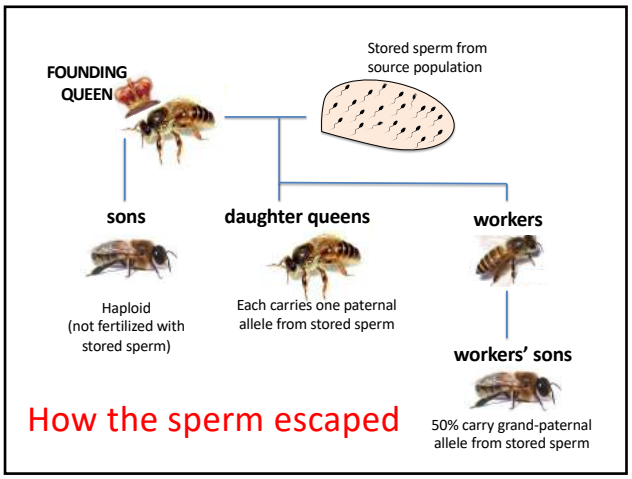

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Conclusions

Genetic evidence is consistent with a single mated queen.

Worker-laid males were critical for releasing genetic diversity

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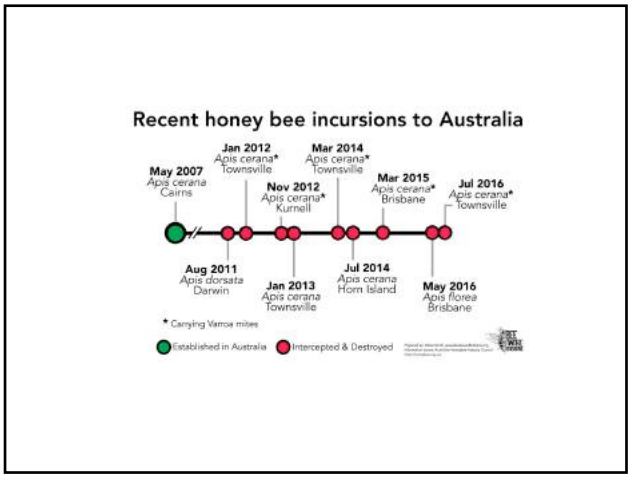

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And they keep on coming….

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INDUSTRY NOTICE NO. 1

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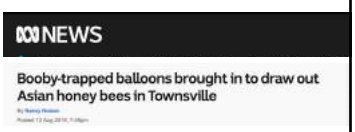
2. Method for trapping drones at mating congregations can be used to identify infested areas



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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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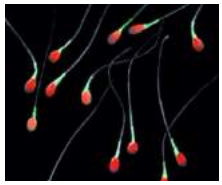
Spermatheca dissections



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

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

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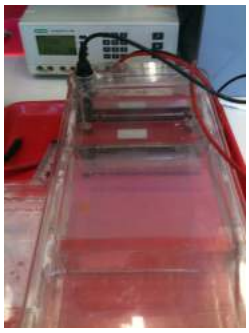
Agarose gel electrophoresis



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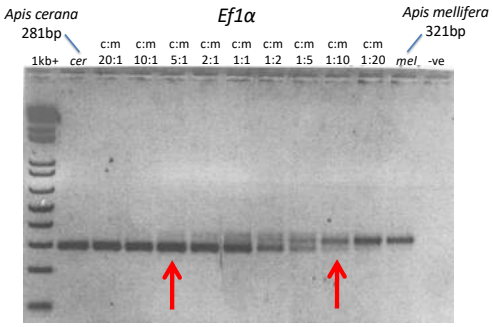
Universal PCR



Detection sensitivity: 1/5-1/10

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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	*A. mellifera*	42	6	14.0
	A. cerana	30	0	0
Cairns, Queensland, Australia	*A. mellifera*	12	4	33.3
	A. cerana	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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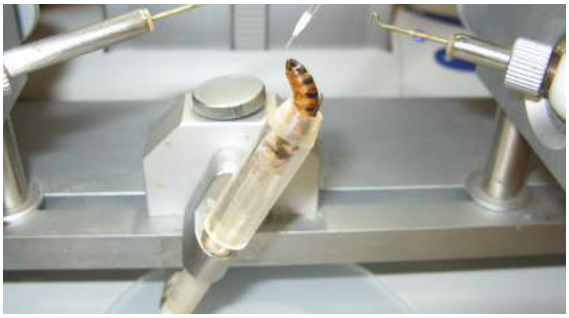

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Artificial insemination in Cairns

	AI Treatment	Offspring
Hive 1	*cerana* sperm	drones + 1 worker*
Hive 2	*cerana* sperm	drones
Hive 3	saline	½ dro
Hive 4	saline	
Hive 5	*cerana* sp	ggs only*
Hive 6		drones
Hive		drones
Hiv	ne	drones

All bees killed at pupal stage

enotyping:

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.
- Gloag, 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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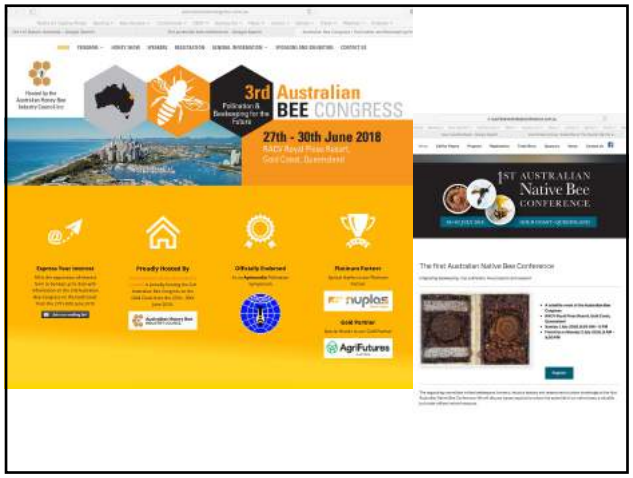

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Thank you.

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