E1 Project Title

Choosing when to be sexual: clonal and sexual reproduction in a population of honey bees

E2 Project Description and Background

The ‘Paradox of sex’ arises from the fact that a mother shares the genome of her sexually-produced offspring equally with her mating partner. Mothers can double their reproductive success by dispensing with the services of males, yet sexual reproduction is near universal in animals [1]. The Paradox remains a conundrum because though most authors would acknowledge that genetic recombination is the key to sex, there is no universal explanation of why recombination is useful [2]. Crucial for exploring the Paradox experimentally are systems where sex is facultative, so the circumstances in which females clone themselves and when they reproduce sexually can be elucidated.

Recent work has revealed that queens of the ant *Cataglyphis cursor* have evolved an interesting system that utilizes the benefits of sex while paying few of the costs [3]. Workers are produced sexually, so that colonies are genetically diverse, providing benefits of disease resistance and an enhanced labour allocation system [4]. In contrast, new queens are almost exclusively produced asexually. The benefit of this system for queens is that they gain genetic immortality by cloning themselves, while colonies reap the main benefit of sex – genetically diverse offspring.

A complicating factor in the Paradox is inter-sexual genomic conflict - mechanisms evolved by males that manipulate the ability of females to choose to clone themselves. For example, in the little fire ant, *Wasmannia auropunctata*, queens do clone themselves, but in this species the males ‘fight back’, and the female genome is eliminated in eggs destined to become males [5].

These ant studies have revealed amazing phenomena, and would be perfect for exploring broader questions concerning the Paradox if they were amenable to experimental manipulations such as artificial insemination and if a sequenced and annotated genome were available. But these advantages are not found in the ants. Fortunately we have recently shown that the Cape honey bee of South Africa (*Apis mellifera capensis*, hereafter ‘Cape’), has a similarly bizarre sexual system to the ants [6], but for these bees a complete genomic sequence and genetic map are available. Additionally, they are manipulable by beekeeping techniques and their behaviour can be observed directly in observation hives.

*The aim of this project is to utilize Cape honey bee queens to discover genetic and social constraints that prevent social insect queens from using parthenogenesis to clone themselves during reproductive swarming.* In achieving this aim we will make a significant contribution to the question of the origins and maintenance of sex in haplo-diploid insects and beyond.

Two types of parthenogenesis. Two kinds of parthenogenesis are observed in Hymenoptera (Figure 1). Arrhenotokous parthenogenesis occurs when one of the haploid pronuclei of Meiosis II divides mitotically and the egg develops as a haploid male. In thelytokous parthenogenesis, two of the pronuclei combine to produce a diploid zygote, as if one of the maternal pronuclei acted as a sperm. In most honey bee populations, only the arrhenotokous form of parthenogenesis is known and when queens or workers lay unfertilised eggs, male bees are produced by this means. In the Cape bee, queens produce males arrhenotokously, but when unmated workers lay eggs the eggs usually develop as females thelytokously.

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Why thelytoky matters. When a honey bee colony reproduces by swarming, the workers construct about 10 ‘queen cells’ that are peanut shaped and hang down from the comb. The queen lays an egg in each of the cells, and the workers lavishly feed the resulting larvae with royal jelly so they develop as queens. In normal honey bee populations the workers never lay eggs in queen cells because their eggs develop as males. Even if they did, the eggs would likely be eaten (‘policed’) by other workers [7]. However, we have recently shown that in Cape colonies, both queens and workers lay in queen cells, and that 23 of 39 new queens were in fact thelytokous daughters of workers [6]. Intriguingly, only 3 of the 16 queen-laid queens in our study were parthenogens, while the rest were produced sexually. So why don’t Cape queens do what the ant queens do and their daughter workers do and reproduce thelytokously when producing daughter queens?

It’s not because they can’t. If virgin Cape queens are anaesthetized with carbon dioxide they will, like all other honey bee queens, commence laying unfertilised eggs. Unlike queens of other subspecies, many eggs of virgin Cape queen develop as females thelytokously. We have genotyped male and female offspring of virgin queens at multiple microsatellite loci on two chromosomes. This study showed some remarkable phenomena (see progress report on DP 0664627). First, while the queens often made mistakes, they mostly laid appropriate eggs in the appropriate cells: arrhenotokous eggs destined to produce males in male-sized brood cells, and thelytokous eggs destined to be workers in worker-sized cells. Consider the implications of this. Because honey bee eggs are laid with their pro-nuclei arrested at metaphase II, arrhenotokous or thelytokous development occurs only after the egg is laid [Fig 1 and ref 8]. Our preliminary study showed that Cape queens are able to influence the kind of parthenogenesis that occurs in their eggs seemingly at will and post partum. Second, we showed that, unlike Cape workers, which have a form of parthenogenesis that greatly reduces meiotic recombination and therefore preserves heterozygosity [9], virgin Cape queens don’t do this: there is no reduction in the rate of recombination.

Sex determination – why recombination matters. In honey bees sex is determined by the combination of paternal and maternal alleles at a single locus, the Complementary Sex Determiner
If the individual is heterozygous at the \textit{csd} it is female. If the individual is homozygous at the \textit{csd} a diploid male develops, but these are removed by workers at the first larval instar and are therefore non-viable [11]. If the individual is haploid and therefore hemizygous at the \textit{csd}, it is male. The \textit{csd} encodes a \textit{Transformer}-type protein, which is enormously polymorphic [12] due to diversifying selection [10]. Beye et al [10] propose that the protein encoded by \textit{csd} must be heteromorphic in order to initiate female sexual development, and monomorphic to produce a male. \textit{Csd} probably acts on an unknown gene, which controls alternative splicing of \textit{double sex}. One splice variant \textit{dsx}$^\text{M}$ initiates male development while two others \textit{dsx}$^\text{F1}$ and \textit{dsx}$^\text{F2}$ initiate female development [13].

\begin{figure}[h]
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\caption{In thelytokous parthenogenesis with central fusion the two central meiotic products fuse while the terminal products (grey) form polar bodies and die. If there is recombination between a locus and the centromere (left) alleles become randomly placed on the four chromatids at meiosis I. (I have drawn the crossover for the middle pair but it could equally be for the outer pair.) Counter-intuitively, this means that for any locus heterozygous in the mother, there is a 1/3 (not 1/2) chance that an offspring will be homozygous. This ratio arises because if we chose any one chromatid at random, two of the three remaining chromatids will carry the alternate allele. If there is no recombination (right), the offspring remain heterozygous. See [14] for details and more discussion of how the 1/3 probability arises.}
\end{figure}

Sex determination via a single complementary sex locus has important consequences. In sexually producing honey bee populations we predict that selection will act to increase recombination rates at the \textit{csd} because recombination increases the probability of heterozygosity at the \textit{csd}. As expected, the region around \textit{csd} shows a four-fold increase in recombination rate relative to the genome-wide average, presumably as a mechanism for generating and maintaining diversity at the \textit{csd}. [15] and preventing the accumulation of deleterious recessives around it. But what is expected in thelytokous populations? Under thelytoky with central fusion of meiotic products we expect a 1/3 reduction in recombination rate at any locus distal to a crossover [Figure 2 and ref 14]. Thus in thelytokous populations we expect reduced levels of recombination to evolve, at least on chromosome 3 which carries the sex locus. Studies of recombination rates in Cape workers show that they are at least an order of magnitude lower than in sexual queen meiosis [9], strongly suggesting that selection for reduced recombination has indeed occurred in thelytokous Cape workers. However, our recent work has shown that this reduction in recombination does not occur when virgin Cape queens reproduce thelytokously (see progress report).

‘Clonal’ thelytokous lineages of reproductive parasites. Thelytoky gives the opportunity for females to forgo sex and produce female offspring like themselves. And indeed, some Cape workers take advantage of their parthenogenetic abilities to found lineages of reproductive parasites that enter nests and parasitize them with their eggs [16]. One lineage, originating from a single
worker who lived in the early 1990s, has killed hundreds of thousands of colonies in what can accurately be described as a social cancer [17]. This lineage is not unique, and others infest the honey bee population of South Africa [6]. All these lineages have an Achilles’ heel, for without sex they will gradually lose heterozygosity via recombination (Figure 2). The current dominant lineage has delayed the inevitable decline into homozygosity by a massively reduced rate of recombination relative to other honey bees [9], but eventually any ancient lineage will end up with too little diversity to compete with younger more heterozygous lineages.

Genetics of thelytoky. In many arthropods thelytoky is induced by endosymbionts such as Wolbachia [18]. However although Wolbachia is present in Cape populations its distribution across sexes demonstrates that it is not responsible for thelytoky [19]. Rather, in Cape workers the thelytoky/arrhenotoky switch is controlled by a single gene, Th, which is most likely a homolog of Grainy Head of Drosophila melanogaster [20]. This gene has multiple pleiotropic effects in workers, including early onset of oviposition, large ovaries, and production of queen like pheromones. Its expression in queens is unknown, and it is not known if it causes the reduced recombination rates observed in the lineage studied by Baudry et al [9].

Summary
This project will use the Cape honey bee to examine the Paradox of Sex. The Cape honey bee is an ideal study species because queens can produce asexual eggs that develop as males or females or sexual eggs that produce females. We can therefore examine the conditions in which queens choose to share their genomes with males – and when they don’t.

E3 SIGNIFICANCE AND INNOVATION

Significance
First raised in the early 1970s [21], the Paradox of Sex remains a central problem in both genetics and evolutionary biology. (An entire issue of Nature Reviews Genetics (vol 3, issue 4) was devoted to it in 2002.) Recent reports of inter-sexual genomic conflict in ants have exposed a new dimension to the complexities of Paradox of Sex in the lives of social insects [22], and this relates to male-female genomic conflict. Resolution of such may in fact be the key to the maintenance of sex. Queen genomes clone themselves, male genomes fight back by eliminating female genomes [5] — astonishing truths that seemed unbelievable as little as 3 years ago. This project will explore what I suspect are similar (but different) phenomena in the genetically tractable Cape honey bee. Importantly, this project will go far further than just revealing astounding phenomena, as has been done in the ants. Rather, we will unravel the genetic mechanisms behind them: the meiotic processes that potentially lead to the elimination of male genomes and the genetic system that reduces recombination in thelytokous lineages of parasitic workers. Thus we will show how the parasites are able to flourish for many generations [9] before (we assume) they finally peter out from the genetic load imposed by increasing homozygosity. I argue that Cape bees provide an outstanding opportunity to examine these remarkable new phenomena using the full suite of genomic tools available, and that this stellar opportunity should not be passed up lightly.

Addressing interesting problems and enhancing the knowledge base of social insects and genetics
We will:

- Make a fundamental advance in the genetics of sex determination by explaining how a homozygous haplo-diploid insect can be female – a genetic impossibility with our current understanding of sex determination in honey bees.
- Determine the meiotic origin of homozygous individuals that are seen in about 5% of queen progeny during reproductive swarming. These individuals probably arise from the duplication of a maternal pro-nucleus, but does this arise after the elimination of the male genome? If not, is the sperm necessary to activate the process? The Journal Club editor of Nature [23] and the authors commissioned by Current Biology [24] to prepare a commentary article on our paper [6]...
emphasized that the origin of these homozygous individuals is an outstanding question that demands a thorough genetic analysis.

- Show how alternative means of parthenogenesis (thelytoky and arrhenotoky) can co-exist in the same species, and indeed the same individual, and how queens can regulate the kind of parthenogenesis that occurs in their unfertilised eggs.
- Discover the genetic mechanisms that suppress recombination in the clonal worker lineages that fail to be expressed in queens. In the wasp, Trichogramma, heterozygosity is maintained because thelytoky is apomictic [25], which is not the case in Cape workers [9] or Cataglyphis ants [14]. Understanding how Cape workers suppress recombination should provide insights into a fundamental aspect of chromosome mechanics, and could potentially provide an important advance in our ability to maintain asexual lineages of automictic bio-control agents from the genus Lysiphlebus. More broadly, genetic recombination is fundamental to meiosis and indeed eukaryotic life. This project is likely to reveal general principles about how rates of recombination are regulated in eukaryotes.

Innovation

The project is innovative because we will be the first to address fundamental aspects of the paradox of sex and genomic conflict and cooperation in insect societies. I believe that the Cape bee is the only social insect that is known to show the fascinating phenomena of thelytoky, asexual and sexual queen replacement, genetic suppression of meiotic recombination and reproductive parasitism by workers. Importantly, unlike ants, Cape bees can be artificially inseminated, and a complete genomic sequence is available so that chromosomal markers and genes related to sex determination are already characterized. Thus we will be able to directly visualize the behaviour of genomes in conflict by fluorescently labeling chromosomes (including that related to sex determination) and observing their fate during meiosis and syngamy.

To our knowledge this project will be the first attempt to artificially inseminate a social insect worker. Unlike all other honey bees, Cape workers have a spermatheca [26], and can thus potentially be inseminated. This project will be the first to use FISH to determine ploidy in the honey bee, and the first to use FISH to observe the cytogenetic events during thelytokous and arrhenotokous meiosis of Cape queens.

Priority area – Safeguarding Australia, Protecting industries from exotic pests and diseases

Thelytokous lineages of Cape workers are reproductive parasites of all other A. mellifera and have caused the death of hundreds of thousands of commercial colonies in South Africa, and continue to cause massive problems [27]. The Australian Honey Bee Industry Council and AQIS list the Cape bee as an exotic pest likely to cause significant damage to the honey industry if established here. Establishment could occur via an illegal importation of breeding stock from South Africa, or (more likely), a queenless swarm arriving in a container on aircraft or shipping. Honey bee swarms are occasionally found in Australian ports. Understanding the genetic mechanisms behind Cape bee thelytoky will help prepare the Australian industry and agencies to deal with an incursion of Cape bees should this occur.

E4 APPROACH AND METHODOLOGY

General cytological techniques. We will follow Yu and Omholt [28]. The colony to be studied will be transferred to an observation hive. The hive will be fitted with an artificial plastic comb that allows individual eggs to be removed straight after an oviposition is observed without opening the colony [i.e. the cell bases are exposed at the back of the colony and can be removed, 29]. Eggs will be removed within a minute of being laid, and either fixed immediately by placing them in a refrigerator, or allowed to develop for various times at 34.5 C and high RH. To visualize both maternal and sperm nuclei (if present), we will inject the eggs with 1 mg/ml of the nuclear stain Hoechst 33342 (Sigma) in 0.9% NaCl solution at the anterior pole and incubate them for > 1.5 hr at 4 C. Ooplasm will be removed from the posterior end of the egg with a pipette using a
microinjector, and the egg gently flattened with a cover slip before observation under a fluorescent microscope.

To determine the ploidy of embryos we will use Fluorescent *In Situ* Hybridization (FISH). Eggs will be prepared as in [30] and probed with fluorescently labeled 4.5 kBP oligonucleotides designed from the honey bee genome. Diploid eggs will reveal two dots per nucleus whereas haploid eggs will show one [30]. We will develop two probes: i) the csd itself and ii) a gene such as *Abdominal-a*, which is unlikely to show variation and is the right length. *Abdominal-a* will provide a definitive test of ploidy. The csd may show duplications or other important phenomenon that cannot be predicted – or may not be suitable at all because it is too variable.

**Mated queen thelytoky.** Last year we found that 3 of 16 queen-laid queen larvae were homozygous at all loci studied, even though these same loci were heterozygous in their mother [6]. We have recently confirmed this finding with independent samples (BP Oldroyd et al unpublished). These individuals are a profound mystery, for homozygosity should lead to the development of a male, and loss of homozygosity at multiple loci in one meiosis is incompatible with the mode of thelytoky observed in virgin queens [31] or in workers [9].

To understand the origin of these individuals we will first need to identify queens that produce them. Thus we will inspect ‘wild’ Cape colonies, during the swarming season, find the queen in each, and take a clipping of their wings for genotyping [32]. From all colonies where queen cells are present we will remove the cells, with a target of obtaining 2-300 queen cells from 20-30 colonies. We shall also sample worker pupae from each colony. Wing clips and all larvae and pupae will be genotyped at 10 microsatellite loci. By this means we will identify i) queens that produce homozygous female offspring; ii) any queens that are themselves homozygous at all loci. This will allow us to obtain reliable estimates of the frequency of parthenogenetic reproduction when queens produce queens, and of the proportion of these (if any) in the adult queen population. Because we will need to exclude the possibility that homozygous individuals are in fact haploid males laid in error, it will be necessary to sex homozygous individuals morphologically. Female pupae are easily distinguished from males by their smaller eyes. Larvae will be sexed by microscopic examination of the sternum of the posterior segments [33].

We will devise a trace back system so that we can return to particular surveyed colonies with either of the two kinds of interesting queens. We will confirm that genotyped queen is in residence by her clipped wing. If present we will transfer her and her colony to Stellenbosch for further study. We will transfer these colonies into modified observation hives as described above, and collect eggs from queen, worker and drone cells. We will breed from these queens and inseminate the offspring with related males in an attempt to develop a line where queens regularly produce offspring parthenogenetically.

These queens will enable us to undertake the following studies:

- **Origin of the homozygous queen cell contents** We know that unmated queens can produce heterozygous eggs by thelytoky, but the only thelytokous eggs that we have observed so far in mated queens were completely homozygous. It seems likely that these arise from fusion of a duplicated maternal pronucleus that forms after competition between maternal and paternal pronuclei within the egg. This hypothesis is extremely difficult to test directly. However two approaches should go a long way towards understanding of the system. First, we will examine the frequency of homozygosity in queen progeny and worker progeny of a queen known to produce queens parthenogenetically. Both castes should be produced by the same sexual syngamy (Fig 1). If the anomalous homozygous individuals are only found among queen progeny, we will infer that queens make an active choice to lay thelytokously in queen cells, but this is somehow derailed by the presence of sperm nuclei. Second, we will examine a time series of eggs laid in queen cells 10, 30, 60 and 90 min post oviposition cytologically. Maternal gamete fusion after competition with sperm pro-nuclei will be inferred if sperm pronuclei are either absent or die during syngamy. Suitable control eggs from an arrhenotokous population will also be examined.
• Progeny of homozygous queens If we find or breed any adult homozygous queens we will induce them to lay in queen cells in a colony set up to mimic conditions prior to swarming. Eggs will be examined genetically to determine their parentage and cytologically to infer the mechanisms by which pro-nuclei fuse. If the queen progeny of homozygous queens are themselves produced thelytokously, then this would indicate that in addition to the clonal lineages of reproductively parasitic workers that infest the Cape population, there are also queen lineages that propagate asexually while still retaining sexually produced workers.

• Do any homozygous queens become adults? The survey and associated genotyping will show if any adult queens are themselves homozygous. This is important because it will show that queens as well as workers can establish parthenogenetic lineages. We will then see if, like C. cursor, the homozygous queens use sex to produce workers, but produce queen offspring asexually.

What is the reproductive strategy of queens that are daughters of workers? We will obtain queenless colonies of Cape workers and rear queens from worker-laid eggs by transferring the eggs into queenless colonies of A. scutellata for rearing. The resulting queens will be allowed to mate naturally. Microsatellite analysis of worker offspring of these queens will allow us to determine if they reproduce thelytokously like their worker mothers or sexually like normal queens.

Does the th locus cause a reduction in recombination? The locus that controls the thelytoky/arrhenotoky switch in workers has several pleiotropic effects that are related to the ability of workers to become reproductively active: queen-like pheromone production and early activation of ovaries [20]. Could another effect of this locus be inhibition of recombination? We will backcross a F1 th/+ queen to a single th male. We will then confine marked individual worker backcross offspring (th/th or th/+) in cages with 50 workers of the A. m. scutellata subspecies, which is universally arrhenotokous [16]. We will record when eggs first appear. We will terminate the experiment after 10 days and genotype each marked worker to find 20 microsatellite loci on each of chromosomes 3 and 13 that are heterozygous. Chromosome 3 carries the csd and chromosome 13 carries th. Individuals that are th/th will lay thelytokous eggs early and these eggs will be heterozygous at some loci. Individuals that are th/+ will produce haploid eggs arrhenotokously. These will be monomorphic at all loci (any eggs with genotypes incompatible with being laid by the marked worker will be discarded). If the th allele causes a reduction in recombination events, all diploid individuals will show an order of magnitude reduction in the recombination frequency expected based on the known map distances [34]. Chromosome 3 is 3.5 Morgans and Chromosome 13 is 2.8 M, so we expect an average of 3.5 and 2.8 crossovers on chromosomes 3 and 13 respectively. We can compare the number of chiasmata observed genetically (see diagram) to the expected number [9]. If th affects recombination frequencies we will expect an order of magnitude reduction in the expected recombination frequency in all diploid offspring. If the recombination suppressor is unrelated to th we will find that not all of the diploid progeny will show a reduction in
recombination frequency. These individuals will have inherited the th/th genotype and so are thelytokous, but will be heterozygous at other loci that control the rate of recombination.

**Artificial insemination of workers.** We will mature 20-30 marked Cape workers each in a cage of 50 *A.m. scutellata* workers until they are 5 days old. We will then use an artificial insemination apparatus to inseminate each worker with 2-3 μl of semen (it will be necessary to modify the ‘queen holder’ so that it will restrain the smaller worker). The fathering drones will be retained for genotyping. After eggs appear we shall sacrifice the marked workers and determine microscopically if semen has entered their spermatheca [35]. We will then genotype the worker and a sample of eggs to determine if they were produced thelytokously or are biparental. If genotyping the eggs reveals that they are produced thelytokously we will examine them cytologically (see above) to determine if sperm cells entered the eggs and if so what their behaviour is. We will also attempt direct fertilization of very young eggs. Fertilization of eggs is possible if they are exposed *in vitro* to semen within 30 min of being laid [36]. Thus if insemination fails we should still be able to observe what happens to sperm cells in an egg which was laid by a worker that is th/th. Will a sperm cell take precedence from one of the central pronuclei and form a zygote, or will central fusion of maternal pronuclei occur?

**How can a homozygous individual be female?** In honey bees an individual must be heterozygous at the sex locus in order to be female — yet we have multiple instances of females homozygous at all loci examined including a locus within the csd region. How? There are several possibilities including a duplication such that there are two different copies of csd within a single genome or a modification of the upstream gene that interacts with csd so that the female splice variants of double sex are always produced. Using primers specific to the csd [15] we will amplify DNA from the homozygous females. The objective then is to show beyond reasonable doubt whether the csd is monomorphic or not. PCR products will be cloned into a microbial vector and multiple clones sequenced at a commercial facility. Sequences will be aligned and compared with each other and to reference sequences to determine if the homozygous individuals are in fact polymorphic at the csd. A potential pitfall with this approach is that the PCR or cloning steps will asymmetrically amplify one allele over another so that one allele is missed. One solution to this problem is digestion of PCR products with restriction enzymes of short recognition sequence. If there is only one allele, the combined lengths of the bands that show after digestion will equal the length of the undigested product. If there are two alleles, the combined length will exceed the length of the undigested product. If we can convincingly show that the csd is indeed monomorphic in these homozygous females we shall assume that a gene downstream of csd has been modified to always produce females. This could be confirmed by reverse transcription of double sex RNA to determine if the female or male-producing splice variants are present [13].

**Breeding queens from thelytokous workers.** We know that workers can be the thelytokous mothers of queens [37]. We will raise queens from workers and examine their progeny genetically to determine if these queens produce female offspring sexually or asexually.

**E5 National benefit**

- **Fundamental knowledge** Genetics is an enabling science. Just as ‘nothing in biology makes sense except in the light of evolution’, so too very little in evolutionary biology makes sense without genetics. The thelytokous honey bees of South Africa provide a magnificent opportunity to address fundamental questions in evolution and genetics: Why is sex important? How are asexual lineages maintained? What are the alternative reproductive strategies available to queens and workers? How did haplodiploidy evolve? What stops asexual lineages from replacing sexual ones? The project will provide insights into a new form of parthenogenesis that results in homozygosity at all loci, and new insights into sex determination in bees – how can individuals that are homozygous at the sex locus also be female?
• **International collaborations** This project will feature South African-Australian collaborations and will act to promote and strengthen research ties between Australia and South Africa. Our project will attract international interest, drawing talented students and postdoctoral fellows from overseas (we currently have interest from French, British and Indian students for thesis work on this project).

• **Building on world-class research capacities** Our lab is one of the largest honeybee research groups in the world. This project will provide us the means to pursue a new direction into honey bee cytogenetics. In Australia there is a lack of expertise in insect cytogenetics. Our project will provide an ideal environment for training cytogeneticists, molecular ecologists, entomologists, apiculturalists and population geneticists, producing a new generation of experimentally and theoretically sophisticated biologists, capable of performing high-quality scientific research that is competitive internationally. Our graduates will have the breadth of entomological, evolutionary and molecular training to play leadership roles in agriculture and fundamental biology. Australia needs people with such training and knowledge to support agricultural industries, to manage our natural environment, and to inspire the next generation of Australian biologists.

• **Leadership** This project will ensure our lab’s place at the forefront of international honey bee research. The project will ensure access by Australian researchers at all levels to experimental systems that will lead to conceptually innovative science.

• **Protecting the Australian honey industry** In South Africa, intra-specific parasitism by offspring of a single thelytokous worker [9] led to the loss of 20-50% of commercial colonies within two years [27]. Such a disaster could happen in Australia if *A. m. capensis* were inadvertently introduced, or if an equivalent mutation occurred naturally here. Our project will generate an understanding of the genetic mechanisms that led to the large-scale parasitism event in South Africa. We therefore anticipate that we will determine the best strategies for controlling an outbreak of parasitic workers. Thus this project falls within the priority area of ‘Safeguarding Australia’ and priority goal ‘Protecting Australia from invasive diseases and pests’.

**E6 COMMUNICATION OF RESULTS**

The applicant has a strong record of publication in top scientific journals including *Nature*, *Science* and *PLoS Biology* and reporting results at pre-eminent scientific congresses such as the international meetings of the IUSSI and the International Congresses of Entomology and Genetics. This project will allow our laboratory to continue generate exciting results that we will submit to the best journals. Furthermore, we will maintain our active links with the honey industry, attending their conferences and writing for their industry journals. We regularly give talks to a general public and have extensive experience with writing articles on scientific issues for the lay reader.

**E7 DESCRIPTION OF PERSONNEL**

**Oldroyd** will lead the project and will take responsibility development of FISH, most fieldwork and artificial inseminations, and development of new theory.

We will involve the following collaborators whose contributions (time, expertise and logistical support) will be funded by their respective agencies and will greatly enhance the value of this project. All participants will be intimately involved in the planning and conduct of these experiments.

**Dr. Victor Rambau** (Lecturer, University of Stellenbosch) is part of the strong Evolutionary Genomics group at the University of Stellenbosch. He has expertise in FISH and other cytogenetic techniques relevant to this project. He will collaborate by providing access to his fluorescent microscopy facilities, and his expertise in the study of chromosomes.

**Mr. M.H. Allsopp** (Research Scientist, Agricultural Research Council, Stellenbosch, South Africa): Allsopp will provide facilities for the observation hives and access to his Cape colonies for
survey work. Mr. Allsopp is arguably the world authority on Cape bee biology, and has over 10 years experience working with them. The Stellenbosch location of his lab minimizes problems that can arise further north in South Africa, where the Cape bee hybridises with other subspecies. 

Dr. T.C. Wossler (Lecturer, Department of Zoology and Botany, University of Stellenbosch, South Africa) will host the Australian students and provide expertise in the biology of Cape bees and logistical support. 

A/Prof M. Beekman (QEII Fellow, University of Sydney) will provide expertise in Cape bee biology and will collaborate in all aspects. 

E8 References

34. Solignac, M. et al. (2007) Genome Biology 8