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Original article

Wolbachia is present in *Apis mellifera capensis*, *A. m. scutellata,* and their hybrid in Southern Africa

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Abstract – *Apis mellifera capensis*, *A. m. scutellata* and their hybrids were screened by a sensitive Long PCR protocol for *Wolbachia* because this endosymbiont has been implicated in causing thelytoky in other Hymenoptera. *Wolbachia* was found in all workers of *A. m. capensis* examined, and in workers and drones of *A. m. scutellata* and in hybrid workers of these two subspecies. Cloning and sequencing indicated that all contained the same unique *Wolbachia* strain, named *w*Cap-B1, which belongs to the Con Group because it displayed less than 2.5% sequence divergence from the reference strain from *Tribolium confusum*. *w*Cap-B1 is closely related to *Wolbachia* from *Diaphorina citri*, *Solenopsis invicta*, *Coleomegilla maculata lengi*, *Plutella xylostella*, and *Bemisia tabaci*. Because no sequence differences were found among these bee populations, infection with this *Wolbachia* strain is unlikely to be associated with thelytoky in *A. m. capensis.*

***Apis mellifera capensis* / *Apis mellifera scutellata* / *Wolbachia* / Long PCR / thelytoky**

1. INTRODUCTION

In most populations of *Apis mellifera* L., females are produced from fertilized queen-laid eggs while males are haploid and are produced from unfertilized queen-laid eggs. Unfertilized eggs also can be produced by worker honeybees under queenless conditions. In all races of *Apis mellifera* except the Cape honeybee, *A. m. capensis* Escholtz, these unfertilized eggs are produced by arrhenotokous parthenogenesis and give rise to haploid males (Ruttner, 1988). In the case of workers of the Cape honeybee, however, unfertilized eggs are produced by thelytokous parthenogenesis and give rise to diploid females (Onions, 1912; Ruttner, 1988). Some colonies of *A. m. capensis* and hybrids of *A. m. capensis* and *A. m. scutellata* Lepeletier exhibit both arrhenotokous and thelytokous worker reproduction (Hepburn and Crewe, 1991).

The ability of Cape honeybee workers to produce female progeny is central to the 'Cape Honeybee Problem' in South Africa where, since 1990, *A. m. capensis* workers have invaded *A. m. scutellata* colonies, causing the loss of many tens of thousands of colonies (Allsopp, 1992; Oldroyd, 2002). Research into the unique features of Cape honeybees has included investigating the genetic basis of thelytoky in *A. m. capensis* (Crewe and Allsopp, 1994). The basis of the *capensis* phenotype

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has been studied by many (Oldroyd, 2002). The genetics basis of the thelytoky could be due to either nuclear or cytoplasmic genetic factors, or interactions between them. One possibility is that the thelytoky is caused by *Wolbachia* in *A. m. capensis*.

Wolbachia is one of the most common cytoplasmically inherited microorganisms in arthropods and its effects on their hosts include altering sex ratio or inducing thelytoky (Rigaud and Rousset, 1996; O'Neill et al., 1997; Werren, 1997; Cook and Butcher, 1999). *Wolbachia* are gram-negative bacteria and are widespread, with 17 to 76% of all arthropod species infected with one or more strains (Werren et al., 1995; Werren, 1997; Jeyaprakash and Hoy, 2000). *Wolbachia* have been found in numerous species of Hymenoptera, including parasitoids and ants (Stouthamer, 1997; Cook and Butcher, 1999; Shoemaker et al., 2000; Wenseleers et al., 1998; Jeyaprakash and Hoy, 2000). *Wolbachia* induces thelytoky in at least 40 species of Hymenoptera (Cook and Butcher, 1999); in a number of cases, the thelytokous insects have been 'cured' of *Wolbachia* after treatment with antibiotics or heat shock and arrhenotokous reproduction has been restored (Zchori-Fein et al., 1992; Cook and Butcher, 1999; Stouthamer, 1997). However, the presence of *Wolbachia* in hymenopteran species does not always induce thelytoky and a full understanding of the evolution and physiological and phenotypic effects of *Wolbachia* on their arthropod hosts is lacking (Rigaud, 1999; Weeks et al., 2002). Recently, for example, Dedeine et al. (2001) found that *Wolbachia* were necessary for oogenesis in the parasitoid *Asobara tabida* and its removal led to sterility, suggesting that the presence of *Wolbachia* in this species is obligatory.

The presence of *Wolbachia* in social Hymenoptera, including the Cape bee, was investigated by Wenseleers and Billen (2000) using a Standard PCR protocol to amplify 16S rDNA sequences from *A. m. capensis* and several species of ants, but they were unable to find *Wolbachia* in *A. m. capensis.* Because we had discovered that a Standard PCR protocol was six to eight orders of magnitude less sensitive than a Long PCR protocol in amplifying *Wolbachia* sequences from a diverse array of arthropod species (Jeyaprakash and Hoy, 2000), we decided to reinvestigate the status of *Wolbachia* in *A. m. capensis*. If *A. m. capensis* contained *Wolbachia*, but *A. m. scutellata* did not, further research into the possible role of *Wolbachia* as the causal agent of the thelytoky seen in *A. m. capensis* would be warranted.

This paper compares the sensitivity of the Long and Standard PCR protocols in amplifying a portion of a *Wolbachia* surface coat protein gene (*wsp*) from southern African populations of *A. m. capensis, A. m. scutellata,* and hybrids between them. The *Wolbachia wsp* sequences obtained were cloned and sequenced. The role of *Wolbachia* in inducing thelytoky in *A. m. capensis* is discussed.

2. MATERIALS AND METHODS

2.1. Colony sources

Bees were collected into 95% EtOH by M. Allsopp from colonies of *A. m. scutellata, A. m. capensis* and hybrids of these two subspecies in southern Africa (South Africa and Zimbabwe) and shipped to the University of Florida for analysis (Tab. I). *A. m. capensis* samples were collected from Robben Island and Stellenbosch in the Western Cape of South Africa. Because the 'Capensis Problem' has resulted in *A. m. capensis* being present through much of South Africa that was inhabited formerly only by *A. m. scutellata*, the *A. m. scutellata* sample was collected from northern Zimbabwe to ensure its purity. A sample of hybrids of the two races was collected from Grahamstown in the Eastern Cape of South Africa (Hepburn and Crewe, 1991).

2.2. DNA extraction and PCR protocols

Single bees were used to extract DNA using Puregene reagents (Gentra Systems, Minneapolis, MN) and the genomic DNA was resuspended in 50 μL of sterile water. 1 μL genomic DNA preparation was used for PCR amplification.

Primers (30-mers, Wsp-F, 5'-TGGTCCAA TAAGTGATGAAGAAACTAGCTA-3' and Wsp-R, 5'-AAAAATTAAACGCTACTCCAGCTTCTGC AC-3') were designed from the *Wolbachia wsp* gene sequence of *Drosophila simulans* (GenBank [http://www.ncbi.nlm.nih.gov] Accession AF020070) to amplify a variable region of about 0.6 kb (Braig et al., 1998).

Standard PCR was performed by the hot start method in a 25 μl volume containing 10 mM Tris

Table I. Colony source and type of *A. mellifera* subspecies from southern Africa tested for the presence of *Wolbachia* by the Long PCR protocol using *wsp* primers.

Site number	Source (type)
1	Robben Island drones (*capensis* or hybrids)*
2	Robben Island workers (*capensis* or hybrids)*
3	Stellenbosch1 drones (pure *capensis*)
4	Stellenbosch1 workers (pure *capensis*)
5	Zimbabwe160 workers (pure *scutellata*)**
9	Stellenbosch2 workers (pure *capensis*)
17	Grahamstown1 workers (hybrids)

* Robben Island bees tested should be pure *capensis*. Although *A. m. scutellata* were kept there 25 years ago, and there are minute traces of *A. m. scutellata* on the island, the bees are "basically 100% pure *capensis*" (M. Alsopp).

** All other bees were collected in South Africa.

(pH 8.3), 1.5 mM MgCl2, 50 mM KCl, 200 μM dATP, dGTP, dCTP, dTTP, 400 picomoles of primers and 0.8 unit *Taq* DNA polymerase (Roche Molecular Biochemicals, Indianapolis, IN) (Saiki, 1989). The PCR was performed using 35 cycles, each consisting of denaturation at 94 °C for 30 s and annealing and extension at 72 °C for 1 min.

Long PCR was performed in a 50 μL volume containing 50 mM Tris (pH 9.2), 16 mM ammonium sulfate, 1.75 mM $MgCl_2$, 350 μM dATP, dGTP, dCTP, dTTP, 800 picomoles of primers (Wsp-F and Wsp-R), 1 unit of *Pwo* and 5 units of *Taq* DNA polymerases (Barnes, 1994). The DNA template, buffer and enzymes were mixed in 25 μL volume and combined with a 25 μL volume containing the primers and dNTPs; both mixes were kept on ice prior to starting the amplification. The Long PCR was carried out using three linked profiles over 36 cycles; (i) 1 cycle of denaturation at 94 °C for 2 min, (ii) 10 cycles each consisting of denaturation at 94 °C for 10 s, annealing at 65 °C for 30 s and extension at 68 °C for 1 min, and (iii) 25 cycles each consisting of denaturation at 94 °C for 10 s, annealing at 65 °C for 30 s and extension at 68 °C for 1 min, plus an additional 20 s added for every consecutive cycle between 11 and 36.

The PCR products were electrophoresed on 2% agarose gels with TBE buffer with a Roche DNA size marker VI producing bands ranging from 2176 to 154 bp in lane I.

Table I lists the site numbers and bees evaluated. DNA isolated from five bees each from sites 1, 2, 3, and 4 were amplified by both Long and Standard PCR protocols on February 3, 2000 by A. Jeyaprakash, using the same DNA to compare the relative efficiency of the protocols. On September 6, 2000, J.M. Alvarez isolated DNA from two bees each from sites 5, 9 and 17 and amplified the DNA by the Long PCR protocol. On August 3, 2001 DNA from two additional bees each from sites 5, 9, and 17 were tested by the Long PCR protocol by A. Jeyaprakash. On October 12, 2001, DNA from five bees from site 5 was amplified by both the Standard and Long PCR by A. Jeyaprakash.

Long PCR products were purified using QIAquick PCR purification column (QIAGEN Inc., Valencia, CA) and cloned into the plasmid pCR2.1-TOPO using the procedure suggested by the manufacturer (Invitrogen Corporation, Carlsbad, CA). DNA sequencing was performed at the University of Florida ICBR Core Facility using a PERKIN-ELMER Applied Biosystems ABI PRISM Automated DNA Sequencer. Clean laboratory practices, sealed pipette tips, and fresh reagents were used to avoid contamination. Negative controls (consisting of all components except the DNA template) were conducted on each date to detect potential contamination, but positive controls were not carried out to reduce the likelihood of contamination.

The *wsp* sequences obtained were compared with *wsp* sequences found by a BLAST search of GenBank.

3. RESULTS AND DISCUSSION

3.1. *Wolbachia* DNA amplification by Standard and Long PCR protocols

Amplification of *wsp* sequences from *A. m. capensis, A. m. scutellata* and a hybrid between them by the Standard PCR protocol with the *wsp* primers never produced a product (partial data shown in Fig. 1). Comparisons were made using the same DNA as a template for both the Standard and Long PCR protocols

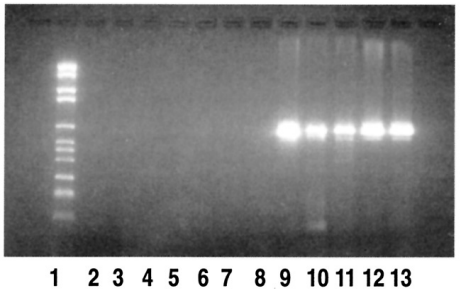

Figure 1. Long PCR was successful in amplifying the 0.6 kb *wsp* sequence of *Wolbachia* from five individuals of *A. m. scutellata* (lanes 9-13) from southern Africa, although the same DNA template when used in a Standard PCR protocol failed to yield a product (lanes 3-7). Lane 1 is DNA Marker VI and shows bands; lanes 2 and 8 are 'no template DNA' controls.

on February 3, 2000 and again on October 12, 2001. None of the 26 bees analyzed by Standard PCR yielded a product. By contrast, DNA from 36 of 37 bees examined by the Long PCR provided a PCR product of the expected length. The failure to obtain a PCR product by Standard PCR and *wsp* primers confirms the negative results obtained by Wenseleers and Billen (2000) with 16S rDNA primers for a population of *A. m. capensis*. By contrast, the Long PCR protocol gave positive results, consistently producing a band approximately 0.6 kb in size, as expected.

Long PCR products were obtained from populations of *A. m. capensis, A. m. scutellata* and the hybrid of these two races on each of the three dates the bees were tested and by two different people. PCR products were never obtained in the no-DNA controls, indicating that contamination was not responsible for these positive results (partial data shown in Fig. 1).

A sensitivity analysis conducted previously by Jeyaprakash and Hoy (2000), using cloned *wsp* sequences, indicated that Long PCR is approximately six to eight times more sensitive than Standard PCR in amplifying *Wolbachia wsp* and 16S rDNA sequences from diverse arthropod species. Although Standard PCR protocols have been used widely to amplify *Wolbachia* sequences, little information is available on the frequency of false negatives. Long PCR amplifies cloned *Wolbachia* *wsp* sequences consistently when mixed with insect DNA, even when there are as few as 100 copies of the plasmid present (Jeyaprakash and Hoy, 2000). Likewise, the Long PCR protocol was more sensitive than the Standard PCR when the *nus*G-*rpl*K segment of a plant-pathogenic bacterium (*Liberobacter*) was amplified from insects and citrus foliage (Hoy et al., 2001). The level of sensitivity obtained in these two prior experiments, along with the results of this survey of southern African bees, suggests that Long PCR is substantially more effective in amplifying microbial DNA when mixed with insect genomic DNA. Long PCR is effective in increasing sensitivity and fidelity (Barnes, 1994); whether the failure to detect *Wolbachia* in these bees by Standard PCR is due to inhibition of the reaction or to a low titer of *Wolbachia* is unknown.

3.2. *Wolbachia* sequence analysis

A Long PCR product from a site-1 bee (Robben Island drone, *capensis*) was cloned by A. Jeyaprakash and three clones were sequenced in February 2000. All three sequences were identical, indicating that there was only one type of *Wolbachia* present in this individual (Tab. II). Long PCR products from a bee from site-5 (Zimbabwe160 worker, pure *scutellata*) were cloned by J.M. Alvarez in September 2000 and two clones had identical sequences (Tab. II). The *wsp* sequences obtained on October 12, 2001 by A. Jeyaprakash were cloned and six independent transformants from each population (site-5, Zimbabwe 160 worker, pure *scutellata*; site-9, Stellen-bosch2 worker, pure *capensis*; and site-17, Grahamstown1 worker hybrids) were sequenced; all sequences were identical (Tab. II). Only one *wsp* sequence was obtained from the Long PCR products cloned from *A. m. scutellata, A. m. capensis*, and hybrids between them (sites 1, 5, 5, 9, 17) on three different dates by two different people. This consistency indicates that all populations and individuals tested had a single *Wolbachia* infection.

The *Wolbachia wsp* sequence is unique and was named *w*Cap-B1 following the guidelines used by Zhou et al. (1998). The sequence was submitted to Genbank under accession number AF510085. The *w*Cap-B1 *wsp* sequence belongs

Table II. All *Wolbachia wsp* sequences obtained from *A. m. capensis, A. m. scutellata* and a hybrid between them from Zimbabwe and South Africa were unique, but identical to each other.

CTACGTTCGTTTACAATACAACGGTGAAATTTTACCTTTTTATACAAAAGTTGATGG
TATTACAAATGCAACAGGTAAAGAAAAGGGTAGTCCCTTAACAAGATCTTTTATAG
CTGGTGGTGGTGCATTTGGTTATAAAATGGATGACATTAGAGTTGATGTTGAAGGG
CTTTACTCAAAATTGGCTAAAGATACAGATGTAGTAAATACTTCTGAAACAAATGT
TGCAGACAGTTTAACAGCATTTTCAGGATTGGTTAACGTTTATTACGATATAGCGAT
TGAAGATATGCCTATCACTCCATACGTTGGTGTTGGTATTGGTGCAGCATATATCAGC
AATCCTTCAAAAGCTGATGTAGTTAAAGATCAAAAAGGATTTGGTTTTGCTTATCA
AGCAAAAGCTGGTGTTAGCTATGATGTAACTCCAGAAATCAAACTCTTTGCTGGA
GCTCGTTACTTCGGTTCTTATGGTGCTAGTTTTGATAAGGCAGCTAAGGATGATACT
GGTATCAAAAATGTTGTTTACAGCACTGTTG

to the Con Group because it displayed less than 2.5% sequence divergence from the B-*Wolbachia* reference strain from *Tribolium confusum* (Coleoptera). The BLAST search indicated the *w* Cap-B1 *wsp* sequence is closely related to *wsp* sequences from *Diaphorina citri,* Homoptera; *Solenopsis invicta*, Hymenoptera; *Coleomegilla maculata lengi*, Coleoptera; *Plutella xylostella*, Lepidoptera; and *Bemisia tabaci*, Homoptera (Zhou et al., 1998; Jeyaprakash and Hoy, 2000; Nirgianaki et al., unpublished).

The fact that the *wsp* sequences are identical could be due to relatively recent horizontal transfer between *A. m. scutellata* and *A. m. capensis* or to vertical transfer to these subspecies from a common ancestor. Because *A. m. scutellata* and *A. m. capensis* can interbreed (and their hybrid had an identical sequence) the evidence for horizontal transfer appears stronger than the evidence for vertical transfer from a common ancestor because we would expect some sequence variation, especially in the third codon through genetic drift, if the *Wolbachia* were present in the common ancestor. Additional species of *Apis* and subspecies of *A. mellifera* from different geographic regions will have to be examined to resolve this issue. Natural horizontal transfer of *Wolbachia* appears to be common, but little is known about the mechanism(s) (Cook and Butcher, 1999).

3.3. The *Wolbachia* phenotype

Because individuals of *A. m. scutellata, A. m. capensis*, and their hybrid all tested positive for a single strain of *Wolbachia*, this strain of *Wolbachia* is unlikely to be the causal agent of thelytoky in *A. m. capensis*. Although we found no evidence that these bees contained more than one strain of *Wolbachia*, multiple infections are relatively common in arthropods (Jeyaprakash and Hoy, 2000) and thus additional strains of *Wolbachia* might be detected in future analyses of *A. m. capensis*. If *A. m. capensis* is found to contain a second strain of *Wolbachia*, it is possible that thelytoky could be due to it. However, parthenogenesis-inducing *Wolbachia* in parasitoids cause a failure in segregation during anaphase I, which restores diploidy with complete homozygosity (Stouthamer, 1997) and this is a different cytological mechanism than that reported for *A. m. capensis* (Verma and Ruttner, 1983). Because *Wolbachia* is reported to cause other cytological distortions, including the terminal fusion of the pronucleus and polar body in the parasitoid *Aphytis mytilaspidis* (Rossler and DeBach, 1973; Stouthamer, 1997), *Wolbachia* could be potentially involved in thelytoky in *A. m. capensis*.

Although some strains of *Wolbachia* are associated with cytoplasmic incompatibility, thelytoky, male killing and feminization, many arthropods infected with *Wolbachia* have no obvious phenotype associated with its presence (Jeyaprakash and Hoy, 2000; Weeks et al., 2002). Unfortunately, investigations of the role this *Wolbachia* strain plays in the biology of its hosts will have to be deferred until it can be eliminated from the bees. Preliminary efforts (M. Allsopp, unpublished) to cure *A. m. capensis* colonies by feeding queens with 20 mg/mL of rifampicin or 20 mg/ml tetracycline failed due to the complete mortality of the treated individuals.

Thelytoky in *A. m. capensis* could be induced by microorganisms other than *Wolbachia*. Recent investigations by Zchori-Fein et al. (2001) indicate that bacteria other than *Wolbachia* can be associated with parthenogenesis in the hymenopteran parasitoids *Encarsia pergandiella* and *E. hispida*. The ability to induce thelytokous parthenogenesis thus is not unique to *Wolbachia* or even to the Proteobacteria to which *Wolbachia* belong because the bacteria causing thelytoky in *Encarsia* were affiliated with the Cytophaga-Flexibacter-Bacteroid (CFB) group (Zchori-Fein et al., 2001). Additional analyses of microbial symbionts of *A. m. capensis* could reveal similar surprises.

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Résumé – *Wolbachia* est présent chez *Apis mellifera capensis*, *A. m. scutellata* et chez leurs hybrides. Des populations d'*Apis mellifera capensis*, d'*A. m. scutellata* et de leurs hybrides sont présentes en Afrique du Sud. Bien qu'une étude précédente ait échoué à détecter la présence de *Wolbachia* par un protocole standard de l'amplification en chaîne par polymérase (PCR) et par des amorces 16S, un protocole plus sensible de PCR long, utilisant des amorces pour la séquence *wsp*, a été utilisé pour déterminer si *Wolbachia* pouvait être présent (Tab. I). *A. m. capensis* a cette caractéristique inhabituelle qu'elle se reproduit par thélytokie, ce qui est parfois induit chez les Arthropodes par *Wolbachia. Wolbachia* a été mis en évidence par le protocole de PCR long chez toutes les ouvrières d' *A. m. capensis* examinées, chez les ouvrières et les mâles d'*A. m. scutellata* et chez les hybrides des deux races. Comme l'on s'y attendait, le protocole standard moins sensible de PCR n'a pu fournir de produits *wsp* détectables (Fig. 1). Le clonage et le séquençage des produits de PCR ont indiqué que tous les individus et les populations d'Afrique du sud testés renfermaient la même souche de *Wolbachia* (Tab. II). La séquence *wsp* était inédite et a été nommée *w*Cap-B1. Elle appartient au groupe Con de *Wolbachia* parce qu'elle présente moins de 2,5 % de divergence avec la souche de référence *Wolbachia*-B provenant de *Tribolium confusum* (Coleoptera). La séquence *w*Cap-B1 est étroitement apparentée à *Wolbachia* de *Diaphorina citri* (Homoptera), de *Solenopsis invicta* (Hymenoptera), de *Coleomegilla maculata lengi* (Coleoptera), de *Plutella xylostella* (Lepidoptera) et de *Bemisia tabaci* (Homoptera).

Puisqu'on n'a pas trouvé de différences de séquence chez la souche de *Wolbachia* de ces populations d'abeilles, il est peu vraisemblable que ce *Wolbachia* soit associé à la thélytokie chez *A. m. capensis*. Les spéculations concernant le rôle que cette souche de *Wolbachia* joue dans la biologie de son hôte devront être repoussées jusqu'à ce qu'il puisse être éliminé des abeilles. Des efforts préliminaires (M. Allsopp, non publié) pour soigner les colonies d'*A. m. capensis* en nourrissant les reines avec 20 mg/mL de rifampicine ou 20 mg/mL de tétracycline ont échoué en raison de la mortalité de tous les individus traités.

Les infections multiples par *Wolbachia* sont relativement communes chez les Arthropodes (Jeyaprakash et Hoy, 2000) et d'autres souches de *Wolbachia* pourraient donc être détectées lors de futures analyses d'*A. m. capensis.* Si une seconde souche de *Wolbachia* est trouvée chez *A. m. capensis,* il se peut que la thélytokie lui soit due. A l'inverse, la thélytokie chez *A. m. capensis* pourrait être causée par la présence d'autres micro-organismes. Les recherches récentes sur les guêpes parasitoïdes par Zchori-Fein et al. (2001) indiquent que d'autres bactéries sont susceptibles de provoquer la thélytokie et l'on a montré que de nombreux microorganismes agissaient sur la production de mâles chez les insectes (Weeks et al., 2002).

La capacité à induire la thélytokie n'est pas restreinte à *Wolbachia,* ni même aux Proteobacteria, auxquelles *Wolbachia* appartient ; une bactérie nouvellement découverte est affiliée au groupe Cytophaga-Flexibacter-Bacteroid (CFB) (Zchori-Fein et al., 2001). D'autres analyses de symbiotes microbiens d'*A. m. capensis* pourraient révéler des surprises semblables.

***Apis mellifera capensis* / *Apis mellifera scutellata* / *Wolbachia* / PCR long / thélytokie**

Zusammenfassung – Nachweis von *Wolbachia* in *Apis mellifera capensis*, *A. m. scutellata* und ihren Hybriden in Süd Afrika. In Südafrika gibt es Populationen von *Apis mellifera capensis*, *A. m. scutellata* und deren Hybriden. In einer früheren Untersuchung bei *A. m. capensis* gelang es nicht, *Wolbachia* mit einem Standard PCR Protokoll und 16S Primern nachzuweisen. Jetzt wurde die empfindlichere Methode des Long PCR Protokolls mit Primern für die *wsp* Sequenz zur Überprüfung des Vorkommens von *Wolbachia* benutzt (Tab. I).

Die Vermehrung der *A. m. capensis* ist ein Sonderfall, da sie auch durch Thelytokie erfolgen kann. Thelytokie wird manchmal bei Arthropoden durch *Wolbachia* ausgelöst. *Wolbachia* wurde mit dem Long PCR Protokoll in allen untersuchten Arbeiterinnen von *A. m. capensis* nachgewiesen, allerdings auch in Arbeiterinnen und Drohnen von *A. m. scutellata* sowie bei den Hybriden der beiden Rassen. Wie erwartet erhielten wir mit der Standard PCR Methode keine erkennbaren *wsp* Produkte (Abb. 1). Clonierung und Sequenzierung der PCR Produkte wies darauf hin, dass bei allen überprüften südafrikanischen Einzeltieren und Populationen dieselben Linien von *Wolbachia* vorkamen (Tab. II). Die *wsp* Sequenz war neuartig und wurde *w*Cap-B1 genannt. Sie gehört zu der Con Gruppe von *Wolbachia* und weist weniger als 2,5 % Abweichung von der B-*Wolbachia* Referenzlinie von *Tribolium confusum* (Coleoptera) auf. Die *w*Cap-B1 Sequenz ist nah verwandt mit *Wolbachia* von *Diaphorina citri,* Homoptera; *Solenopsis invicta*, Hymenoptera; *Coleomegilla maculata lengi*, Coleoptera; *Plutella xylostella*, Lepidoptera; und *Bemisia tabaci*, Homoptera.

Da keine Sequenzunterschiede in der *Wolbachia* Linie in diesen Bienenpopulationen gefunden wurde, ist es unwahrscheinlich, dass *Wolbachia* mit der Thelytokie bei *A. m. capensis* in Zusammenhang steht. Spekulationen über die Bedeutung dieser *Wolbachia* Linien in Bezug auf die Biologie ihres Wirtes müssen zurück gestellt werden, bis es gelingt die Bienen befallsfrei zu machen. Vorläufige Bemühungen (M. Allsopp, nicht publiziert) *A. m. capensis* Völker durch Fütterung der Königinnen mit 20 mg/ml Rifampicin oder 20 mg/ml Tetracyclin zu heilen, blieben durch das Absterben aller behandelten Tiere ohne Erfolg. Mehrfache Infektionen mit *Wolbachia* sind relativ häufig in Arthropoden (Jeyaprakash and Hoy, 2000) und so könnten noch zusätzliche Linien von *Wolbachia* in zukünftigen Analysen von *A. m. capensis* entdeckt werden. Wenn der Nachweis einer zweiten Linie *Wolbachia* in *A. m. capensis* gelingen sollte, wäre es möglich, dass Thelytokie auf einer solchen Linie beruht. Alternativ könnte die Thelytokie in *A. m. capensis* auch von der Anwesenheit anderer Mikroben abhängig sein. Neuere Untersuchungen von parasitären Wespen durch Zchori-Fein et al. (2001) weisen darauf hin, dass andere Bakterien Thelytokie auslösen können und bei einer Vielzahl von Mikroorganismen konnte ein Einfluss auf die Produktion von Männchen bei Insekten gezeigt werden (Weeks et al., 2002). Die Fähigkeit zur Induktion von Thelytokie ist demnach nicht auf *Wolbachia* beschränkt, noch nicht einmal auf Proteobakterien zu denen *Wolbachia* zählt. Ein neu entdecktes Bakterium wird der Gruppe der Cytophaga-Flexibacter-Bakteroiden (CFB) zugeordnet (Zchori-Fein et al., 2001). Zusätzliche Analysen von mikrobiellen Symbionten bei *A. m. capensis* könnte zu ähnlichen Überraschungen führen.

***Apis mellifera capensis* / *Apis mellifera scutellata* / *Wolbachia* / Long PCR / Thelytokie**

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