Thelytoky in Cape honeybees (*Apis mellifera capensis*) is controlled by a single recessive locus

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Abstract – Worker reproduction in *Apis mellifera* typically leads to haploid males produced via arrhenotokous parthenogenesis. An exception are laying workers of the South African Cape honeybee *Apis mellifera capensis*. Due to an abnormal spindle rotation during meiosis *A . m . capensis* workers are able to produce female progeny via thelytokous parthenogenesis. This trait has been suggested to be genetically controlled by a recessive allele at the *thelytoky* locus (*th*), but this conclusion was recently challenged by Chapman et al. (2015). To clarify the mode of inheritance for thelytokous parthenogenesis in Cape honeybees, we determined the sex of the offspring of 74 *A . m . capensis* workers of a single queen from a colony of the endemic wild population at the Cape of Good Hope Nature Reserve. When we tested individual worker reproduction, parthenogenesis was dimorphic, segregating in a Mendelian fashion supporting the single locus model. We could exclude maternal or paternal effects determining the mode of parthenogenesis. A careful re-analysis of the data of Chapman et al. (2015) also revealed that their data do not contradict the one locus model for the inheritance of thelytoky.

thelytoky / worker reproduction / reproductive dominance / asexual reproduction / gemini

1. INTRODUCTION

The female worker caste of the honeybee (*Apis mellifera spp.)* typically does not reproduce (Lattorff and Moritz 2013) in the presence of the pheromones produced by the queen (Butler 1959; Slessor et al. 1988; Plettner et al. 1993; Winston and Slessor 1998) and the brood (Arnold et al. 1994). Whenever the queen and her pheromonal control are absent, workers are able to activate their ovaries and parthenogenetically produce haploid male offspring (arrhenotoky) (Crozier 1975; Ruttner and Hesse 1981; Winston 1987; Page and Erickson 1988; Visscher 1989). A rare exception to worker arrhenotoky is the parthenogenetic production of female offspring (thelytoky) by laying workers, which is a characteristic of *Apis mellifera capensis* (Eschscholtz, 1822), the Cape honeybee (Onions 1912; Anderson 1963). In this subspecies, an incomplete meiotic division based on an abnormal spindle orientation in anaphase II results in a central fusion of the meiotic products restoring egg diploidy (automixis with

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central fusion; Verma and Ruttner 1983) and hence female offspring.

This exceptional trait was claimed to be under genetic control of a single recessive allele because of suggestive Mendelian segregation (Ruttner 1988), which was subsequently confirmed in a controlled laboratory experiment (Lattorff et al. 2005). Based on a backcross mapping population and using the segregation of a set of 566 microsatellites, a recessive locus (*th*) on chromosome 13 was identified to be significantly linked with thelytokous parthenogenesis (Lattorff et al. 2007). This region spans 11.4 cM (180 kb), comprises 15 genes (NCBI; Genome assembly Amel 4.5) and was genetically linked to rapid ovary activation and the production of high levels of the queen substance pheromone 9-oxo-2-(E)-decenoic acid (9-ODA). Finally, Jarosch et al. (2011) showed that alternative splicing of *gemini*, a CP2 transcription factor in the target region, correlates with different reproductive states in *A. m. carnica* and also with the different modes of parthenogenesis. Moreover, RNAi knock-down studies proved the alternatively spliced exon 5 to control ovary activation in *A. m. carnica* workers. An adjacent intronic deletion of 9 bp (thelytoky associated element; *tae1*), present in the parasitic thelytokous *A. m. capensis* clone (Baudry et al. 2004), was proposed to control the process of alternative splicing and serves as the genetic switch determining the mode of parthenogenesis. More recently, however, Chapman et al. (2015) reported that thelytoky in *A. m. capensis* workers is not under single locus control. Specifically, they report the segregation of alleles at various markers flanking *gemini* (Shaibi et al. 2008; Jarosch et al. 2011) not to be associated with thelytoky.

In order to clarify the mode of inheritance of thelytoky in *A. m. capensis* workers, two complementary strategies were used in this study:

1. The segregation of different modes of parthenogenesis was assessed in a new mapping population using individual workers from a single naturally mated *A. m. capensis* queen. As a result of the high mating frequencies in *A. m. capensis* (Kraus et al. 2004), the experimental workers represent a reasonable subsample of the *A. m. capensis* population as they comprise not only the queen's genotype but also those of many siring drones. To avoid a pheromonal arms race resulting in reproduction of the most dominant thelytokous worker (Moritz et al. 1996, 2000, 2004, 2008; Härtel et al. 2011), the workers were tested in an experimental setting free of intra-colonial selection for reproductive dominance to determine the mode of parthenogenesis of individual workers.

2. A careful re-analysis of the genotype data provided by Chapman et al. (2015) was conducted to test its consistency with a single locus model.

2. METHODS

2.1. *A. m. capensis* laying workers

Sealed worker brood frames of a wild *A. m. capensis* colony from the Cape of Good Hope section of the Table Mountain National Park (34°14′45.0″S 18°24′15.0″E) were brought to Stellenbosch and kept in an incubator (37 °C, 60% r. h.). Freshly emerged workers were collected daily, individually labelled with Opalith bee tags (Graze) on their thorax and colour marks on their abdomen (painted with uniball POSCA markers PC5M) and their wings clipped in order to prevent drifting into other colonies. These workers were individually introduced into a small group of newly emerged *A. m. scutellata* (Lepeletier, 1836) host workers where the *A. m. capensis* worker develops into a pseudoqueen to become the only reproductive individual (Neumann and Hepburn 2002).

2.2. *A. m. scutellata* host colonies

Twenty *A. m. scutellata* colonies from Douglas (28°48′30.0″S 23°50′30.0″E) were brought to Stellenbosch. Multiple frames of emerging *A. m. scutellata* brood were placed in an incubator and emerged workers were collected every 24 h. About 1000 individuals each were used to set up small queenless colonies in Apidea® mating nucs, provided with three combs containing

honey, pollen and empty space for eggs. A single freshly emerged, individually marked and wing clipped *A. m. capensis* worker was introduced into every colony ($N = 74$). Colonies were kept inside (but closed) in a room at 28 °C and 60% RH for 2 days. Thereafter, the colonies were transported and opened in bee-tight netted cages to exclude invasion of foreign parasitic workers into the experimental colonies (Moritz et al. 2008, 2011). Every 2 days, the colonies were checked for the presence of the marked *A. m. capensis* worker and any brood. Newly emerged *A. m. scutellata* workers were added to the colonies when necessary to make sure that the *A. m. capensis* worker is accompanied by a sufficient number of *A. m. scutellata* host workers all the time. Bee-collected pollen and sugar candy were added when necessary to sustain the colonies. As soon as the first larvae hatched, but at the latest after 14 days, the marked *A. m. capensis* worker and all the brood (eggs and larvae) were collected and stored at −20 °C for subsequent microsatellite genotyping to assess maternity and to identify the ploidy of the offspring to discriminate between male (haploid) and female sex (diploid). If the *A. m. capensis* worker died before collection all *A. m. scutellata* workers were sacrificed and the nuc was re-started.

2.3. Genotyping

DNA was solvent extracted from half of the flight muscle of each *A. m. capensis* worker ($N = 74$) and their brood (1–9 eggs or larvae) using phenol-chloroform (Kirby 1956). Genotyping was done using seven polymorphic unlinked microsatellite markers (A107, A79, A113, A14, A28, A88, A35; Solignac et al. 2003) and five linked microsatellite markers flanking *gemini* (AT012, K1363, Hb-the 2, 3, 4; Shaibi et al. 2008) and the *tae* intronic region of *gemini* (tae; Jarosch et al. 2011). PCRs were conducted following standard protocols (Solignac et al. 2003) and subsequently analysed in a MegaBace®1000 (GE Health Care) automatic capillary sequencer following the instructions of the manufacturer. Sizing of fragments was done using the MegaBACE Fragment Profiler 1.3.

The laying *A. m. capensis* mother workers and the sampled brood per colony were analysed according to their relatedness and ploidy level. Arrhenotokously produced individuals show a single allele at all loci. Individuals showing heterozygosity at any of the tested loci are diploid and were thus produced via thelytoky. The genotype of the mother queen and the siring fathers of the laying workers were derived by Mendelian inference from the laying worker genotypes (Estoup et al. 1995).

2.4. Statistical analyses

Statistical analyses were done using Statistica 7.0 (StatSoft, Tulsa, OK, USA) and G*Power 3.1.9.2 (Faul et al. 2007). To estimate how accurately the sampling reflects the total number of patrilines, the non-sampling and non-detection errors were calculated following Boomsma and Ratnieks (1996). Chi-squared tests were used to test for deviations of the frequency of arrhenotokous and thelytokous workers from a Mendelian segregation assuming a single recessive locus control of thelytoky. Since the within-patriline sample sizes might be small, Fisher's exact tests were used to test for deviations from such a segregation within patrilines and to analyse the association between the mode of parthenogenesis and the occurrence of microsatellite marker haplotypes within the region of the *th* locus (Lattorff et al. 2007). A power analysis using a generic binomial test was performed to assess whether the experimental sample size was sufficient to gain a power of ≥80%. The calculation of the frequency of the *thelytoky* allele and the exact confidence interval was done based on a binomial distribution (Clopper and Pearson 1934).

2.5. Re-analysis of Chapman et al. (2015) data

We re-evaluated the genotype tables provided by Chapman et al. (2015) retaining the original notation of all experimental colonies and all individuals. Individuals with genotypes lacking a maternal and/or a paternal allele at a locus where recombination seemed unlikely (double cross-over events over a physical distance of ca. 7 kb within the *th*-locus) were classified as not belonging to the pedigree (i.e. an invaded individual).

After removing these individuals, the raw data were re-analysed to infer the minimum number of laying workers within the sample set using Mendelian inference. It is crucial to use the number of arrhenotokous and thelytokous laying workers rather than the actual number of offspring produced to assess the mode of inheritance of the mode of worker parthenogenesis. Since there were multiple laying workers in all colonies, using the overall number of female and male offspring in the colony is inappropriate for determining the mode of inheritance of worker parthenogenesis, unless all workers lay exactly the same number of offspring.

3. RESULTS

3.1. Mode of parthenogenesis

The controlled introduction of *A. m. capensis* workers from a single wild colony into the *A. m. scutellata* host colonies resulted in 74 workers for which the mode of parthenogenesis was determined. Thirty-three individuals showed thelytokous and 41 arrhenotokous parthenogenesis (genotypes in Online Resource 1, Tab. S1-S3). This phenotypic distribution did not significantly deviate from a 1:1 ratio (Chi-squared test, $p = 0.51$). A power analysis revealed that the analysed sample size was sufficient to gain a power of $\geq 80\%$ (generic binomial test, power = 86%). No amphitokous workers showing both modes of parthenogenesis were found.

3.2. Patrilines and queen alleles

The 74 thelytokous or arrhenotokous workers could be assigned to 25 patrilines (Fig. 1; genotypes in Online Resource 1, Tab. S4). The low non-detection error (3.3×10^{-5}) suggests that the number of markers used for genotyping and their polymorphism allows for an unambiguous separation of different individuals. The estimated non-sampling error was 1.5 indicating that we may have missed only up to two patrilines; hence, the sample represents at least 92% of all present patrilines. Eleven patrilines showed a single mode of parthenogenesis. These patrilines were all represented by a small sample size, mostly with a single tested worker only. Fourteen patrilines comprised both thelytokous and arrhenotokous workers (patrilines 3–16) with a median of 3.5 sampled workers per patriline.

Under the assumption of a single recessive locus control of thelytoky, the father drones either carried the thelytoky or the arrhenotoky allele. Depending on the genotype of the mother queen, this results in different modes of parthenogenesis of the offspring workers, which is shown in detail in Table I. Because the thelytoky allele is recessive (Ruttner 1988, Lattorff et al. 2005), under a single locus model, the occurrence of both types of parthenogenesis within one patriline can only be due to a heterozygous queen (*Ar/th*) with males carrying the thelytoky allele. Under this assumption, a 1:1 segregation of the two modes of parthenogenesis would be expected per patriline as well as over all patrilines if most workers have a father drone that carried the thelytoky allele. Testing this expected 1:1 segregation within
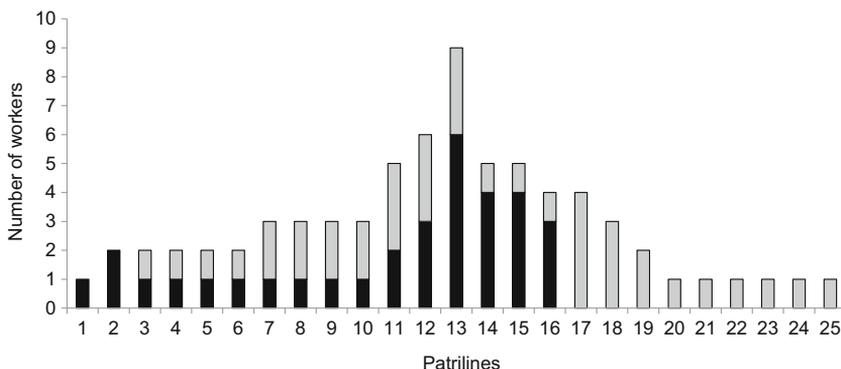
Figure 1. The detected patrilines of the *A. m. capensis* colony (numbered from 1 to 25) and the number of thelytokous (*black*) and arrhenotokous (*grey*) workers per patriline (NDE = 3.3×10^{-5}).

Table I. The expected genotypes and modes of parthenogenesis of the offspring of drones that either carry the thelytoky (*th*) or the arrhenotoky allele (*Ar*) and queens of all possible genotypes (*th/th*, *Ar/Ar*, *Ar,th*) under a single recessive locus control of thelytoky.

	Queen *th/th*	Queen *Ar/Ar*	Queen *Ar/th*
Drone *th*	100% thelytokous workers (*th/th*)	100% arrhenotokous workers (*Ar/th*)	50% thelytokous workers (*th/th*) + 50% arrhenotokous workers (*Ar/th*)
Drone *Ar*	100% arrhenotokous workers (*Ar/th*)	100% arrhenotokous workers (*Ar/Ar*)	100% arrhenotokous workers (*Ar/Ar* and *Ar/th*)

patrilines using Fisher's Exact tests for each patriline separately ($p \geq 0.21$) and a subsequent Fisher's combined probability test ($p = 0.997$) over all patrilines resulted in no significant deviation from this expected 1:1 distribution.

Based on the 27 chromosomal sets sampled (2 from the queen, 25 from the fathers), we estimated the frequency of the *thelytoky* allele in the population as well as the exact confidence interval (CI) based on a binomial distribution (Clopper and Pearson 1934). Under a single locus model the queen carried one *thelytoky* and one *arrhenotoky* allele. Sixteen of the siring drones unambiguously carried the *thelytoky* allele resulting in a minimum frequency of *thelytoky* of $p_{th} = 0.63$ (17/27, CI 0.42–0.81). The genotypes of the drones siring exclusively arrhenotokous workers were not certain and can either be *thelytoky* or *arrhenotoky*. These workers may have inherited their *arrhenotoky* allele either by the queen or by the male. The small sample size of these patrilines (17–25) precludes a precise assessment. As we cannot exclude all siring fathers to carry the *thelytoky* allele, the maximum frequency of *thelytoky* within the population would be $p_{th} = 0.96$ (26/27, CI: 0.81–1.00).

3.3. *Tae* length polymorphism and allele segregation of the markers flanking *gemini* in arrhenotokous and thelytokous workers

The markers flanking *gemini* did not co-segregate with thelytokous parthenogenesis (genotypes in Online Resource 1, Tab. S5) both over the whole sample set (Chi-squared test, $p = 0.52$) as well as within single patrilines containing both thelytokous and arrhenotokous individuals (Fisher exact test, p between 0.20 and 0.75). The putative functional mutation of 9 bp (*tae1*) adjacent to the alternatively spliced exon 5 of *gemini*, the expected thelytoky switch, was found in a homozygous state in only 45% of the thelytokous workers. Equally, 12 workers homozygous for *tae1* were arrhenotokous instead of thelytokous (genotypes in Online Resource 1, Tab. S6).

3.4. Re-analysis of Chapman et al. 2015 data

As Chapman et al. (2015) acknowledge their experiment suffered from recurrent invasions of foreign laying workers into the test colonies, whose offspring was removed from the dataset by using the microsatellite data. However, carefully scrutinizing the genotype data provided in their supplementary tables revealed that this process had not always been fully successful and needed some critical adjustments (see Online Resource 2, Tab. S18-S22, S25-S28, S33-S35). We re-evaluated the genotype tables provided by Chapman et al. (2015) by exactly retaining the original notation of all experimental colonies and all individuals. Individuals with genotypes lacking essential maternal and/or paternal alleles at a given locus were classified as not belonging to the pedigree (i.e. an invaded individual; details can be found in Online Resource 2 comprising both the original genotypes of Chapman et al. (2015) and our re-analysis). The corrected sample sizes are given in Table II. The revised data were re-analysed to determine the minimum number of laying workers within the sample set using Mendelian inference (Table II). In particular within the backcross colonies, we inferred a 1:1 segregation for arrhenotoky and thelytoky of laying

Table II. Corrected numbers of thelytokous and arrhenotokous offspring and the number of inferred reproductive workers in each F1 and backcross (BC) colony respectively

Colony	Cross type	Cross direction (queen × drone)	Thelytoky expected	Arrhenotoky expected	Thelytokous offspring	Arrhenotokous offspring	Laying worker *th*	Laying worker *arrh*
3	F1	SxC	No	Yes	0	47	0	12
4	F1	SxC	No	Yes	0	31	0	8
7	F1	CxS	No	Yes	0	20	0	7
9	F1	CxS	No	Yes	1	16	1	8
11	F1	CxS	No	Yes	0(1)	15	0	5
14	F1	CxS	No	Yes	0	58	0	7
10	BC	(SxC)xS	No	Yes	0	8	nd	nd
15	BC	(SxC)xC	Yes	Yes	19(20)	8	4	4
16	BC	(SxC)xC	Yes	Yes	1(26)	1	1	1
21	BC	(SxC)xC	Yes	Yes	0(2)	0	0	0
19	BC	(CxS)xS	No	Yes	0	0(6)	0	0
20	BC	(CxS)xS	No	Yes	17(21)	0	12	0
22	BC	(CxS)xC	Yes	Yes	22(23)	0	15	0
26	BC	(CxS)xC	Yes	Yes	12(14)	21	5	5

Original data (Chapman et al. 2015) are shown in parentheses

workers in the colonies 15, 16 and 26 matching the predicted segregation under a single locus control (Fisher exact test, p between 0.68 and 1). One hybrid colony (colony 9) showed thelytokous offspring where it is not plausible under a single allele inheritance of thelytoky when following the expectations of Chapman et al. (2015). We here inferred nine laying workers. Eight of them contribute to the production of 15 male offspring (arrhenotokous parthenogenesis), and a single individual

Table III. Alleles and their frequencies in the experimental *A . m . scutellata* and *A . m . capensis* populations carried by drone 20 used for breeding backcross colony 20.

		A107	A14	A29	A79	B124	CSD	Hb-the2	Hb-the3	Hb-the4	tae
Drone 20	**Allele (bp)**	175	214	129	87	232	-	242	189	235	110
	af Sc.	0.1	0.2	0	0.2	0.1	-	0.4	0.2	0.2	0.3
	af Cap.	0.023	0.023	0.013	0	0	-	0.12	0.02	0.33	0.56

Subspecies-specific alleles in the drone are highlighted in grey

af Sc allele frequency within experimental 'Scutallata' population; *af Cap* allele frequency within experimental 'Capensis' population

Table IV. Comparison between the original analysis of Chapman et al. (2015) and the re-analysis of their data set and this study.

	Chapman et al. (2015)	This study and re-analysis of Chapman et al. (2015)
Method to assess mode of inheritance of worker parthenogenesis	Counting the number of offspring produced per colony	Determining the number of laying workers per colony based on the genotypes of the offspring produced per colony
Rejection of single locus control of thelytoky	Yes	No
Rejection of maternal effects	No	Yes
Rejection of paternal effects	Yes	Yes
Rejection of epigenetic mechanisms	No	Yes
Rejection of tae/gemini (Jarosch et al. 2011)	Yes	Yes

reproduced thelytokously. This worker, which seems to be a native worker, produced just a single female pupa. Within the backcross colonies, the segregation of thelytoky and arrhenotoky was only inconsistent with the single locus model in colony 20. We therefore present the re-analysis of this colony in more detail here. According to the provided backcross scheme ((Capensis × Scutellata) × Scutellata) and under single locus control, this colony should not generate the observed 13 reproductive thelytokous individuals. Unfortunately, the phenotype of the F1 hybrid colony (colony 6) used to generate this backcross is unknown as no natal workers started egg-laying here. Additionally, to test if drone 20 used for insemination of the hybrid queen shows indications of uncertain genotypic origin, we compared its genotype with the alleles of the corresponding 'Capensis' and 'Scutellata' populations, including the genotype data on *A . m . capensis* from our study. The majority of the alleles were inconclusive as they were present in both subspecies (see Table III). However, although two alleles (locus A79 (87 bp) and B124 (232 bp)) are exclusively found in the *A . m . scutellata* workers sampled by Chapman et al. (2015), this drone also carries one allele exclusively found in the *A . m . capensis* population (A29; 129 bp), also

present in both F0 *A . m . capensis* queens used by Chapman et al. (2015).

3.5. Similarities and differences between the original analysis of Chapman et al. (2015) and the re-analysis of their data set

A comparative overview between the original analysis of Chapman et al. (2015) and the re-analysis of their dataset and this study is shown in Table IV.

4. DISCUSSION

Thelytoky is a rare phenomenon resulting in worker-derived female offspring from unfertilized eggs. In the Cape honeybee, *A . m . capensis*, this mode of parthenogenesis is tightly linked to some other reproductive traits like an early onset of egg-laying (Anderson 1963; Ruttner and Hesse 1981; Moritz and Hillesheim 1985; Plettner et al. 1993) and the production of queen-like amounts of the queen mandibular gland pheromones (Hemmling et al. 1979; Crewe and Velthuis 1980; Moritz et al. 2002; Wossler 2002). Thus, under natural conditions with intra-colonial selection, thelytokous individuals producing the strongest queen-like pheromone signal with the highest amounts of 9-ODA will establish themselves as pseudoqueen and suppress the reproduction of other workers (Moritz et al. 1996,

2000, 2004; Härtel et al. 2011; Okosun et al. 2015). To avoid this intra-colonial arms race in our experiment, every laying *A . m . capensis* worker was tested individually, which is essential to eventually reveal a Mendelian segregation. We here show that workers produced by a multiply mated *A . m . capensis* queen heterozygous at the *th* locus (arrhenotoky ; thelytoky) reproduce either by arrhenotoky or thelytoky. The two modes of parthenogenesis segregate well in a Mendelian 1:1 ratio predicted by a single recessive locus control.

In contrast, Chapman et al. (2015) reported that thelytokous parthenogenesis in *A . m . capensis* is under multi-locus control. However, a careful analysis of their dataset revealed their results largely confirm our findings and thus also the one locus model for worker thelytoky. The contrasting interpretation of the same dataset results from using the number and genotypes of the actual laying workers within a colony instead of the number of the overall offspring produced in the colony to assess the mode of inheritance of worker parthenogenesis. Using the overall number of female and male offspring produced within the whole colony will suffer from the introduction of a strong bias as not every worker lays exactly the same number of offspring in the colony and worker reproduction can be inhibited or suppressed by intra-colonial selection (Moritz et al. 1996, 2000, 2004; Härtel et al. 2011). Furthermore, re-counting the offspring of an individual will inflate the estimate for the allelic version present in the reproductive worker and hence is a pseudo-replication. If the actual number of reproductive individuals is considered, a distinct 1:1 segregation of both modes of parthenogenesis within colonies showing both arrhenotokous and thelytokous parthenogenesis can be observed. Only two experimental colonies (colony. 9 and 20) of Chapman et al. (2015) do not support the single locus model. According to the crossing scheme for colony 9 (Capensis × Scutellata) and the back-cross scheme for colony 20 ((Capensis × Scutellata) × Scutellata) and under single locus control, these colonies should not produce any thelytokous offspring. Whereas in colony 9 (F1 generation) just a single individual did not match the single locus mode by reproducing via

thelytoky, 13 individuals in colony 20 (F2 generation) produce female offspring when they should not according to the single locus model. Although we cannot exclude this outcome to be due to an incomplete penetrance of the thelytoky trait or due to a multi-locus control, we suspect this outcome to be most likely caused by insufficient control of the breeding individuals. In particular, the genotype of the putative *A . m . scutellata* drone 20 seems to be questionable. If this drone had carried the *thelytoky* allele instead of the *arrhenotoky* allele, the entire dataset is in agreement with the one locus model. Indeed, there is reason for concern because this drone carries an allele otherwise only found in the *A . m . capensis* lineage. Hence, we have to conclude that the choice of individuals, supposed to have a certain genotype at the *th*-locus, just based on their geographical origin may be insufficient at best. It ignores the possibility that the putative *thelytoky* allele may have been present in the Douglas (*A . m . scutellata*) region due to introgression of *thelytoky* alleles from the parasitic clone present there (Neumann et al. 2011). It is crucial to test the incidence of the *thelytoky/arrhenotoky* alleles in the mother colony, and it is crucial to confirm that the chosen drone is offspring of the queen heading the colony. Furthermore, the choice of breeding individuals by geography ignores the occurrence of the *arrhenotoky* allele in the *A . m . capensis* population at the Cape of Good Hope. Although its frequency might be low, two independent studies found a queen that carries the *arrhenotoky* allele at the *th*-locus (Beekman et al. 2009; this study). Therefore, it might be possible that the *A . m . capensis* and *A . m . scutellata* queens and drones used for crossing may have carried either the *arrhenotoky* or the *thelytoky* allele respectively.

Taken together, we see little merit in rejecting the single-locus inheritance of thelytoky in *A . m . capensis* . First, because it requires the fewest assumptions it remains the most parsimonious explanation following Occam's razor *'Pluralitas non est ponenda sine neccesitate'* . Only if it is falsified by evidence it should be refused. Second, the single locus control is further supported by the lack of amphitoky (intermediate mode of parthenogenesis) of laying workers in both studies discussed here, as well as in Ruttner (1988) and Lattorff et al.

(2005). Third, the clear-cut 1:1 segregation pattern and the occurrence of both types of parthenogenesis within a single patriline rule out epigenetic mechanisms or paternal factors determining the mode of parthenogenesis in *A. m. capensis*. Also, maternal effects other than the alleles of the Mendelian locus can safely be excluded, because otherwise there should be no polymorphism at all among the workers tested in our study. Moreover, a single locus control has also been suggested in other thelytokous Hymenoptera. The allelic origin of asexual reproduction in the parasitoid wasp *Lysiphlebus fabarum* appears to be controlled by a single locus (Sandrock and Vorburger 2011).

However, we also found that the previously claimed genomic region around *gemini* on chromosome 13 (Lattorff et al. 2005, Jarosch et al. 2011) does not harbour the locus switching between thelytoky and arrhenotoky in the current study. Nevertheless, although *gemini* is not the genetic switch for thelytoky in this experimental population, both *gemini* and the 9-bp deletion (*tae1*) may be involved in controlling the reproductive capacity in female honeybees which is tightly interwoven in the complex phenotype of the 'thelytoky syndrome' (Lattorff and Moritz 2013). The detection of the actual 'thelytoky switch' will require a concise mapping population coupled with a much higher marker coverage than possible with microsatellite techniques, which is now entirely feasible using SNP marker-based methods (e.g. Stolle and Moritz 2013).

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La thélytoquie chez les abeilles du Cap (*Apis mellifera capensis*) est contrôlée par un seul locus récessif

Thélytoquie / reproduction des ouvrières / dominance reproductive / reproduction asexuelle / gémini

Die Thelytokie bei der Kapbiene (*Apis mellifera capensis*) wird durch einen einzigen rezessiven Lokus Kontrolliert

Thelytokie / Arbeiterinnenreproduktion / reproduktive Dominanz / asexuelle Reproduktion / gemini

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