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Whole-Genome Scan in Thelytokous-Laying Workers of the Cape Honeybee (*Apis mellifera capensis*): Central Fusion, Reduced Recombination Rates and Centromere Mapping Using Half-Tetrad Analysis

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ABSTRACT

While workers of almost all subspecies of honeybee are able to lay only haploid male eggs, *Apis mellifera capensis* workers are able to produce diploid female eggs by thelytokous parthenogenesis. Cytological analyses have shown that during parthenogenesis, egg diploidy is restored by fusion of the two central meiotic products. This peculiarity of the Cape bee preserves two products of a single meiosis in the daughters and can be used to map centromere positions using half-tetrad analysis. In this study, we use the thelytokous progenies of *A. m. capensis* workers and a sample of individuals from a naturally occurring *A. m. capensis* thelytokous clone to map centromere position for most of the linkage groups of the honeybee. We also show that the recombination rate is reduced by >10-fold during the meiosis of *A. m. capensis* workers. This reduction is restricted to thelytokous parthenogenesis of *capensis* workers and is not observed in the meiosis of queen within the same subspecies or in arrhenotokous workers of another subspecies. The reduced rate of recombination seems to be associated with negative crossover interference. These results are discussed in relation to evolution of thelytokous parthenogenesis and maintenance of heterozygosity and female sex after thelytoky.

THE honeybee, like other hymenopteran species, is characterized by a haplodiploid system of reproduction. Fertilized oocytes generally produce diploid females (workers and queens) whereas unfertilized eggs produce haploid males (drones) through arrhenotokous parthenogenesis (Dzierzon 1845). However, in the honeybee and in various species of the hymenoptera, sex is not determined directly by ploidy level but by the genotype at the so-called sex locus (Cook and Crozier 1995; Beye *et al.* 2003). Heterozygosity at this multiallelic locus is necessary to produce a female whereas hemizygosity (for a haploid genome) or homozygosity (for a diploid genome) produces males. Diploid drones are sterile but the genetic load they generate in a colony is prevented by their early destruction by workers (Woyke 1963). In addition to the general arrhenotokous parthenogenesis, some cases of thelytokous parthenogenesis (*i.e.*, female-producing parthenogenesis) are known in hymenoptera (Slobodchikoff and Daly 1971). The proteobacterium Wolbachia is one of the possible agents of this thelytoky (Rousset *et al.* 1992; Stouthamer *et al.* 1999) and induces parthenogenesis in at least 40 species of Hymenoptera (Cook and Butcher 1999). However, the mode of restoration of diploidy through gamete duplication (Stouthamer and Kazmer 1994; Plantard *et al.* 1998) does not seem compatible with heterozygosity at the sex locus, which is necessary to produce females in the honeybee.

Thelytoky is known to occur at a low frequency in several subspecies of the domestic honeybee (Tucker 1958; Verma and Ruttner 1983) but it is the norm only in *Apis mellifera capensis*, a subspecies restricted to a small geographic area around the Cape of Good Hope (Onions 1912; Anderson 1963; Verma and Ruttner 1983). In queenright colonies (colonies that have a queen present) of this subspecies, workers do not produce offspring. However, in queenless colonies, workers may lay unfertilized eggs that develop into females and can be reared as either workers or queens (Onions 1912; Ruttner 1977).

The cytological analysis of thelytokous *A. m. capensis* workers by Verma and Ruttner (1983) showed that diploidization follows a central fusion, *i.e.*, the fusion of two of the four meiotic products that have a central position on the spindles and were separated at the first meiotic division, whereas the two terminal nuclei degenerate. In the queen meiosis, at the first anaphase, the spindle rotates 90°. In addition, two of the polar bodies fuse in the so-called *Richtungskopulationskern* (RKK), which later degenerates. In pseudo-queens meiosis, the first

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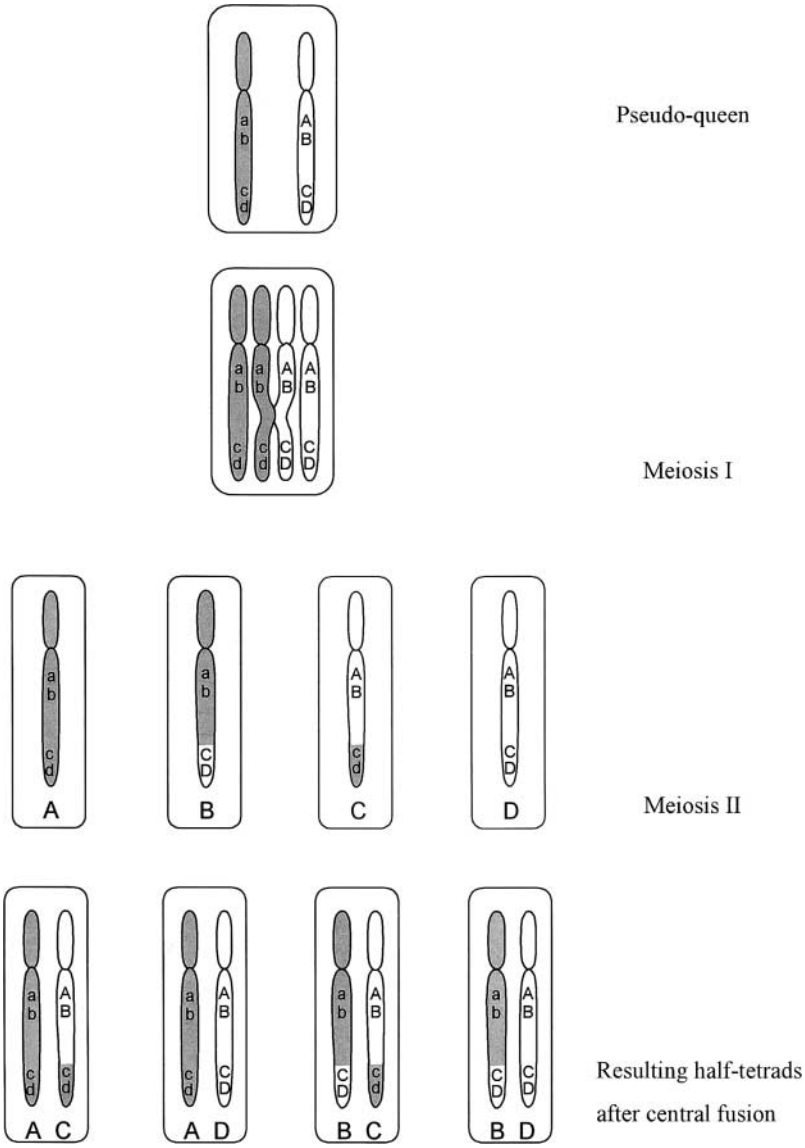


FIGURE 1.—Diploid regulation of thelytokous-laying workers (pseudo-queens) in the Cape honeybee (*Apis mellifera capensis*). In the absence of chiasma, all the diploid progeny have exactly the same genotypic structure as the parent (not shown). For a unique chiasma shown, the four products of meiosis are named A (not recombined), B (recombined), C (recombined), and D (not recombined). The A or B product as well as the C or D product may occupy a central position. Among the four possible equiprobable diploid combinations, (i) the fusion AC produces homozygosity distally to the chiasma for one allelic phase, (ii) the fusion BD produces homozygosity distally to the chiasma for the other allelic phase, (iii) the fusion AD restores the parental structure, and (iv) the fusion BC restores heterozygosity but the allelic phase has been changed distally to the chiasma.

anaphase spindle remains parallel to the axis of the egg, an orientation that is conserved by the two spindles at the second division. The authors speculate that the fusion of polar bodies in the RKK might be a preadaptation to automictic parthenogenesis through central fusion.

This peculiarity preserves two of the meiotic products (half-tetrad) in the offspring, allowing direct observation of some of the recombination events that occurred in the mother. A pseudo-queen that is heterozygous at a locus will produce daughters that are homozygous at this same locus in half of the cases where a recombination event took place between the locus and the centromere of the chromosome (Figure 1). The number of recombination events between a locus and the centromere depends on their linkage distance. Therefore, the percentage of daughters homozygous at a locus in the progeny of a pseudo-queen should allow the centromere to be placed on a linkage map. Similarly, it is possible to calculate the linkage distance between pairs of markers. Our genetic results (see RESULTS and DISCUSSION) are in agreement with central fusion as described above.

Our main goal in this study was to use the thelytokous parthenogenesis of the Cape honeybee to position the centromeres of each chromosome onto the linkage map of the honeybee genome (SOLIGNAC *et al.* 2004, accompanying article in this issue). For this purpose, we used several first-generation progenies of *A. m. capensis* pseudo-queens. However, we observed very few recombination events in this sample, which did not allow us to map the centromere precisely. We thus decided to take advantage of a situation offered by a natural clone of Cape bees, whose main biological characteristics are described below. *A. m. capensis* is distributed over a limited geographic area around the Cape of Good Hope.

The subspecies is in contact with *A. m. scutellata* (the subspecies at the origin of the Africanized honeybees in the Americas). A stable hybrid zone exists in these natural zones of contact, where no particular biological problems have been noticed (Hepburn and Crewe 1991). However, after relocation of Cape colonies in southern and northern South Africa, *capensis* workers became social parasites of local *scutellata* colonies (the so-called Capensis problem) and destroyed a considerable number of colonies (Allsopp 1992; Allsopp and Crewe 1993; Martin *et al.* 2002). Recent evidence from the genetic profiles of these social parasites gathered on few loci indicates that they all belong to a single clone (Kryger 2001). The study of a high number of microsatellites in the present work showed that, at each locus, all individuals either present the same two alleles or are homozygous for one of these two alleles, except for a few mutations. This is the genetic signature of a clone derived from a single individual by uninterrupted generations of thelytokous parthenogenesis.

In this study, using microsatellite markers, we performed a whole-genome scan on two *A. m. capensis* samples: first-generation progeny of pseudo-queens reared in experimental hives and individuals from the multiple-generation clone, taken in nature. The two samples are complementary. The first one provides estimates of recombination rates and both, but mainly the second one, allow the observation of gradients of homozygosity along the chromosomal arms. The high number of generations that elapsed since the birth of the clone allowed accumulation of recombination events, a favorable situation that counterbalances the low recombination rates. Results obtained in these two whole-genome scans were used to confirm genetically the occurrence of central fusion during thelytokous parthenogenesis, to map the centromeric regions onto most of the linkage groups of the linkage map, and to compare the recombination rate of the pseudo-queens *capensis* to that of other honeybee meioses.

MATERIALS AND METHODS

Biological material and DNA extraction: We used four different kinds of samples in this study, two of them for mapping centromeres and studying the recombination pattern during thelytokous parthenogenesis, the other two as control samples to determine whether recombination rates vary with subspecies (*capensis vs. mellifera*) or caste (worker *vs.* queen).

An experimental population of *A. m. capensis* is maintained in Oberursel (near Frankfurt-am-Mein, Germany). To obtain pseudo-queens, freshly emerged Cape honeybee workers were isolated with a queenless group of young European *A. m. carnica* workers. A total of 153 female individuals were obtained from these *A. m. capensis* pseudo-queens. However, preliminary genetic analyses showed offspring admixture was probably caused by apicultural drift (beekeepers' term for the change of hive or colony). Individuals have been reassigned to their respective families on the basis of their microsatellite genotypes. Four progenies composed of 10, 23, 64, and 11 individuals, respectively, were retained for further analyses and comprise the first sample.

Workers from the invasive *capensis* clone were collected in the field from four regions in South Africa, representing a substantial cross-section of commercial beekeeping activity in the "*capensis*-infected" region. These samples were collected from different queenless colonies, the worker brood emerging being laid by workers. Only newly emerging bees were collected, to eliminate the possibility of drifting bees. Samples were collected from three laying worker colonies in Richmond, Kwazulu-Natal (colonies 4, 5, and 6), nine from Pretoria (colonies 23, 26, 27, CL, CS, CC, SB, UP, and TdK), two from Alberton (colonies 29 and 30), three from Piet Retief (colonies PR10, 11, and 18), one from Hazyview (colony JW), and one from White River (colony 25). Parasitized hives contained an admixture of *A. m. capensis* (dark body) and *A. m. scutellata* (light-color body) workers. The study of a few microsatellite loci confirmed identification of individuals of the Cape bee clone on the basis of body color. Forty-one individuals from 19 parasitized colonies (1–5 individuals per colony) comprise the second sample.

Two other types of progenies were also analyzed for control purposes. One sample is composed of 33 male eggs produced by an *A. m. mellifera* worker by arrhenotokous parthenogenesis. The other one consists of 65 drones produced by an *A. m. capensis* queen, also by arrhenotokous parthenogenesis. Freshly emerged drones were used to avoid apicultural drift.

DNA from the pseudo-queens' progenies was extracted from the head with a phenol-chloroform extraction (Kocher *et al.* 1989). The chelex method (Estoup *et al.* 1996) was used for the other samples, choosing the slightly modified version adapted to low DNA content for the egg sample.

Microsatellites: For this study, microsatellites were chosen from those published for the honeybee (Solignac *et al.* 2003) and mapped (see Solignac *et al.* 2004, accompanying article). In the first sample, the heterozygosity of the four pseudo-queens was tested prior to the analysis of their offspring. A total of 229 loci were assayed and we identified 101 informative loci (mother heterozygous) for at least 33 individuals. For the study of the Cape bee clone, 350 loci were tested for heterozygosity on a subsample of 11 individuals. A total of 161 loci were selected and analyzed with 30 additional individuals. For the arrhenotokous progenies of an *A. m. mellifera* worker and that of an *A. m. capensis* queen, four pairs of linked loci were genotyped.

Data analysis: Data obtained with the Cape bee parthenogens could in principle allow direct mapping of the centromeres. However, in the pseudo-queen progenies, recombination events were too rare to allow accurate map construction. Furthermore, if in *A. m. capensis* recombination events can accumulate over numerous generations, they are not independent and cannot be used to calculate linkage distances. Consequently, instead of constructing a map with these data, we have used them to localize the centromeres on the microsatellite linkage map (Solignac *et al.* 2004, accompanying article). We applied the following criterion: if chiasmata are more or less randomly distributed along the arms of the chromosomes, a central fusion generates a gradient of homozygosity from the centromeric region toward the telomeric ones. The coherence of the results obtained with thelytokous *A. m. capensis* and the linkage map was carefully checked and showed congruencies for linkage and order of loci.

The other goal of this study was to compare the recombination rates observed in various meioses. With the pseudo-queen progenies, we have calculated linkage distances between each marker of linkage groups I, II, III, and VIII (the numbers refer to the current state of the linkage map of the bee genome; see Solignac *et al.* 2004, accompanying article) and we compared

them with distances along the linkage map. These four linkage groups were chosen for the recombination study because they had a large number of markers heterozygous in the pseudo-queens. Half-tetrads resulting from central fusion allow calculation of linkage distance between a marker and the centromere. The linkage distance D is related to the proportion of homozygote individuals H by the Rizet and Engelmann (1949) formula: $D_{RE} = -\frac{2}{3}\ln(1 - 3H)$, which assumes that the number of crossovers follows a Poisson distribution (no interference). When H is small, as is always the case in the first-generation progeny, D_{RE} approximates $2H$. This formula can also be used to calculate the linkage distance between two markers. In this case, H is the proportion of individuals that are homozygous at one marker locus and heterozygous at the second locus.

We also computed the linkage distances for four pairs of linked markers with the progeny of an *A. m. mellifera* worker and that of an *A. m. capensis* queen. For these two progenies, linkage distances were estimated from recombinant fraction r using Haldane's distance function $D_H = -\frac{1}{2}\ln(1 - 2r)$.

RESULTS

Localization of centromeric regions: The gradient of homozygous recombinants both of first-generation Cape honeybees and from the clone was used to orient the chromosome arms and map the centromeres on the microsatellite map established with queen-laid workers (Solignac *et al.* 2004, accompanying article). Figure 2 shows the results for a selection of linkage groups (the localization of the centromere using this rationale is presented for most of the linkage groups in Solignac *et al.* 2004, accompanying article). Note that the simplicity of the situation described above is frequently modified. For instance, numerous cases of multiple crossing over occurred during the same meiosis; they produced a homozygosity array followed distally by a heterozygosity array (*i.e.*, individuals CC 22 and 30 7; Figure 2A, right arm.). In other cases, the allelic phase was modified distally to the crossing over but because heterozygosity was preserved this became apparent only when an additional recombination appeared later, generating non-concordant succession of homozygosity (for instance, individuals CC 17, 30 7, and TdK 2; Figure 2A, markers *Am*081 and *Am*389 on the left).

Figure 2 provides three examples of centromere localization. In group I (Figure 2A), homozygosity increases with distance from a central region where five markers present no recombinant at all. This central region is a good candidate for the position of the centromere. Linkage group I is thus likely to be chromosome 1, which is the longest of the complement and the only metacentric one. A second example concerns linkage group II (Figure 2B), which has a very terminal location of the centromere and is probably telocentric. This linkage group is not assigned to a particular chromosome. Finally, gradients on the sex chromosome (Figure 2C), *i.e.*, the chromosome bearing the sex locus, would have been difficult to interpret without the information of the subterminal location of the sex locus (Beye and Moritz 1995; Beye *et al.* 1996); recombination events that occurred on this chromosome between the centromere and the sex locus were conserved only if they were accompanied by a second recombination restoring heterozygosity in its vicinity; otherwise diploid drones would be produced and destroyed (see Introduction). This group is our linkage group IV and it corresponds cytologically to chromosome 8 (Beye *et al.* 1996).

Recombination in thelytokous parthenogens: The linkage distances between markers observed during the thelytokous parthenogenesis of Cape bee pseudo-queens (first sample) have been compared with the distances between the same markers in the linkage map of the honeybee genome (Solignac *et al.* 2004, accompanying article). Figure 3 presents a plot of the distances calculated in parthenogens *vs.* the map distances for 78 pairs of adjacent linked markers. Among the 78 linkage distances that we compared, 77 are shorter in the thelytokous parthenogens than in the linkage map based on the queen progenies. The reduction during thelytoky is highly variable. For example, two markers that are >70 cM apart in the linkage map present no recombination at all in the pseudo-queen progenies, although one pair of markers recombines more in the latter. On average, as estimated by linear regression, recombination is reduced in the pseudo-queen progenies by a factor of 12.8. Figure 4 shows the same representation for the four dense linkage groups chosen for a more detailed study. Among these four groups, recombination rates are reduced in Cape pseudo-queens by a factor of 5.6–16.8 (Table 1). The reduction of the rate is not uniform: in the centromeric regions of each chromosome, recombination is almost absent in the Cape bee pseudo-queens but it increases markedly in the telomeric regions.

To determine if this reduction of recombination could be related to the subspecies (*A. m. capensis*) or the caste (worker) of the pseudo-queens, the linkage distances observed in two other types of meioses were also contrasted with the distances of the linkage map. Table 2 compares the distances of the linkage map constructed with regular queen meioses with the distances observed during the meiosis of an *A. m. capensis* queen and an *A. m. mellifera* worker, for four pairs of linked markers. It has not been possible to use the same panel of loci in both cases due to the different heterozygous loci of the mothers. The linkage distances observed in both cases are very comparable with the map distances [except for the pair of loci (*Am*0191-*Am*0097) that presents a significantly smaller recombination rate in the *A. m. mellifera* worker meiosis]. This suggests that the reduction of recombination rates observed in the *capensis* pseudo-queen progenies is not associated with the subspecies *A. m. capensis* or to the meiosis of workers but is particular to thelytokous parthenogens. The excess of double or multiple recombination events suggests the

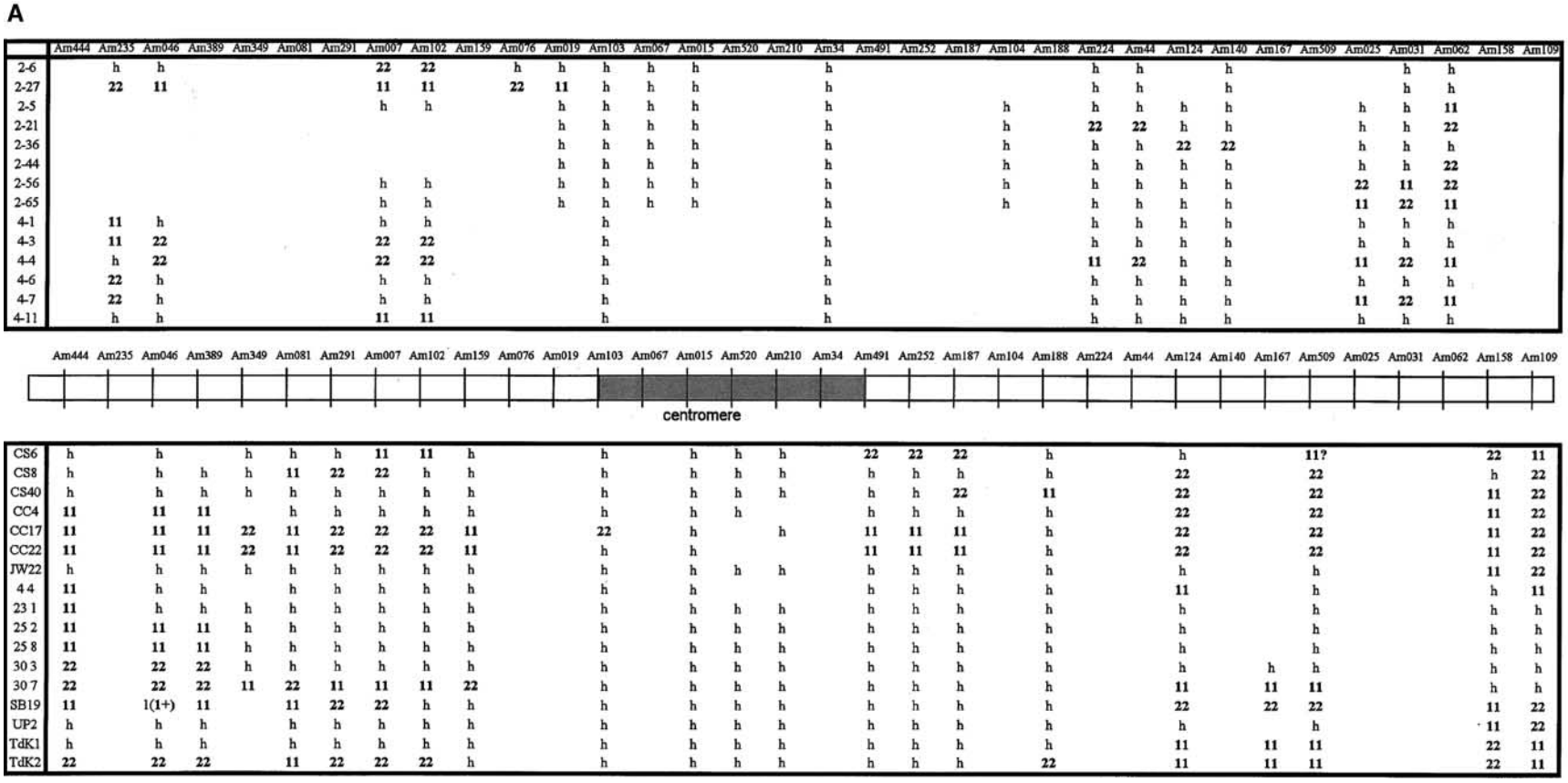


FIGURE 2.—Pattern of homozygosity observed in the pseudo-queens' offspring (top, one-generation progenies; bottom, individuals of the clone) and deduced position of the centromere for three linkage groups. Individuals that were heterozygous for all loci assayed are not shown but their number can be obtained by substracting the number of shown individuals from the total number of individuals in each sample (up to 108 for the top box and 41 for the bottom one). (A) Linkage group I. The centromeric region encompasses the five loci at the center of the group. This group is likely to correspond to the large metacentric chromosome 1 of the complement. (B) Linkage group II. (C) Linkage group IV (sex chromosome). h, heterozygote; 11 and 22, two types of homozygotes. On the left, family (top) or colony (bottom) code is followed by the number of the individual.

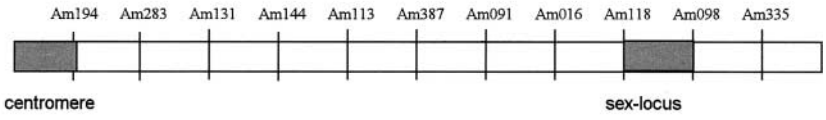
B

	Am383	Am361	Am59	Am382	Am321	Am527	Am148	Am306	Am407	Am434	Am154	Am281	Am504	Am086	Am257	Am221	Am149	Am178
2-1			h				**11**							**11**	**11**		**11**	**11**
2-4			h				h							**22**	**22**		h	h
2-5			**22**				**11**							**11**	**11**		**11**	**11**
2-12			h				h							h	h		**11**	**11**
2-27			h				**11**							h	h		h	h
3-13			h				h				h				h		**11**	h
3-19			h				**11**				**22**				**11**		**11**	**22**
3-58			h				**11**				**22**				h		h	h
4-1			h				h							**22**	**22**		**22**	**22**
4-3			h				h							h	**11**		**11**	**11**

CS1	h	h	h		h	h		**11**	**11**	**22**	**22**	h	h			h		**11**
CS6	h	**22**	**11**		h	h		**11**	**11**	**22**	h	h	h			h		h
CC8	h	h	h		**11**	**11**		h	h	h	**11**	**22**	**11**			h		h
CC17	h	h	h		**11**	**11**		h	h	h	**11**	**22**	**11**			h		h
CC22	h	h	h		**11**	**11**		h	h	h	**11**	**22**	**11**			h		h
44	h	h	h		h	h		**11**	**11**	**22**	h	h	h			**22**		11
307	h	h	h		h	h		h	h	h	h	**22**	**11**			**11**		**22**
PR10-1	h	h	h	h	h	**11**		**11**	**11**	**22**	**22**	**11**	**22**			**11**		**22**
PR10-2	h	h	h	h	h	**11**		**11**	**11**	**22**	**22**	**11**	**22**			**11**		**22**
PR11-1	h	h	h	h	h	**11**		**11**	**11**	**22**	**22**	**11**	**22**			**11**		**22**
PR11-2	h	h	h	h	h	**11**		**11**	**11**	**22**	**22**	**11**	**22**			**11**		**22**
PR18-2	h	h	h	h	h	**11**		**11**	**11**	**22**	**22**	**11**	**22**			**11**		**22**

C

	Am194	Am283	Am131	Am144	Am113	Am387	Am091	Am016	Am118	Am098	Am335
2-6			h	h			h		**11**		
3-36			**11**	**11**			h			h	



CS1	**22**	**11**	h	h	h	h	h	h	h	h	h
CS6	**11**	**22**	h	h	h	h	h	h	h	h	h
CS8	h	h	h	h	h	h	h	h	h	**11**	**11**
CC8	h	h	**11**	**22**	**11**	**11**	**11**	h	h	h	h
CC17	h	h	**11**	**22**	**11**	**11**	**11**	h	h	h	h
CC22	h	h	**11**	**22**	**11**	**11**	**11**	h	h	h	h
44	h	h	h	h	**22**	**22**	**22**	h	h	**11**	**11**

FIGURE 2.—*Continued.*

possibility of a negative interference but recombination rates and sample sizes were too small to analyze it.

DISCUSSION

This formal genetics, established in part on results from a natural laboratory (*i.e.*, the parasitic clone), brings some light to the genetics of the honeybee but also to the more general problem of parthenogens, which until recently have been studied more at the cytological level than at the genetical level.

Central fusion: The work of VERMA and RUTTNER (1983) provided cytological evidence for a central fusion accounting for diploid restoration of the thelytokous parthenogens in the Cape honeybees. Genetic results expected from this mode of diploid restoration are gradients of homozygosity from the centromere toward the tip of the chromosomal arm(s). Gradients were actually observed for almost all long linkage groups. However, these gradients may also be produced by terminal fusion, *i.e.*, fusion of the two meiotic products that were separated only at the second meiotic division. In that case, gradients of homozygosity should have the reverse direction; *i.e.*, they should increase from the telomere to the centromere. A support for central fusion is provided by linkage group I, most probably assigned to

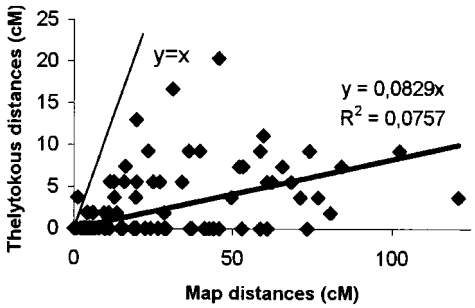


FIGURE 3.—Genetic distance in *capensis* pseudo-queens as a function of the linkage distances on the map constructed with regular meioses. All 78 pairs of adjacent markers in the data set are shown. With only one exception, all points are located well below the line of equation $y = x$, which indicates an important reduction of the recombination rate during thelytokous parthenogenesis.

chromosome 1. Heterozygosity is observed for all individuals at five loci located in the middle of the group, while homozygosity increases with the distance to these markers. This pattern is what is expected for a metacentric chromosome under central fusion. Less direct evidence advocating for central fusion emerges from the high level of heterozygosity that has been preserved in the clone over numerous generations. A terminal fusion cannot maintain high heterozygosity in presence of low levels of recombination.

Centromere mapping: The number of species where centromeres can be genetically mapped is relatively limited because it is necessary to have access to several products of the same meiosis. The most favorable material is obviously fungi, where tetrads give access to the four meiotic products (PERKINS 1953). More common are species where only half-tetrads, *i.e.*, two meiotic products, can be recovered. The first well-studied half-tetrads analyses were attached-X chromosomes in Drosophila (BEADLE and EMERSON 1935). Other examples include induced chromosome doubling at meiosis in maize (RHOADES and DEMPSEY 1966), gynogenesis in zebrafish (JOHNSON *et al.* 1995), and automictic parthenogenesis in *Venturia canescens* and in honeybee (VERMA and RUTTNER 1983; BEUKEBOOM and PIJNACKER 2000). In mammals, autosomal trisomies (MORTON *et al.* 1990) or ovarian teratomas, where meiosis second division is suppressed (CHAKRAVARTI *et al.* 1989), can be used for half-tetrad analysis.

Assuming central fusion, as justified above, we have used homozygosity gradients to map the centromeric regions on the linkage groups of *A. mellifera*. Depending on the group, these regions are more or less extended, as a function of the number of recombinant individuals observed (see SOLIGNAC *et al.* 2004, accompanying article). It must be noted that in some instances the clarity of the gradient is obscured toward the tips of the chromosomes and this evanescence of homozygosity is attributable to the high abundance of multiple chiasmata and to the subterminal preferential location of crossing over.

Recombination in the Cape bee: The analyses on the Cape honeybees by MORITZ and HABERL (1994) using

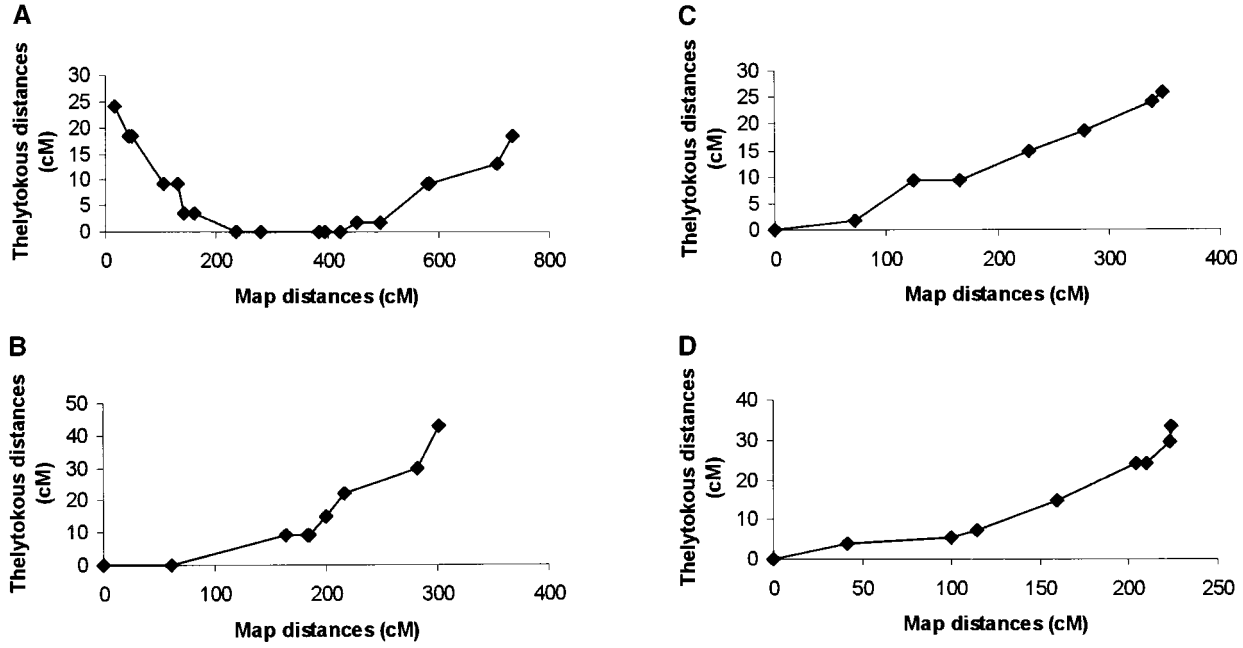


FIGURE 4.—Cumulative linkage distance in the *capensis* pseudo-queens, from centromere to telomere(s), as a function of the cumulative linkage distances of a map constructed with regular meioses. Four linkage groups are shown. (A) Linkage group I; (B) linkage group II; (C) linkage group III; (D) linkage group VIII. The group numbers refer to the current state of the linkage map of the honeybee, see SOLIGNAC *et al.* (2004, accompanying article). Note that the regression is not linear and that most of the chiasmata are localized at the tips of the chromosomes in the thelytokous parthenogens.

TABLE 1

Comparison of the total genetic lengths of four linkage groups during the thelytokous parthenogenesis of the *Apis mellifera capsensis* pseudo-queens with those of the linkage map

Linkage group	Total linkage distance in thelytokous parthenogenesis (cM)	Map linkage distance (cM)	Reduction
I	42.6***	715.1	16.8
II	25.8***	276.6	10.7
III	42.6***	240.2	5.6
VIII	33.4***	223.8	6.7

***Significantly different from the map linkage distances at the 0.001 level.

fingerprinting (12 dominant markers) led them to conclude that recombination was absent in pseudo-queens. Our results show that recombination is not totally suppressed. This difference may be attributed to the low number of markers used by the two authors, to their dominance (only homozygotes for the absence of bands are detectable), or to a centromeric location of the loci.

Verma and Ruttner (1983) have cytologically observed an average of two chiasmata per bivalent during the meiosis of the Cape laying workers. This is equivalent to a length of 100 cM per chromosome, *i.e.*, a total of 1600 cM for the 16 chromosomes of the genome (Beye and Moritz 1995 and references therein). The "normal" genomic length of the honeybee is ~4500 cM (see Solignac *et al.* 2004, accompanying article; this is slightly longer than the randomly amplified polymorphic DNA map published by Hunt and Page 1995). By comparing linkage distances in pseudo-queens and in the linkage map, we found that linkage length is reduced on average by a factor of 12.8 in the pseudo-queens. This means a linkage length of the genome of ~310 cM for the pseudo-queens. This value is much lower than the one inferred from the cytological distribution of chiasmata. A possible explanation could be that many chiasmata occur very close to the telomeres of chromosomes and hence are rarely detected in our data.

Kauhausen (1978), using five morphological characters on the thelytokous progeny of pseudo-queens of *capensis* ancestry, observed a high number of recombinant individuals homozygous for recessive alleles. The linkage distances to the centromeres deduced from her values are 36.0, 25.7, 18.2, 3.6, and 1.6 cM for *chartreuse*, *cream*, *cordovan*, *bayer*, and an eye color mutation, respectively. The position of these loci is unknown but their average distance to the centromere is relatively high compared to the "thelytokous" distances (see Figure 4). This might be due to a preferential telomeric location of these morphological markers but can also result from the fact that the pseudo-queens were not pure Cape bees but were produced by two or three recurrent backcrosses of *capensis* and *carnica* hybrid drones with *capensis* queens.

Even if from these results the exact linkage length of the map of the *capensis* pseudo-queens cannot be determined, it is clear that there is a strong reduction of recombination during their meiosis. This raises the problem of its origin. Ruttner (1988) has concluded that thelytoky in *A. m. capensis* is under the control of a single Mendelian gene with incomplete penetrance. It is highly improbable that the reduction of recombination is a pleiotropic effect of the factor responsible for thelytoky. More probably several genes are involved, as suggested by the intermediate recombination rates observed in interracial hybrids between *carnica* and *capensis* (Kauhausen 1978).

Thelytoky and recombination: Thelytokous parthenogenesis (the production of diploid females from unfertilized eggs) is relatively rare in the animal kingdom with hardly >1500 thelytokous species (White 1984). In Hymenoptera, it also does not appear to be a frequent phenomenon (reviewed in Slobodchikoff and Daly 1971; Schilder 1999), although, on the basis of the sporadic appearance of thelytoky in several parasitoid families (Crozier 1975), it has been argued that Hymenoptera might quite easily make the transition from arrhenotoky to thelytoky (Cornell 1988). The various cytological mechanisms of thelytoky are traditionally divided in two groups. When offspring are produced without meiosis, the parthenogenesis is called apomixis. When reproduction involves chromosomal reduction and restoration of the original ploidy (by fusion of two meiotic products or through gamete duplication), the parthenogenesis is called automixis. The majority of Hymenopteran parthenogens for which the cytological mechanism has been investigated are automictic (Cook 1993). Automixis leads to complete homozygosity or at least to an increase in the homozygosity of the offspring compared to the mother. In *A. m. capensis*, the increase of homozygosity is much reduced because of the low recombination rate during thelytoky. It is currently not known whether the other automictic Hymenopteran species also have mechanisms to control the homozygosity increase associated with automixis. In a few automictic insect species, it has been shown that most individuals are heterozygous for chromosomal inversion (Stalker 1956; Carson 1962; Suomalainen *et al.* 1987), which may be a way to preserve heterozygosity.

This reduction, limited to the *capensis* pseudo-queens, appears as a specific genome-subspecies-caste interaction. It would be interesting to investigate crossover rate

TABLE 2

Comparison of linkage distances using Haldane correction with the linkage map distances

Loci	Linkage group	Linkage distance in an *A. m. capensis* queen (cM)	Map linkage distance (cM)
	Estimated with the progeny of an *Apis mellifera capensis* queen		
*Am*062-*Am*031	I	22.4	25.0
*Am*091-*Am*118	IV	19.5	18.7
*Am*191-*Am*097	VIII	47.5	37.9
*Am*097-*Am*125	VIII	27.2	19.1
Loci	Linkage group	Linkage distance in an *A. m. mellifera* worker (cM)	Map linkage distance (cM)
	Estimated with the progeny of an *Apis mellifera mellifera* worker		
*Am*062-*Am*109	I	6.5	17.7
*Am*091-*Am*118	IV	31.4	18.7
*Am*191-*Am*097	VIII	15.5*	37.9
*Am*043-*Am*087	XIV	19.3	36.5

*Significantly different from the map linkage distances at the 0.05 level.

in laying workers in other subspecies where thelytoky is observed at low frequency (Tucker 1958).

Maintenance of heterozygosity: The reduction of recombination in laying workers is a way to preserve most of the heterozygosity present in the parthenogens. A rate similar to the one observed in the queens would produce in a few generations a progeny that is mostly homozygote (autozygote). Even in a single generation, the reduction of heterozygosity would cause damage because the honeybee is very sensitive to inbreeding for many characters (Brueckner 1976a,b).

Reduced recombination is probably a compromise between the necessity of chiasmata to ensure a faithful chromosome segregation (aneuploids are produced when crossing over is absent; Bascom-Slack *et al.* 1997; Moore and Orr-Weaver 1998) and the requirement to reduce or suppress recombination to maintain heterozygosity. This maintenance is enhanced by the location of the "residual" chiasmata: as long as the linkage map is approximately the reflection of the physical distance on the chromosomes, our results indicate that crossing over is preferentially located at the tips of the chromosomes in the Cape bees. This means that fewer loci become homozygous per recombination event.

The sex locus is located on one of the groups we have studied. Suomalainen *et al.* (1987) have predicted that, because Cape bee pseudo-queens are able to produce heterozygous females parthenogenetically, this locus must be localized near a centromere. This prediction has not been verified by subsequent analysis: Beye *et al.* (1996) have located the sex locus in subtelomeric position on the large arm of chromosome 8. In the Cape honeybee clone, the chromosomal region that encompasses this locus has regularly conserved heterozygosity thanks to double crossing over.

In spite of the high number of generations elapsed since the origin of the clone, as testified by the observation of mutations at 10 loci, the reduction of heterozygosity by a central fusion process was rather moderate. Considering the 161 loci analyzed, which were heterozygous in the foundress, the average reduction of heterozygosity per individual is only 19.1% (including 7 loci that were homozygous in all individuals for two alternate alleles and the few mutations being taken into account).

Several researchers took advantage of the thelytokous pseudo-queens in the Cape bee to study the heritability or the phenotypic variance of quantitative traits (Moritz and Hillesheim 1985; Moritz and Klepsch 1985; Brandes 1988, 1991; Radloff *et al.* 2002). The situation (assuming the absence of recombination) seemed favorable because the parthenogen and its daughters were thought to have exactly the same genotype. In fact, this is only approximately true, every meiosis generating daughters that are homozygous for a fraction of the genes that are heterozygous in the mother. It remains that the daughters of a laying worker are highly related (far more than true sisters that are sexually produced). However, a calculation of relatedness in thelytokous families has to take into account the position of genes (centromeric or telomeric) on the chromosome.

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