

# A New Metazoan Recombination Rate Record and Consistently High Recombination Rates in the Honey Bee Genus *Apis* Accompanied by Frequent Inversions but Not Translocations

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## Abstract

Western honey bees (*Apis mellifera*) far exceed the commonly observed 1–2 meiotic recombination events per chromosome and exhibit the highest Metazoan recombination rate (20 cM/Mb) described thus far. However, the reasons for this exceptional rate of recombination are not sufficiently understood. In a comparative study, we report on the newly constructed genomic linkage maps of *Apis florea* and *Apis dorsata* that represent the two honey bee lineages without recombination rate estimates so far. Each linkage map was generated *de novo*, based on SNP genotypes of haploid male offspring of a single female. The *A. florea* map spans 4,782 cM with 1,279 markers in 16 linkage groups. The *A. dorsata* map is 5,762 cM long and contains 1,189 markers in 16 linkage groups. Respectively, these map sizes result in average recombination rate estimates of 20.8 and 25.1 cM/Mb. Synteny analyses indicate that frequent intra-chromosomal rearrangements but no translocations among chromosomes accompany the high rates of recombination during the independent evolution of the three major honey bee lineages. Our results imply a common cause for the evolution of very high recombination rates in *Apis*. Our findings also suggest that frequent homologous recombination during meiosis might increase ectopic recombination and rearrangements within but not between chromosomes. It remains to be investigated whether the resulting inversions may have been important in the evolutionary differentiation between honey bee species.

**Key words:** crossover, linkage map, social evolution, genome structure, recombination rate, chromosome evolution.

## Introduction

Crossovers leading to meiotic recombination are required for proper segregation of homologous chromosomes and also serve an important evolutionary role by generating new allelic combinations within chromosomes. Abundant evidence for substantial variation within genomes exists (Coop and Przeworski 2007; Comeron et al. 2012; Webster and Hurst 2012; Hunter et al. 2016). Inter-specifically, meiotic recombination rates largely vary as a function of genome size and chromosome number because crossovers occur only once or twice per chromosome in most Metazoan species (Lynch

2006). However, evidence for adaptive inter-specific variation is growing (Dumont and Payseur 2008). Social insects, particularly the Western honey bee (*Apis mellifera* L.), represent an important exception to this rule. While a large-scale analysis (Ross, Blackmon, et al. 2015) failed to support the theoretical notion that social insect evolution should be accompanied by an increase in chromosome number (Sherman 1979), the few existing estimates of genome-wide recombination rates indicate that highly social species have exceptionally high rates of recombination among the Metazoa (Beye et al. 2006; Wilfert et al. 2007).

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Despite the wealth of genomic data, relatively few accurate measures of genome-wide recombination rates exist because complete linkage maps are still laborious and costly to produce. The assertion that social evolution is associated with high recombination is thus based on genome-wide estimates of recombination for the leaf cutter ant *Acromyrmex echinator* (Sirviö et al. 2006), the harvester ant *Pogonomyrmex rugosus* (Sirviö, Pamilo, et al. 2011), the wasp *Vespa vulgaris* (Sirviö, Johnston, et al. 2011), the bumblebee *Bombus terrestris* (Stolle et al. 2011), and the two closely related honey bee species *A. mellifera* (Beye et al. 2006; Liu et al. 2015; Wallberg et al. 2015) and *A. cerana* (Shi et al. 2013). Hypotheses to explain the high recombination rates can principally be distinguished into two lines of arguments. The first invokes immediate, short-term benefits of increasing offspring (=colony) genetic diversity to enhance division of labor and disease resistance (Sirviö et al. 2006). However, the theoretical increase of intra-colonial genetic diversity due to recombination is small compared with the effect of multiple mating (Rueppel et al. 2012). Nevertheless, a negative correlation between the varying levels of polyandry in the genus *Apis* (Tarpy et al. 2004) and recombination rate may be predicted if the evolution of both was due to selection for increased intra-colonial genetic diversity: Genetic diversity could be increased by either mechanism without the need for two redundant mechanisms and polyandry is predicted to outweigh any effect of recombination (Rueppel et al. 2012). The second set of arguments to explain high recombination rates in social insects is based on the evolutionary history of strong, divergent selection within the genome or ongoing directional selection coupled with small effective population sizes in social insects (Beye et al. 2006; Sirviö et al. 2006). Selection in highly social insects may be particularly strong due to disease pressure (Wilfert et al. 2007) or functionally independent evolution among castes (Kent and Zayed 2013; Liu et al. 2015). This second hypothesis predicts that all *Apis* species share a similar recombination rate due to their shared queen-worker divergence and caste differences, similar mating biology, and similar social life style (Oldroyd and Wongsiri 2006). Preliminary evidence for a genus-wide high recombination in the honey bee genus *Apis* exists (Mezner et al. 2010), but more conclusive evidence is needed.

Moreover, the long-term consequences of high recombination rates for genome structure and chromosome integrity are not yet understood in general. On the one hand, crossovers are regarded as necessary to ensure proper chromosome segregation (Baker et al. 1976) and consequently more crossovers may stabilize the chromosome tetrads during meiosis. On the other hand, double-strand breaks that are required to initiate recombination events have been associated with chromosomal instability (van Gent et al. 2001). Thus, highly recombining genomes may be more or less prone to chromosomal rearrangements over evolutionary time and genome divergence in the genus *Apis* with a

minimum age of 20 million years (Ramírez et al. 2010; Cardinal and Danforth 2013) presents a good opportunity to test for genome consequences of high recombination.

To investigate the rate of recombination throughout the genus *Apis* and study its consequences for chromosome evolution, we constructed two new linkage maps of the genomes of the giant honey bee (*Apis dorsata*), representing the subgenus Megapis, and the dwarf honey bee (*Apis florea*), representing the subgenus Micrapis, and compared the marker order of the linkage maps to *A. mellifera* to assess synteny.

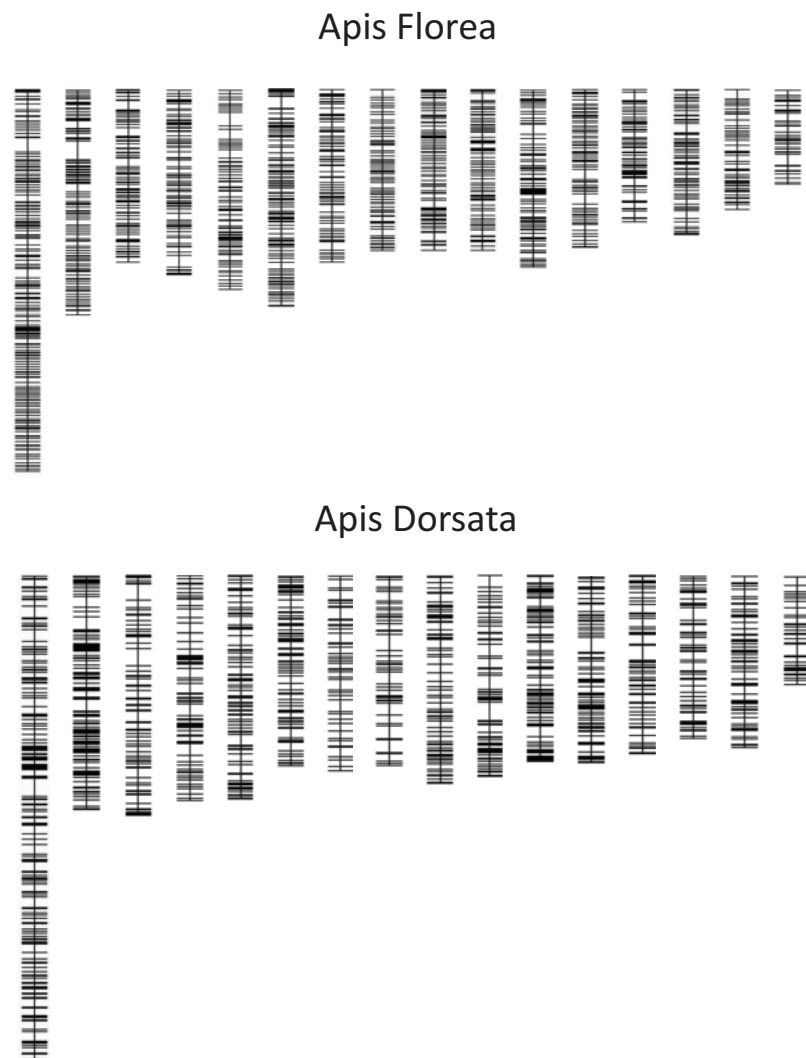
## Results

For *A. florea*, 2906 high-quality SNP and small InDel markers were selected. Due to missing data for individual genotypes, the average number of high quality markers per individual was 2,555 (range: 1,715–2,785) and markers contained data from 72 individuals on average (range: 53–82). For *A. dorsata*, 3,548 high-quality markers were obtained. Each *A. dorsata* drone was covered on average by 3,151 SNP loci (range: 2,760–3,380), and each worker by 2,074 loci (range: 2,021–2,132) after excluding the uninterpretable heterozygous genotypes. Together, markers in *A. dorsata* contained informative data from 77 individuals on average (range: 51–93).

After duplicate markers and markers with biased allele distribution or elevated counts of missing data were excluded, 1,297 markers remained in the *A. florea* dataset for linkage mapping. These markers were genotyped on average in 88.4% of the individuals and mapped to 16 final linkage groups (fig. 1) corresponding to the 16 honey bee chromosomes (Fahrenhorst 1977). After elimination of 135 double crossovers, the final map was determined to be 4781.9 cM long, with linkage groups ranging from 156.0 to 640.7 cM (table 1), resulting in a genome-wide recombination rate estimate of 20.8 cM/Mb.

In *A. dorsata*, workers were excluded after an initial analysis revealed map expansion when workers and drones were analyzed together. A final set of 1,201 non-redundant markers with the least missing data and most even allele distribution were used for linkage mapping. The resulting map contained 16 major linkage groups (fig. 1) and two much smaller groups (8 and 4 markers), which were excluded from the analysis. After elimination of 141 double crossovers, the overall length of the final map was determined to be 5,761.8 cM long, with chromosomes ranging from 184.2 to 830.0 cM (table 1), resulting in a genome-wide recombination rate estimate of 25.1 cM/Mb.

Blast-searches of the *A. mellifera* genome with the sequences of the RAD-tag markers identified homologous sequences for 513 of the 1,279 mapped *A. florea* markers and 641 of the 1,189 mapped *A. dorsata* markers (supplementary table S1, Supplementary Material online). Only 5.3% of these homologous sequences for *A. florea* and 3.6% for *A. dorsata*



**FIG. 1.**—Linkage maps of the dwarf (*A. florea*) and the giant honey bee (*A. dorsata*), with linkage maps ordered according to the homologous *A. mellifera* chromosomes. Each horizontal line indicates a SNP marker and their vertical position indicates recombination distances.

were located on *A. mellifera* chromosomes that did not match the chromosome that corresponded to the respective linkage group (fig. 2). Almost all of these “misplaced” markers matched multiple locations in the *A. mellifera* genome with similar identity scores. Map saturation was confirmed by constructing linkage maps with the subset of markers with homologous sequences on respective chromosomes in the *A. mellifera* genome: These linkage maps were 4,657.7 and 6,811.8 cM long for *A. florea* and *A. dorsata*, containing subsets of 476 and 608 markers, respectively.

Compared to the marker order within the chromosomes of the *A. mellifera* reference genome, the most likely marker order in the *A. florea* linkage groups suggested at least 28 inversions and 19 single markers that violated a syntenic order. In *A. dorsata* the most likely marker order indicated at least 46

inversions and 14 nonsyntenic single markers. Reordering the markers according to the corresponding order of the homologous sequences in the *A. mellifera* genome resulted in a 33.9% map expansion in *A. florea* and 30.1% in *A. dorsata*. All of the 14 rearranged chromosomes in *A. florea* and 14 of 16 rearranged chromosomes in *A. dorsata* were substantially (>10%) expanded in linkage map size and the originally determined marker order was significantly more likely in all of these cases (supplementary table S2, Supplementary Material online).

## Discussion

Our genome-wide recombination rate estimates for dwarf and giant honey bees exceed 20 cM/Mb, providing

**Table 1**

Chromosome Characteristics of the Linkage Maps of the Dwarf and Giant Honey Bees

Linkage Group	Dwarf Honey Bee: <i>Apis florea</i>		Giant Honey Bee: <i>Apis dorsata</i>	
	Length (cM)	Marker Number*	Length (cM)	Marker Number*
1	640.7	173 (87)	830.0	163 (78)
2	374.9	107 (48)	399.1	108 (66)
3	288.3	81 (31)	408.3	68 (37)
4	310.4	80 (37)	377.8	71 (35)
5	332.2	73 (26)	382.0	81 (43)
6	366.8	107 (45)	324.3	83 (54)
7	288.3	71 (28)	331.6	47 (24)
8	266.7	69 (21)	321.6	53 (27)
9	267.6	75 (28)	353.1	76 (35)
10	267.7	73 (25)	341.9	65 (37)
11	296.1	79 (31)	317.2	86 (50)
12	262.5	70 (18)	318.6	74 (42)
13	222.5	65 (23)	303.8	60 (29)
14	241.9	68 (32)	276.1	56 (36)
15	200.5	51 (25)	292.4	62 (30)
16	156.0	37 (8)	184.2	36 (18)

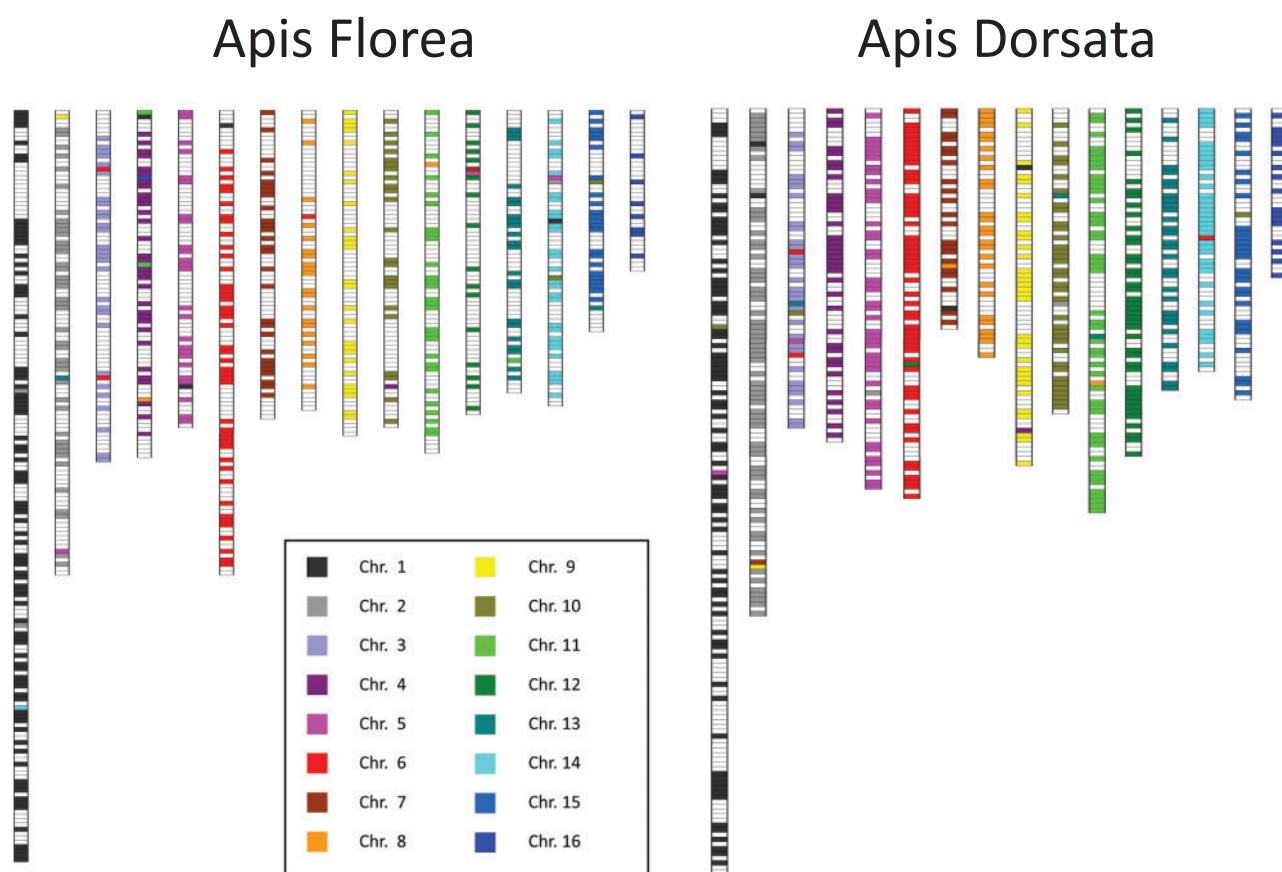
\*Markers with homologous matches in the *A. mellifera* genome are shown in brackets.

unequivocal evidence that high recombination rates are the rule in the honey bee genus *Apis*. The presented results confirm a preliminary study of *A. florea* (Mezner et al. 2010) and complement the findings of high recombination in the cavity-nesting honey bee species *A. mellifera* (Beye et al. 2006) and *A. cerana* (Shi et al. 2013) with data from both remaining subgenera, Megapis and Micrapis. Thus, a common explanation for the evolution and/or evolutionary maintenance of high recombination rates in all honey bees is most parsimonious. Even though the species of honey bees differ in many details of their biology and have presumably diverged over 20 million years ago (Ramírez et al. 2010; Cardinal and Danforth 2013), they share a basic social organization with one polyandrous reproductive female and thousands of functionally sterile workers cooperating in perennial colonies that reproduce by fission (Oldroyd and Wongsiri 2006). The reason for the evolutionary origin and maintenance of the exceptional recombination rates of honey bees is presumably related to these fundamentals and it is most likely that the increase in recombination rate occurred only once before the divergence of the three honey bee lineages. However, we can neither specify the timing and particular biological characteristic, nor exclude a mechanistic and potentially non-adaptive explanation.

The genome-wide estimate of recombination rate in *A. dorsata* is substantially higher than any comparable value among Metazoans (Wilfert et al. 2007). Two studies that report a higher recombination rate for *A. mellifera*, estimating a genome average of 37 cM/Mb (Liu et al. 2015) and

26.0 cM/Mb (Wallberg et al. 2015), differ substantially in methodology. Specifically, both were based on a much higher (> 100×) marker density. A higher marker density typically results in higher recombination estimates because it allows for the detection of crossover events that may be dismissed as genotyping errors or missed entirely with lower marker densities (Liu et al. 2015). All studies with comparable marker densities and analyses have estimated a lower value for the recombination rate of *A. mellifera* (Ross, DeFelice, et al. 2015) than our value for *A. dorsata*. This finding provides evidence against a trade-off between polyandry and recombination rate, as predicted by the genetic diversity hypothesis, because *A. dorsata* also exceeds all other *Apis* species in queen mating frequency (Tarpay et al. 2004). Likewise, our results contradict the prediction of the genetic diversity hypotheses for *A. florea* to exhibit a higher recombination than other *Apis* species. Despite its lowest queen mating frequency in the genus (Tarpay et al. 2004), *A. florea* does not show a higher recombination rate than the other *Apis* species. Instead, our results are consistent with the hypothesis that the high recombination rates of all honey bees determined so far reflect shared ongoing selection processes or the legacy of an evolutionary history of small effective population sizes and strong divergent selection pressures (Kent and Zayed 2013). Our study does not present a conclusive test because the number of independent data points does not allow for statistical testing and the positive correlation between polyandry and high recombination could be due to the influence of other confounding variables. However, it is interesting to note that the other highly recombining social insect genomes in ants and wasps (Sirviö et al. 2006; Sirviö, Johnston, et al. 2011; Sirviö, Pamilo, et al. 2011) are also coinciding with polyandry. Based on our data, we can also not determine whether the high recombination rates in *Apis* are due to current selection or represent the evolutionary legacy of early honey bee social evolution. The closest relatives to honey bees with a recombination rate estimate are bumblebees with a considerably lower recombination rate (Stolle et al. 2011), suggesting that the very high recombination rates reported for *Apis* is genus-specific.

A consistently high genomic recombination rate in the genus *Apis* throughout its evolutionary history is most likely. Therefore, the generated data sets also allowed us to study the long-term consequences of high recombination rates for genome evolution. We found no substantial evidence of inter-chromosomal translocations between the three analyzed *Apis* genomes. The small number of markers that were identified as non-homologous in the linkage groups of *A. florea* and *A. dorsata* were presumably due to misidentification of homology among several similar sequences in the *A. mellifera* genome. In no case were multiple linked markers found on a nonhomologous linkage group. Thus, the high recombination rate in honey bees appears not to increase the likelihood of crossovers between nonhomologous chromosomes, although both processes depend on double strand breaks and



**FIG. 2.**—Chromosomes show general conservation among the three *Apis* species, indicated by the color coding of markers with homologous sequences in the *A. mellifera* genome. Most single markers in the *A. florea* and *A. dorsata* linkage groups that corresponded to sequences in non-homologous chromosomes were likely assigned to the different chromosomes in error due to the presence of multiple homologous sequences in *A. mellifera*. Markers without significant homology to *A. mellifera* or with homology in “ungroups” were left white.

are potentially linked (Sargent et al. 1997; Puchta 1999; Stankiewicz and Lupski 2002; Berg et al. 2010). Conversely, the absence of inter-chromosomal translocations supports the notion that homologous chromosome tetrads are stabilized by crossovers during meiosis (Baker et al. 1976). In contrast, many intra-chromosomal inversions during *Apis* evolution were suggested by our data and the statistical support for inversions was high in most chromosomes. Due to the limited marker density of our study, it is likely that many of the non-syntenic single markers also indicate inversions and that the majority of inversions remained undetected (Feuk et al. 2005). The causation of intra-chromosomal rearrangements by several simultaneous crossover events is mechanistically plausible (Sharakhov et al. 2006). Such inversions facilitate differentiation within species and speciation by locally suppressing recombination (Hoffmann and Rieseberg 2008), which might be particularly important in social insect species (Linksvayer et al. 2013; Wang et al. 2013). However, it remains to be tested which of the chromosomal inversions is associated with

functionally important gene clusters and signatures of accelerated evolution in honey bees.

## Material and Methods

A total of 71 drones and 110 workers were collected from a colony of *Apis dorsata*, located on the grounds of the Agricultural Research Station Tenom (Sabah, Malaysia) in a tree about 20 m above ground. To nondestructively sample bees from one colony, individuals were captured with a net mounted on a pulley system directly next to the top of the comb (Koeniger et al. 2010). To preferentially sample drones, the net was only dropped during the mating flight time in the evening (Koeniger et al. 1994) of February 19–28, 2007. Individuals were killed by chilling and head and thorax stored in RNAlater<sup>®</sup> (Life Technologies). Genomic DNA was extracted using a modified CTAB protocol (Hunt and Page 1995) and diluted to 100 ng/μl after quantification with a Nanodrop 1000 spectrophotometer (Thermo Scientific).

To verify that they were derived from the same mother, samples were genotyped at three microsatellite loci on a DNAnalyzer (LiCor), using previously described methods (Meznar et al. 2010).

*Apis florea* was sampled from Thailand, King Mongkut's University of Technology Thonburi, Ratchaburi Campus on May 24, 2012. A section of drone comb was cut from a single colony and all emerged, adult bees were removed. A cohort of capped drone pupae near emergence was collected using ethanol-washed forceps. Drone heads and thoraces were cut in half along the sagittal plane with a sterile razor and stored immediately in RNAlater® (Life Technologies). Genomic DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega) and diluted to 100 ng/μl after quantification with a Nanodrop 1000 spectrophotometer (Thermo Scientific).

All samples were treated with RNaseA (1 μl of a 10 ng/μl solution), precipitated with 3N sodium-acetate and ethanol, and resuspended in molecular grade water (G-Biosciences). Subsequently, all samples were re-evaluated by Nanodrop spectrophotometry and a small aliquot was analyzed for DNA quality by electrophoresis. Genomic DNA samples that were of sufficient quality and quantity (*A. florea*: 82 drones, *A. dorsata*: 71 drones and 24 workers) were sent to SNPSaurus (Eugene, OR) for genotyping by sequencing libraries using nextRAD markers (<http://snpsaurus.com/nextrad-genotyping/>; last accessed November 14, 2016), a modified, proprietary RAD-tag sequencing protocol (Baird et al. 2008).

The resulting 5,374 *A. florea* SNP markers were quality-filtered, excluding all markers with a minor allele frequency < 0.2 and > 50% missing genotypes. All heterozygous worker genotypes in *A. dorsata* were coded as missing data because these genotypes could not be unambiguously assigned a maternal allele. Of the original 6,193 *A. dorsata* SNP markers only markers with a minor allele count of > 19 in drones were retained. Linkage maps were generated *de novo* because neither species' genome is currently assembled at the chromosome level. Linkage analyses were performed "phase unknown" (Sirviö et al. 2006) because the grandparents were not available for phase determination. Data were processed and analyzed with "r/QTL" (Broman et al. 2003) in the "RStudio v. 0.98.501" environment. Data sets were rechecked for quality of markers and individuals and markers with identical genotype information (duplicate markers) were eliminated. Pairwise recombination frequencies were calculated with *est.rf* and linkage groups were formed based on pairwise linkage results with *formLinkageGroups* with a maximum recombination frequency (rf) of 0.15 and a minimum LOD score of 8 for *A. florea* and  $rf \leq 0.20$  and  $LOD \geq 6$  for *A. dorsata*. For *A. dorsata* this step was repeated without the 24 female offspring, because preliminary analyses indicated that the inclusion of these individuals inflated the genetic map. Resulting linkage groups were symmetrical and one half of these were dismissed to account for the duplication of

markers before mapping "phase unknown" (Rueppel et al. 2004). The marker order in the remaining linkage groups was determined using the *orderMarkers* command, except for the largest linkage groups where this was not possible due to memory limitations. These linkage groups were partitioned into smaller subgroups by using more stringent linkage criteria (maximum recombination frequency reduced by 5 cM and minimum LOD score threshold increased by two). Preliminary linkage maps for these subgroups and the smaller linkage groups were computed. Based on these maps all markers were eliminated that were within 0.01 cM of each other because we regarded their information as largely redundant and potentially inflating the map size. Subsequently, all linkage groups were searched for linkage gaps of > 20 cM and the flanking markers of these gaps and markers at the end of the linkage groups were investigated by manually inspecting their LOD scores with all other such "flanking" markers to identify the correct linkage patterns.

Resulting linkage groups were tested with two *ripple* commands to adjust local marker order where necessary. First, *ripple* was run with a window size of seven based on total crossover counts. The second *ripple* run for each chromosome was based on likelihood, restricting the window size to four. Suggested marker orders from both *ripple* runs were compared and the order selected that resulted in the shortest overall map length. In addition, markers near remaining linkage gaps (> 20 cM) were tested manually for linkage with all markers in the data set to search for better linkages or single markers located in the gaps. Chromosomes were tested with the *allchrSplits* command. Map expansion by individual markers was assessed with the *droponemarker* command and internal markers that expanded the map by > 6 cM were eliminated. Marker order was rechecked with *ripple* analyses before linkage map calculation, using Kosambi's mapping function (Kosambi 1943). In a second step, double crossover events around a single marker were eliminated by recoding the specific genotype as missing data because such double crossovers are considered improbable in honey bees due to local crossover interference (Solignac et al. 2007). The relatively low density of markers does not allow us to distinguish a true double crossover from a genotyping error or a local gene conversion event. Thus, the elimination of the questionable data was the most conservative approach.

Next, we tested maps for synteny with the *Apis mellifera* genome by blast searches of the marker sequences against the Amel\_4.5 reference assembly. We used NCBI's Megablast with default parameters, including repeats and low complexity regions. The chromosome identity and position of the best Blast match was recorded and compared to the marker order of the *A. florea* and *A. dorsata* linkage maps. Excluding markers that matched other chromosomes or unplaced genome fragments (Ungroups), inversions were counted when the linkage map order of  $\geq 3$  markers was inverted relative to the *A. mellifera* order on the homologous

chromosome. Misplaced single markers that were directly adjacent to the inversion points were disregarded but all other single markers that differed from *A. mellifera* in their relative position to other markers were counted as “non-syntenic single markers”. All *A. florea* and *A. dorsata* markers with significant matches in the homologous *A. mellifera* chromosomes were reordered according to their position in *A. mellifera*. The likelihood of this new, syntenous order was compared to the originally determined order (Meznar et al. 2010), and the size between alternative orders was compared, limiting any linkage gap between adjacent markers to 50 cM.

## Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

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