Comparative Linkage Mapping Suggests a High Recombination Rate in All Honeybees

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Abstract

Meiotic recombination is required for proper chromosome assortment, and accordingly, 1–2 chiasmata per chromosome are found in most species. However, observed recombination rates deviate in some cases from neutral expectations between and within genomes and may play an important role in adaptive evolution. One potentially important argument for an adaptive evolution of recombination rates is the exceptionally high genome-wide recombination rates of social Hymenoptera, in particular the Western honeybee, *Apis mellifera*. It has the highest metazoan recombination rate reported so far. Proximate or ultimate causes for this elevated recombination rate have not yet been resolved. In a comparative study, we investigated meiotic recombination in the red dwarf honeybee *Apis florea*. Microsatellite markers developed for *A. mellifera* were genotyped in a natural mapping population of *A. florea*. From these genotypes, we calculated local recombination rates, using the physical distances from *A. mellifera*. In addition to a few comparisons of intervals across the genome, we particularly focused on chromosomes 3 and 12. Confirming marker synteny, we found that recombination rates in *A. florea* are as high as or higher than those in *A. mellifera*. Our results are limited to select genomic regions but suggest that *A. florea* also exhibits an exceptionally high genome-wide recombination rate. This trait may thus occur genus wide. Although our study cannot identify a single explanation for the high rates of recombination in *Apis*, it favors hypotheses that apply to the entire genus. Furthermore, we conclude that the genome structure of the 2 species has been largely conserved, at least in the parts we investigated.

Key words: adaptive evolution, *Apis florea*, comparative genomics, genome structure, microsatellites, recombination, social insects

In most organisms, sexual reproduction is associated with meiotic chromosome pairing, mediated by chiasma formation (Kleckner 2006). Pairing of homologous chromosomes ensures proper segregation of these chromosomes into offspring cells, preventing major genetic defects due to aneuploidy (Kurahashi et al. 2009). At least one chiasma per chromosome arm is necessary, and over a wide range of organisms, the number of genome-wide crossover events correlates with chromosome number (Baker et al. 1976). Larger genomes are usually partitioned into larger, rather than more numerous, chromosomes. Therefore, a general negative correlation between genome size and recombination rate is observed (Lynch 2006). This provides a general, structural explanation for the variation of genome-wide recombination rates, which range over several orders of magnitude from 0.07 cM/Mb in the pine tree, *Pinus pinaster* (Chagne et al. 2002), to >50 cM/Mb in some protozoans (MacLeod et al. 2005).

However, adaptive evolution of meiotic recombination frequency in specific cases cannot be excluded based on these general observations. Chromosome number itself may evolve adaptively to modify genome-wide recombination rates (Sherman 1979; Imai et al. 1999), and different theoretical arguments suggest adaptive reasons to explain the evolution of meiotic recombination (Otto and Lenormand 2002; Agrawal 2009). On the one hand, recombination increases offspring diversity, resulting in potential short-term benefits (Bell 1985). On the other hand, it enhances adaptive evolution by reducing genetic linkage across the genome, thereby reducing genetic hitchhiking and facilitating multi-locus selection (Hill and Robertson 1966).

Several empirical studies indicate that genome-wide meiotic recombination rates can evolve adaptively. For example, the number of chiasmata in excess of the required minimum (=number of bivalents) in mammals correlates with generation time, which has been interpreted as an evidence of selection for elevated recombination in species with longer generation times to adapt to their (biotic) environment (Burt and Bell 1987). Recombination rate
responds to strong, short-term selection, for example, via
domestication (Ross-Ibarra 2004; Groenen et al. 2009). Several
studies have shown elevated local recombination rates near genes under strong selection, indicating that
intragenomic patterns of recombination rate can be explained by selection (Beye et al. 1999; Marais and
Charlesworth 2003; Presgraves 2005, but compare Kulathinal et al. 2008). Nevertheless, the most compelling evidence for
a genome-wide adaptive evolution of recombination rate seems to be found in social insects, such as ants and
honeybees, that have relatively high recombination rates (Beye et al. 2006; Wilfert et al. 2007), even after accounting
for high chromosome numbers and haplo-diploidy (Gadau et al. 2000).

To date, recombination rate has been studied in 4
social insect species, representing 2 independent origins of
advanced sociality. In general, the social species have higher
genomic recombination rates than the solitary, haplo-diploid
Hymenoptera (Beye et al. 2006; Wilfert et al. 2007). Two ant
species with relatively complex, large, and perennial colonies
show high genomic recombination rates of 6.7 cM/Mb for
Aenonymoxex echination, a leaf cutter ant, and of 14 cM/Mb for
Pogonomymox rugosus, a harvester ant (Servio et al. 2006).
The recombination rate of the primitively social bumblebee,
Bombus terrestris, has been computed as 4.4 cM/Mb (Wilfert
et al. 2006). This species lives in annual colonies of smaller
size and is single mated (Baer and Schmidt-Hempel 1999), in
contrast to the other social insects studied so far. The
related, but socially more complex Western honeybee, Apis
mellifera, displays the highest recombination rate of all
metazoans studied so far, with estimates ranging between 19
and 22 cM/Mb (Beye et al. 2006; Solignac et al. 2007). This
corresponds to a total linkage size of >4100 cM (Solignac
et al. 2007) and about 5 recombination events per
chromosome on average (Beye et al. 2006). Moreover,
recombination rate and chromosome size in A. mellifera are
not correlated (Beye et al. 2006).

Apis mellifera is a model organism for the study of the
evolution of sociality. It is the best studied of 10 recognized
honeybee species (Arias and Sheppard 2005), and many
details of its genome are known due to its completed
genome project (Honey Bee Genome Sequencing Consorti-
um [HGBSC] 2006). Apis mellifera has long been exploited
by humans through nectar gathering and more recently
domesticated beekeeping worldwide (Crane 1999). Today,
most A. mellifera colonies are probably managed by humans.
However, the remaining honeybee species live overwhel-
mingly in natural populations in Asia (Oldroyd and Wongsiri
2006). All honeybees share an advanced social lifestyle in
kin-structured colonies that comprise 1 reproductive queen,
female workers that are functionally sterile but perform all
other tasks, and haploid males (drones) that are produced
from unfertilized queen eggs (Winston 1987). The queens
mate multiply and genetic diversity within colonies has
demonstrated benefits for the efficiency of their labor
system (Oldroyd and Fewell 2007) and disease resistance
(Seeley and Tarpy 2007). However, the degree of multiple
mating ranges within the genus from an effective paternity
in A. florea of 7.9 to more than 44 in Apis dorsata, with
Apis mellifera intermediate (Tarpy et al. 2004). Honeybees also
differ in body size and colony size, nesting habits, the details
of their dance language, and the degree of female caste
divergence (Oldroyd and Wongsiri 2006). All 4 Apis species
studied so far, including A. mellifera and A. florea, share the
same karyotype of n = 16 chromosomes and the presence of
1 large, metacentric chromosome, indicating a conserva-
tion of important structural features of the genome
(Fahrenhorst 1977).

The main hypotheses that have been proposed to explain
the exceptionally high genomic recombination rate found in
A. mellifera are either based directly on sociality or invoke
more general causes. The general explanations include
haplo-diploidy, exposing recessive mutations (Hunt and
Page 1995), and strong selection, such as exerted by
domestication (Ross-Ibarra 2004) or rapid population
expansions (Zayed and Whitfield 2008). The effects of
haplo-diploidy and strong selection are not mutually
exclusive and can select for higher recombination rate
particularly in populations of small effective population size
(Otto and Barton 2001) and species with relatively long
generation times (Burt and Bell 1987). Honeybees display
a small population size (Baudry et al. 1998) and long
generation times (Solignac et al. 2007) compared with
solitary Hymenoptera. Selection for a high recombina-
tion rate could also be based on the benefits of multifili-
ous genetic diversity within colonies to increase societal disease
resistance (Seeley and Tarpy 2007), improve division of
labor (Oldroyd and Fewell 2007), or buffer colonies in
general against genetic stochasticity (Rueppell et al. 2008).
Recombination-induced reduction of individual genetic
variance has also been suggested to reduce within-colony
genetic conflict (Gadau et al. 2000). Alternatively, the
observed, high recombination rates might have evolved by
drift and not be adaptive: Experimentally induced increases
in recombination rate have shown direct detrimental effects
(Susiarjo et al. 2007) and high recombination might disrupt
evoled gene complexes (Pepper 2003).

More comparative data on genome-wide recombination
rates in social insects and their close relatives will be needed
to distinguish among these hypotheses for the evolution of
high metazoan recombination rates. An important question
in this regard is whether the very high recombination rate of
A. mellifera is unique to this species or occurs genus wide and
thus may be ancestral. To resolve this question, we
investigated recombination rates in the red dwarf honeybee
A. florea, which is basal in the genus Apis (Alexander 1991;
Arias and Sheppard 2005; Raffiudin and Crozier 2007) and
diverged from A. mellifera more than 8–10 Ma (Engel 2006).

Based on the A. mellifera value, we predicted a relatively
high recombination rate in A. florea compared with the
nearest out-group, B. terrestris, perhaps as a result of
the common biology and shared ancestry of all Apis species.
Compared with A. mellifera, we did not have a unique
prediction. Apis florea could display a lower recombination
rate because it has never been domesticated, is basal in the
genus, and has a smaller and more ephemeral nest
organization (Oldroyd and Wongsiri 2006). Its recombination rate could be higher because its open nests are more exposed to parasites and pathogens (Seeley et al. 1982) or to compensate for its lower effective mating frequency (Tarpy et al. 2004). Finally, the recombination rate could be similar between the species due to phylogenetic inertia or because they share a common social structure and division of labor system.

**Materials and Methods**

**Materials**

The mapping population of *A. florea* for this project was collected in Mahasararakham (Thailand). Individual drone pupae were directly extracted from a single comb of 1 large nest and stored in 95% ethanol in separate microcentrifuge tubes. After transport to University of North Carolina, Greensboro, the samples were transferred to −80 °C until DNA extraction. As confirmed by microsatellite genotyping later, these drones were the offspring of a single queen. Due to the haplo-diploidy of bees, the drones directly represent the recombined gametes of their mother and therefore constitute an ideal linkage mapping population, with results equivalent to single sperm typing in diploid organisms.

The thoraces of 120 drones were homogenized with disposable polypropylene pestles and DNA was extracted, exactly following the manufacturers recommendations (DNeasy Blood and Tissue Kit; Qiagen, CA). Resulting DNA concentrations were checked for purity and quantified by 2 duplicate spectrophotometric (N-1000 Nanodrop) measures, and the best 96 samples were diluted to a working concentration of 10 ng/µl in TE buffer for inclusion in our study.

**Genotyping**

We used microsatellite loci that had previously been characterized in *A. mellifera* (Solignac et al. 2004; Solignac et al. 2007). A tailed-primer approach (Schuelke 2000), labeling microsatellite products with a universal M13-primer coupled to IRD700 or IRD800, was employed for detection on a DNA Analyzer 4300 (Licor Inc., Lincoln, NB). All microsatellites were amplified using a touchdown polymerase chain reaction (PCR) protocol, gradually decreasing the annealing temperature from 68 to 48 °C (Schug et al. 2004). PCRs were performed in 15 µl as follows: 1 µl of 10 ng/µl of template DNA was added to a master mix that contained 1.5 µl of 2 mM dNTPs, 0.375 µl of 10 µM forward primer, 0.75 µl of 10 µM reverse primer, 0.75 µl of 1 µM of IRDye®-labeled M13 primer, 1.5 µl of 10× PCR buffer (20 mM MgCl₂, 100 mM Tris–HCl [pH 8.3], 500 mM KCl), 0.05 µl of 5 U/µl Taq DNA polymerase (from various sources), and 9.2 µl dH₂O.

We amplified 8 individuals for each locus to assess whether the primer binding sites were sufficiently conserved in *A. florea* to amplify a unique product. PCR amplification and approximate product size were confirmed by agarose gel electrophoresis (25-cm gels consisting of 0.5% Synergel [Diversified Biotech, MA]) and 0.3% agarose dissolved in 0.5× TBE), staining with EnVISION dye (Amresco, OH). Loci with 1 unique amplification product were then screened on the DNA Analyzer to exclude loci with nonspecific amplification and to test whether they were polymorphic in the 8 amplified individuals. Nondetection probability of an existing polymorphism was $P < 0.01$. Loci that resulted in 1 specific, polymorphic product were included in the analysis and subsequently genotyped in all 96 drones. All DNA Analyzer gels were prepared and run according to the manufacturer’s recommendations, using the Long Ranger polyacrylamide premixed gel solution (Lonza, ME), 25-cm gels, 1500 V, and 45 °C for 90–120 min. Scoring of the 2 alternative alleles for each locus was performed manually, visualizing the gel with the Gene Profiler 4.05 software. All loci were independently genotyped twice to calculate our experimental error rate, and inconsistent genotype scores were resolved by a third genotyping or excluded from the analysis. A few loci were excluded altogether because they could not be reliably amplified a second time.

Initially, we screened 460 microsatellite loci located throughout the *A. mellifera* genome and 146 primers successfully amplified. Of these, 18 were identified as polymorphic markers and amplified in the complete mapping population. Subsequently, we focused on microsatellites located on chromosomes 3 and 12 in the *A. mellifera* genome. Chromosome 3 was selected because it contains the sex determination locus, which is characterized by a very high recombination rate in *A. mellifera* (Beye et al. 1999), and chromosome 12 was selected as a comparative chromosome of similar physical size but lower recombination rate (Beye et al. 2006). We screened an additional 101 previously characterized *A. mellifera* microsatellite loci (Solignac et al. 2004, 2007) and designed 16 primer pairs for new additional loci from the *A. mellifera* genome sequence with Primer3 (Rozen and Skaletsky 2000) to improve the coverage of chromosome 3. This resulted in 10 polymorphic loci that were genotyped in all individuals. Similarly, we tested all additionally available 77 microsatellite primer pairs from chromosome 12, resulting in 12 polymorphic loci that were then amplified and scored in the entire mapping population. We evaluated the influence of PCR product length, microsatellite length, and repeat motif length on the probability of loci to amplify and to be polymorphic with separate binary regressions. We also analyzed the effect of chromosome number (#3, #12, all others) with a 2 × 3 contingency table.

**Analyses**

Pairwise distances for linked loci from the initial genome-wide primer screen were calculated using JoinMap 4.0 (http://www.joinmap.nl), a program designed to automatically identify genetic linkage groups (Stam 1993). We had to map phase-unknown because the grandparents were not available for the determination of the ancestral haplotypes.
marker data was entered in both linkage phases, resulting in 2 syntenic linkage maps. Linkage was determined using the default program settings of a logarithm of the odds (LOD) score >2.0, and map distances for linked markers were determined using the Kosambi function, justified by its fit to empirical data in Apis (Solignac et al. 2007). LOD scores for linkage between adjacent markers were used to calculate standard errors of recombination distances (Van Ooijen 2006).

The map distances between linked markers in A. florea were additionally calculated in MapMaker3.0 (Lincoln et al. 1993) using Kosambi's map function and then retransformed to recombinational rates. In A. mellifera, we used the recombinational distances and sample sizes (n ranged from 92 to 187) from the latest, >2000 marker linkage map (Solignac et al. 2007) to calculate the number of recombinants and nonrecombinants for each corresponding interval. The number of recombinant and nonrecombinant individuals was then compared between species using 2 × 2 contingency tables. P values were calculated using Fisher's Exact test, and Bonferroni correction was used to account for multiple tests. Assuming conservation of physical marker distances, we calculated descriptive statistics of recombination rates in both species by dividing recombinational distances by physical distances as determined in the A. mellifera genome (HBGSC 2006; Solignac et al. 2007).

We created separate data sets for chromosomes 3 and 12 to determine linkage order and distances between markers by manual linkage mapping, using MapMaker3.0 (Lincoln et al. 1993). We made no a priori assumptions of marker order and tried to map all markers, in both phases. First, all pairwise distances between markers were investigated with the "near" command. Based on these results, 1 linkage group per chromosome was established, excluding single markers that displayed no linkage to other markers. Linkage groups were analyzed for best marker order by evaluating all possible orders manually, using the "compare," "ripple," "map," "sequence," "drop marker," and "try" commands, according to the programs manual. Linkage distances were calculated using the Kosambi function and multipoint analyses to integrate the genotypic information from multiple adjacent markers. As described above, the recombination rate of each syntenous interval was compared between A. mellifera and A. florea with Fisher’s Exact tests.

In addition, we evaluated the differences between the 2 species over all intervals with a paired Wilcoxon signed-rank test to assess overall differences.

### Results

**Genome Wide**

Duplicate genotyping resulted in an overall estimate of experimental error rate of 0.8%, varying between loci with 0% to 1 locus with 1.6%. We did not find a significant effect of product length, microsatellite length, motif length, or chromosome location on the probability of a locus to amplify or to be polymorphic (Table 1).

#### Table 1: Tests revealed no influence of structural variables on the success of microsatellite markers between species

<table>
<thead>
<tr>
<th>Variable</th>
<th>Amplification</th>
<th>Polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product length (bp)</td>
<td>B = 0.00, P = 0.928</td>
<td>B = 0.003, P = 0.337</td>
</tr>
<tr>
<td>Microsatellite length (bp)</td>
<td>B = 0.004, P = 0.876</td>
<td>B = 0.04, P = 0.295</td>
</tr>
<tr>
<td>Repeat motif length (bp)</td>
<td>B = 0.192, P = 0.395</td>
<td>B = -0.01, P = 0.982</td>
</tr>
<tr>
<td>Chromosome ID</td>
<td>( \chi^2 = 0.55, P = 0.759 )</td>
<td>( \chi^2 = 5.0, P = 0.081 )</td>
</tr>
</tbody>
</table>

Our genome-wide screen amplified 5 informative microsatellite pairs that were linked in A. mellifera. Combined, these 5 regions spanned 83.7 cM, corresponding to 3.1 Mb, in A. mellifera. Four of these pairs were also significantly linked in A. florea, but 1 pair was not (K1168–B1001: 89.4 cM; LOD < 0.5). Although the distance between K1168 and B1001 in A. florea could be due to a significantly increased recombination rate, this interval was conservatively excluded from the analysis. After Bonferroni correction, 1 of the remaining intervals between linked loci (AC003–B1251) showed a significantly higher recombination rate in A. florea than in A. mellifera (Figure 1), with recombination occurring more than twice as often in A. florea. The significant interval is only 300 kb long on chromosome 2 (scaffold 2.36) and is characterized in both species by a high recombination rate.

In A. mellifera, it contains 7 putative genes, including 2 hypothetical gene predictions, 2 genes of unknown function, 1 protein tyrosine phosphatase, a protein serine/threonine
kinase involved in wnt signaling (gish-homolog) and a gene involved in homophilic cell adhesion (CG32387-homolog).

The nearest known single nucleotide polymorphism under selection in *A. mellifera* (Zayed and Whitfield 2008) is 1 Mb away.

Chromosomes 3 and 12

Two of the 10 scored loci of our markers for the third chromosome were excluded from the analysis because they failed to link to any other marker in the linkage group. The remaining 8 markers covered 243.3 cM, corresponding to 9.3 Mb. The null model for these markers (conserved marker order between *A. mellifera* and *A. florea*) and the most probable model calculated from *A. florea* genotypes alone were not significantly different (Figure 2). Therefore, the null model was used for further analyses.

For chromosome 12, 2 of the 12 scored loci had to be excluded because they lacked linkage to any other marker in the chromosome. The remaining 10 loci spanned 205.7 cM, corresponding to 7.8 Mb. The null model, statistically most likely model, and an intermediate, 1-inversion model were not significantly different from one another (Figure 3), and therefore, the null model was used for further analysis.

*Apis florea* displayed a higher overall recombinational size of chromosomes 3 and 12 than *A. mellifera* under all chromosome models (Figures 2 and 3). Across all data, the recombination rate was significantly correlated between the 2 species (Spearman’s \( R = 0.47 \), \( n = 19 \), \( P = 0.042 \)). In both chromosomes, *A. florea* showed elevated recombination rates and 1 short interval with particularly high values (Figures 4 and 5). However, the recombination rate was not significantly different between species in any specific interval on chromosome 3 or 12 (Bonferroni-corrected Fisher’s Exact tests > 0.05). Overall, 11 of all 19 genome-wide intervals were higher in *A. florea*, which was not significant (Wilcoxon signed-rank test: \( Z = 1.3 \), \( P = 0.184 \)).

**Discussion**

Our *A. florea* mapping population demonstrated recombination rates in the investigated genome regions that were at least as high as those in *A. mellifera*. Due to our limited data, we pursued a conservative analysis. If the identified trend were confirmed with a genome-wide data set, *A. florea* would display the highest recombination rate of all metazoans.

**Figure 2.** Most likely linkage models for chromosome 3. Eight of 153 markers from the third chromosome in *Apis mellifera* amplified, were polymorphic, and fell into 1 corresponding *Apis florea* linkage group. Marker orders, interval distances, and likelihood scores were determined by MapMaker3.0. The null model in *A. florea* represented the conserved *A. mellifera* marker order. In contrast, the most likely model postulated 1 inversion event between SV197 and K0320, but did not increase the model likelihood significantly. Thus, the null model was adopted.

**Figure 3.** Most likely linkage models for chromosome 12. Ten of 104 markers from the 12th chromosome in *Apis mellifera* amplified, were polymorphic, and fell into 1 corresponding *Apis florea* linkage group. Marker orders, interval distances, and likelihood scores were determined by MapMaker3.0. The null model represented the conserved *A. mellifera* marker order. The most likely model with 2 inversion events (see arrows) and an intermediate model with 1 inversion are also shown. Likelihood scores of the 3 models were not significantly different, favoring the null model.
study only covered 10.8% of the recombinational and 12.9% of the physical genome map. However, it seems plausible that our results are general to the extent that the high recombination rate in *A. mellifera* holds true equally for all chromosomes (Beye et al. 2006; Solignac et al. 2007). At minimum, our results suggest similarly high recombination rates in 2 *Apis* species that are separated by a minimum of 8–10 My of independent evolution (Engel 2006).

The discovered linkage patterns, together with the conserved karyotype (Fahrenhorst 1977) and physical genome size (Jordan and Brosemer 1974) in the genus *Apis*, suggest broad evolutionary conservation between the genomes of *A. mellifera* and *A. florea*. This supports our assumption that the physical genome structure and genome size do not differ significantly in comparison with the recombination rate between the 2 species, justifying our direct comparison of recombination rates (Dawson et al. 2007). The *A. mellifera* genome is less diverged from vertebrate genomes than other insect genomes, indicating a generally slow rate of molecular evolution (HBGSC 2006). It is devoid of most known transposable elements (HBGSC 2006) that generally make up a large proportion of the variation of physical genome size among closely related species (Gregory 2005). Furthermore, we did not detect any sign of recent polyploidization in *A. florea* because none of the microsatellite loci resulted in multiple amplification products.

We are also confident in other respects that our results are not an artifact of our experimental procedures. All drones in the mapping populations could be assigned to a single mother based on the microsatellite genotypes. Although we lack allele frequency estimates to calculate the exact probability for this assignment, typically only 6–10 microsatellite loci are required to achieve a highly accurate parental analysis in honeybees (Tarpy et al. 2004) and we genotyped our mapping population at 40 polymorphic loci. Our experimental genotyping error rate was low, and the exclusion of any questionable data points further lowered our error rate significantly. Our main conclusion of a recombination rate in *A. florea* that is similar to or higher than that of *A. mellifera* is independent of different chromosome models employed. To exclude the possibility that the high marker density or other procedural differences of the comparative data set (Solignac et al. 2007) were responsible for our conclusions, we validated our results with 2 of our own *A. mellifera* linkage maps that were generated with the same procedures and had a similar marker density (Ruepell O, unpublished data).

The alternative models of chromosomes 3 and 12, generated by MapMaker, were evaluated carefully, and we did not automatically accept the most likely or shortest chromosome models. In both cases, we retained the null model of synteny between *A. mellifera* and *A. florea* because the alternative models were not significantly more likely. In all cases, we decided that the probability advantage of rearranged marker orders was more than outweighed by the
additional requirement of a chromosomal mutation. In particular, the debatable chromosomal rearrangements fall into relatively large linkage intervals, which makes the linkage analysis susceptible to otherwise unlikely problems, such as double crossovers. Undetected double crossovers can lead to errors in determining the most likely marker order (Lincoln and Lander 1992). Several markers were excluded from the model because they were essentially unlinked to the remaining markers and led to map expansions (Hunt and Page 1995). The genotypes of most of these markers were also more problematic to verify independently through a second round of amplification.

The high recombination rate of *A. florea* adds another record of a social insect species with a very high recombination rate (Beye et al. 2006; Wilfert et al. 2007), strengthening the argument for the exceptional evolution of genomic recombinational rates in social insects. The basal position of *A. florea* in its genus (Arias and Sheppard 2005; Raffiudin and Crozier 2007) suggests that high recombination occurs throughout the genus. The consistent association between high recombination rate and advanced sociality in ants and bees has several potential explanations. Our study does not rule out any nonadaptive arguments. It is possible that a single, early evolutionary transition from low to high recombination before speciation within the *Apis* lineage, combined with subsequent phylogenetic inertia (Felsenstein 1985), accounts for our observations. Because the number of phylogenetically independent contrasts are limited, random genetic drift cannot be excluded. However, recombination rate is an evolutionarily labile character (Dumont et al. 2009) that is readily increased by selection (Ross-Ibarra 2004). Estimates of the size of the *A. mellifera* linkage map vary (Hunt and Page 1995; Rueppell et al. 2004, Ruepell et al. 2006, Solignac et al. 2007; Ruepell O, unpublished data), which indicates genetic variation for genomic recombination rate in this species. Nevertheless, our results for *A. florea* suggest that an excessive recombination rate has been evolutionarily maintained over >8 million years, which may be interpreted as tentative support for adaptive maintenance of a high recombination rate in the genus *Apis*.

The explanation of the high recombination rate in social insects might be related to an explanation of differences in genomic recombination rates within the genus *Apis*. However, our study has to remain inconclusive with respect to the explanation of differences within the genus *Apis* because we only included 2 species in our study and many relevant, biological parameters have not been compared between the 2. For example, we lack good studies of population genetic structure and pathogen pressure. It could be argued that the open-nesting *A. florea* is more exposed to parasites and pathogens and environmental fluctuations (Seeley et al. 1982), which may both select for higher genetic diversity within colonies, and thus higher recombination rate. However, temperate races of *A. mellifera* constantly reuse their comb over many generations and display sophisticated hygienic behavior (Rothenbuhler 1964), which could be interpreted as evidence for higher pathogen pressure in this species. More quantitative research in these regards is clearly needed.

*A. florea* displayed higher recombination rates than *A. mellifera* in 11 of 19 intervals tested. The possibly higher genomic recombination rate in *A. florea* may compensate for its lower effective mating frequency (Tarpy et al. 2004) to realize colony genetic diversity when high mating costs select against multiple mating (Hayworth et al. 2009). The invariably high recombination rate in both species could also suggest nonadaptive explanations, such as unknown structural features of the *Apis* genomes or phylogenetic inertia. In any case, our additional results obtained in *A. florea* eliminate explanations for high recombination rate that are specific for *A. mellifera*, such as domestication or rapid population expansion into temperate climates (Whitfield et al. 2006; Zayed and Whitfield 2008).

The only single interval that exhibited a significantly higher recombination rate in *A. florea* than in *A. mellifera* was located in one of the hotspots detected. It contained several genes but we were unable to identify a putative reason for its extreme recombination rate. Our spatial resolution did not reveal the predicted recombinalional hotspot surrounding the sex determination locus near marker *SLE* on chromosome 3 (Beye et al. 1999). Chromosome 3 has a higher overall recombination rate than chromosome 12. However, both chromosomes showed a similar heterogeneity in recombination rate and recombination was slightly reduced near the center of both chromosomes, although they are presumably acrocentric (HBGSC 2006). It is possible that the recombination rate of chromosome 3 is an overestimate due to gaps in the physical sequence assembly, while chromosome 12 was manually assembled to completion (Robertson et al. 2007).

In conclusion, our results from a limited portion of the genome indicate that *A. florea*, the red dwarf honeybee, exhibits a genomic recombination rate that is at least as high as the previous metazoan record of *A. mellifera*. The genomes of the 2 honeybee species seem to be similar overall, which should facilitate the imminent genome project of *A. florea*. We suggest that high recombination is a genus-wide phenomenon in the honeybee genus *Apis*, but the proximate and ultimate reasons for this remain to be resolved.

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