# High recombination frequency creates genotypic diversity in colonies of the leaf-cutting ant *Acromyrmex echinatior*

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## Abstract:

Honeybees are known to have genetically diverse colonies because queens mate with many males and the recombination rate is extremely high. Genetic diversity among social insect workers has been hypothesized to improve general performance of large and complex colonies, but this idea has not been tested in other social insects. Here, we present a linkage map and an estimate of the recombination rate for *Acromyrmex echinatior*, a leaf-cutting ant that resembles the honeybee in having multiple mating of queens and colonies of approximately the same size. A map of 145 AFLP markers in 22 linkage groups yielded a total recombinational size of 2076 cM and an inferred recombination rate of 161 kb $cM^{-1}$ (or 6.2 cM $Mb^{-1}$). This estimate is lower than in the honeybee but, as far as the mapping criteria can be compared, higher than in any other insect mapped so far. Earlier studies on *A. echinatior* have demonstrated that variation in division of labour and pathogen resistance has a genetic component and that genotypic diversity among workers may thus give colonies of this leaf-cutting ant a functional advantage. The present result is therefore consistent with the hypothesis that complex social life can select for an increased recombination rate through effects on genotypic diversity and colony performance.

**KEYWORDS**

*Acromyrmex echinatior* • division of labour • genetic linkage map • recombination frequency • social insects

## Article:

INTRODUCTION

Recombination rate is a genetically regulated trait that can be changed by selection without significant constraints (Otto & Lenormand, 2002), as natural populations of closely related species have been reported to differ in their crossover frequency (True *et al.*, 1996). Within species, recombination rates can also vary among individuals of the same sex (e.g. Broman *et al.*, 1998; Kong *et al.*, 2004), between the sexes (see Lynn *et al.*, 2004) and among different parts of the genome (Jensen-Seaman *et al.*, 2004). Artificial selection has led to increased recombination rates in domesticated animals (Burt & Bell, 1987) and Otto & Lenormand (2002) estimated that the recombination frequency (number of chiasmata) increased by 24.6% over a median of 50

generations of artificial selection for traits unrelated to recombination. Recombination directly affects the multilocus genotypic diversity among offspring and also influences population level genetic diversity through background selection or selective sweeps that depend on linkage disequilibria.

One hypothesis for the evolution of high recombination rates is the coevolutionary struggle between parasites and hosts. This hypothesis predicts elevated recombination rates in social insects whose large colonies offer potentially suitable platforms for the rapid spread of epidemics (Seger & Hamilton, 1988). In accordance with this expectation, the first genomic map of a social insect, the honey bee (*Apis mellifera*), showed that recombination frequency was the highest known for any eukaryote species (Hunt & Page, 1995). There are, however, alternative hypotheses put forward to explain the high recombination rate of the honeybee, and putatively of other highly social insects, which can be divided into three main classes:

1. Hypotheses referring to genomic features: most social insects are hymenopterans (ants, bees and waSPS) and have a male-haploid sex-determining system. High recombination rate in females could thus compensate for the lack of recombination in the haploid males. However, as the recombination rates in other hymenopteran insects are lower than in the honey bee, male haploidy has not been considered a sufficient explanation for the elevated recombination frequency in honey bees (Gadau *et al.*, 2000). The same applies to structural features of the genome, such as the number and size of the chromosomes.
2. Hypotheses depending on the variance-reducing effects of recombination: when many heterozygous loci are spread throughout the maternal genome, recombination reduces the variance of the number of shared alleles between two offspring that are identical by descent. In other words, the kinship (or relatedness) coefficient between the members of the colony measured over the complete genome is more uniform than without recombination (Barash *et al.*, 1978; Sherman, 1979; Templeton, 1979). This factor can potentially be important in the evolution of sociality.
3. Hypotheses referring to the diversity-increasing effects of recombination: recombination creates new allelic and genotypic combinations among progeny. This feature has been hypothesized to be beneficial in social insects when colonies function better with higher genotypic diversity at loci influencing polygenic traits. Such traits include pathogen resistance (Sherman *et al*., 1988; Seger & Hamilton, 1988), division of labour (Page & Smitchell, 1991) and tolerance to environmental variation (Pamilo *et al.*, 1994). The same selection pressures have been predicted to favour polyandry (Crozier & Page, 1985), but polyandry also increases genotypic diversity at single loci and simultaneously decreases relatedness among the worker individuals of the colony.

The only social insects for which marker maps have been made so far are the honey bee *A. mellifera* (Hunt & Page, 1995; Solignac *et al.*, 2004) and the bumble bee *Bombus terrestris* (Gadau *et al.*, 2001). These studies showed that the recombination rate in the highly eusocial honeybee is five times higher than in the primitively eusocial bumble bee, which has a recombination rate similar to solitary hymenopteran insects (Gadau *et al.*, 2001). However, it is premature to draw strong conclusions based on just two estimates from the same family (Apidae). We therefore decided to map the genome of a leaf-cutting ant *Acromyrmex echinatior*, which represents a different, phylogenetically independent lineage of highly eusocial insects.

This species resembles the honeybee in its general biology and life history traits. Colonies are large and typically consist of thousands of individuals that are usually headed by a single polyandrous queen, division of labour is strongly developed, queens are long-lived and the pathogen load is significant (Hughes *et al.*, 2002,2004; Hughes & Boomsma, 2004). *Acromyrmex* leaf-cutting ants also have physically polymorphic workers, with minor workers that mainly take care of tasks inside the nest and major workers that are specialized in foraging and guarding. The importance of polyandry and intracolonial genetic diversity was demonstrated by Hughes *et al.* (2003), who showed that different patrilines are unequally represented in the two worker castes and that genetic variation explained a significant amount of the phenotypic variance in the development and specialization of workers. The observed developmental specialization agreed with the stimulus-response-threshold model of Page & Mitchell (1991). Similarly, genotypic variation in honeybee workers is associated with traits such as foraging for pollen and nectar (e.g. Hunt *et al.*, 1995), defence (Breed & Rogers, 1991; Breed *et al.*, 2004) and regulating nest temperature (e.g. Jones *et al.*, 2004), which are all complex physiological traits that are likely to be polygenic. As the genotypic diversity within colonies is influenced by both polyandry and recombination, we have earlier predicted that the leaf-cutting ant *A. echinatior* should resemble the honeybee in having a high recombination frequency (Gadau *et al.*, 2001).

## MATERIALS AND METHODS

Social hymenopteran species are highly suitable for mapping purposes because of their haplodiploid sex-determination system and large family sizes. Haploid males represent meiotic products of their mother, so controlled crosses are not necessary for constructing linkage maps (Gadau *et al.*, 2001). Our mapping material consisted of 96 haploid males produced by a single queen under laboratory conditions. We could be certain of the origin of males because they are produced by arrhenotokous parthenogenesis and are not affected by the number of matings of the mother queen. In addition, workers refrain from laying unfertilized eggs in the presence of the queen (Dijkstra *et al.*, 2005), so all males were queen sons. The laboratory colony was originally dug up in April 1996 in Gamboa Panama as a young colony with a single queen and a single ball-shaped fungus garden with a radius of ca. 5 cm. The colony was kept in the laboratory where it grew to an appreciable size so that males were produced and could be collected in the summer of 1998. The genomic DNA was extracted according to the CTAB-based method (Hunt & Page, 1995).

### AFLP

The AFLP markers were used to genotype the males (see Vos *et al.*, 1995 for the method). These are normally dominant (presence/absence) markers, which normally lead to a loss of information in a diploid population, but which remained fully informative in our study because we used haploid males.

We used the AFLP Core Reagent kit (Invitrogen Life Technologies, USA) and the reactions were done according to the manufacturer's protocol with the following modifications: we used 15 ng $L^{-1}$ of genomic DNA in the initial restriction digest. Subsequently, all reactions were done at 50% of the recommended reaction volumes. We used 64 different combinations of selective EcoRI- and MseI-primers (Alameda, CA, USA) in the second PCR. These primers have earlier been used in mapping studies of the honey bee (Rueppell *et al.*, 2004) so that we had preliminary

information of their suitability. EcoRI primers were labelled with ($P^{33}$) ATP and used at a final concentration of 85 ng $mL^{-1}$. MseI-primers were used at 625 ng $mL^{-1}$. The steps of the preamplification program were 94 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min and preamplification was run for 24 cycles. The products were diluted 1 : 50 to serve as template in the selective amplification. The temperature profile for the selective amplification was 94 °C for 1 min, 65 °C for 1 min (increment of 0.7 °C $cycle^{-1}$), 72 °C for 1 min (cycled 12 times), 94 °C for 30 s, 56 °C for 1 min and 72 °C for 1 min (cycled 22 times). The products were run on 6% polyacrylamide gels (35 cm × 45 cm × 0.4 mm) with a radioactively labelled Sequamark size standard (Research Genetics, Hunstville, AL, USA) for 2.5 h at 1600 V. The gels were transferred onto filter paper, vacuum dried for 1–3 h and exposed against autoradiograph films (Biomax MR film, Kodak, NY, USA) for 2–7 days. All the polymorphic markers that could be screened reliably were typed and second screening was done for those markers. Ambiguous bands were either rerun or marked as missing data.

## LINKAGE ANALYSIS

mapmaker/exp version 3.0 (Lander *et al.*, 1987; Lincoln *et al.*, 1992) was used to construct the linkage map. The haploid male data were coded as an f2 backcross, because it gives the same estimates of cM distances when the data are coded to be haploid (present alleles were coded as H, absent alleles as A and missing information as -). Mapping was done 'phase unknown' because no information was available from the grandparents of the males. In addition to the observed phase the alternative phase was created for each marker. Thus all the markers had a first phase (original data) and an artificially created second phase where allelic information per marker was coded to be the opposite. The Kosambi mapping function (Kosambi, 1944) was used in all steps of map construction because it takes potential double recombination events into account, and thus reduces the map length. The overall mapping procedure followed Gadau *et al.* (2001):

1. The phase was assigned to every marker allele.
2. For each marker an artificial second marker was introduced in the data set with the same genotype but complementary phase.
3. In two-point analysis, the 'Group'-command (LOD 5.0, 24.6 cM) created a preliminary set of linkage groups for the doubled dataset. Because each marker was present twice, we found initially 28 identical pairs of linkage groups. This allowed us to fix the phase for every marker inside a given linkage group and to discard the alternative equally likely linkage group. For all following steps we used only one arbitrarily chosen linkage group of the two mirror linkage groups.
4. The most likely order of markers within each linkage group was determined by multipoint analysis using a combination of commands 'Order' (LOD 5.0, 24.6 cM), 'Compare' and 'Try'.
5. The three point command 'Ripple' (LOD 3.0, window size 3–5) was used to confirm the final marker order in the groups.
6. We used the 'Near' command with the criteria LOD 3.0 and 35 cM to add markers that had remained unlinked in step 3 but which could be linked when several other markers were already clustered and ordered in the linkage groups.
7. Finally, some of the existing groups were combined if the distal markers were within 35 cM distance and the statistical threshold score was at least 3.0. The 'Near'-command

was repeated for every new distal marker in the group until there were no new markers close enough to be attached in the groups with the set criteria (3.0 LOD, 35 cM). The order within these newly formed groups was again tested using the 'Order' and 'Ripple' commands (LOD 3.0, 35 cM). The final map was then run through an error detection process of Mapmaker 3.0, with the error detection option switched on with an a priori error probability of 1% to detect the presence of multiple cross-overs in linkage groups. As a final check, the raw data were inspected manually, and arranged according to the order of markers to allow visual identification of possible falsely scored individuals.

## FLOW CYTOMETRY

Single cell suspensions from worker ants of which the gaster was removed (and/or muscles of honey bees) were obtained by applying a modification of the method of Lamatsch *et al.* (2000) for fish fin clips. The tissue was chopped in 2.1% citric acid/0.5% Tween 20, and incubated at room temperature (RT) with gentle stirring for 10 min.

The cells were resuspended for the propidium iodide measurement directly in staining buffer containing 154 mm NaCl, 100 mm Tris–Cl pH 7.4, 1 mm CaCl2, 0.5 mm MgCl2, 0.2% BSA, 0.1% NP40, 25 U $ml^{-1}$ RNAse A and 50 $\mu g$ $ml^{-1}$ propidium iodide, and stained for approximately 1 h at 4 °C in the dark. Heparinized red blood cells from female chicken (*Gallus gallus*) were used as standard (crbc). Whole blood was diluted approximately 1 : 100 in minimal essential medium (MEM) containing a final concentration of 10% dimethyl sulfoxide, aliquoted and stored at −20 °C. After centrifugation the cells were treated like the sample cells. The concentration of the samples was approximately 2 × 105 cells $mL^{-1}$, the concentration of crbc slightly higher. Sample cells and reference cells were mixed at a ratio of 2 of 3 to 1 of 3 to obtain optimal results. Immediately before analysis the samples were filtered through a 50 mm nylon mesh to prevent obstruction of the flow chamber with chitin. The measurements were performed on a BD-L3R (Becton Dickinson, NJ, USA) equipped with a 488 nm argon-ion laser with 20 mW power output. At least 10 000 cells were measured per sample.

To determine the nuclear DNA content, the ratio of the channel numbers from the sample and chicken is multiplied by the known DNA content of crbc (2.5 pg $nucleus^{-1}$; Vinogradov, 1998). Our estimate for the honey bee exceeded by 10% the value given with the release (Amel_3.0) of the draft genome sequence so we transformed our estimates to correspond to the genome size of 238 Mb in the honey bee.

## RESULTS

Out of a total of 64 selective primer pairs, 59 produced 216 variable markers. We discarded markers as qualitatively questionable when their segregation was more biased than 1 : 3 or 3 : 1. This was done because the applicability of segregation distortion markers in constructing the linkage map is not unambigious (e.g. Kuittinen *et al.*, 2004) especially when using Mapmaker that cannot analyse data using distortion models (Hui Liu, 1998). We therefore discarded 38 markers from mapping and discuss them separately below. It has also been suggested that the segregation distortion loci should have little effect on the estimate of genome length (Hackett & Broadfoot, 2003).

Of the remaining 178 informative markers, 145 linked in groups with the final linkage criteria of LOD 3.0 and 35 cM (Kosambi). The mean frequency of the presence alleles for the markers in linkage groups was 0.44 (Fig. 1). The band sizes ranged from 68 to 600 bp and the detected polymorphisms were exclusively presence/absence polymorphisms. On average, we scored 3.4 markers per primer pair (216/64), or 2.8 markers (178/64) if only the markers used for the map are taken into account. This is in the range of AFLP-marker information from many other species (e.g. Parsons & Shaw, 2002; Zhong *et al.*, 2004). Marker names refer to the combination of selective EcoRI- and MseI-primers used and the last part indicates the inferred fragment size of the marker.

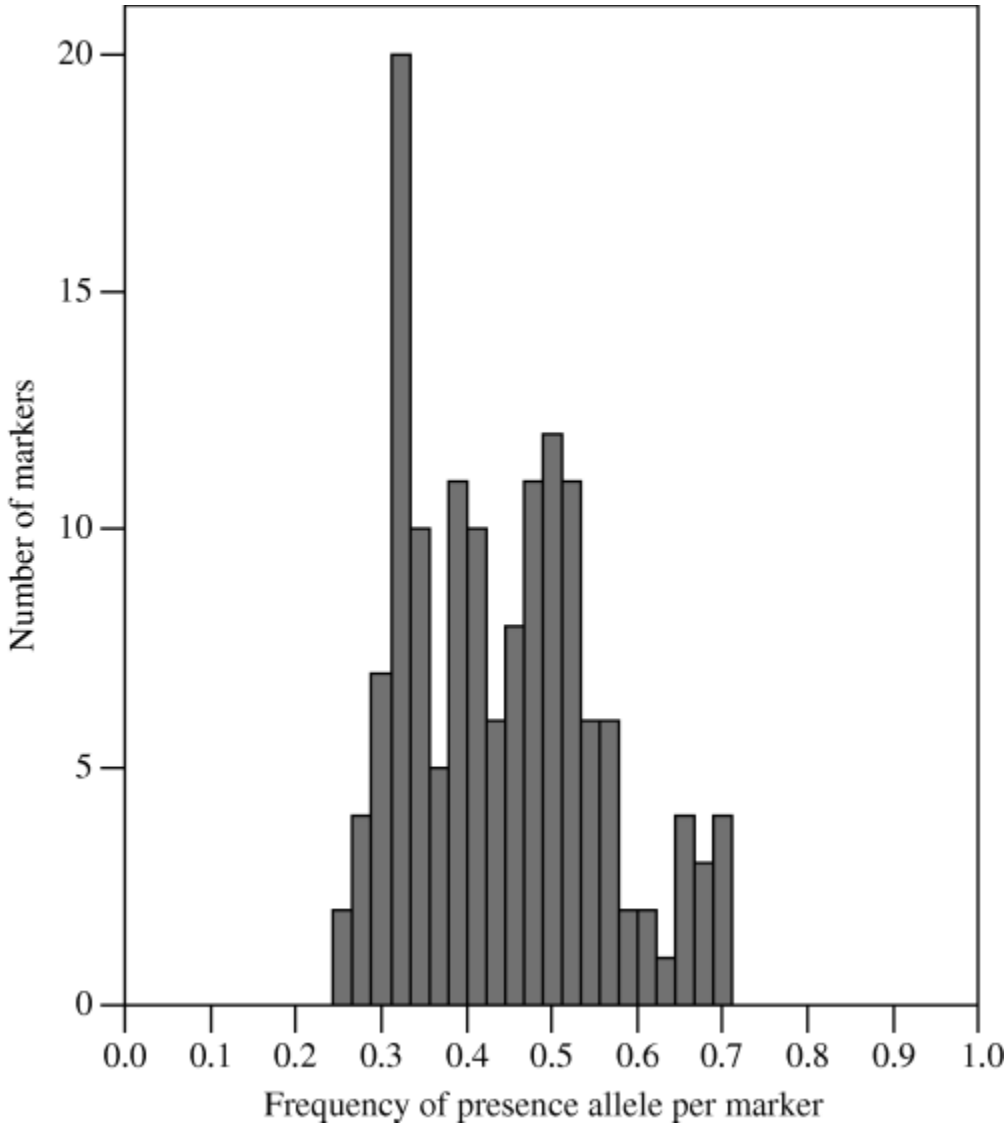


**Fig. 1** Distribution of presence alleles of linked markers. On *X*-axis are frequencies for each allele per marker and in *Y*-axis are counts of markers.

The frequency of scoring errors and processing mistakes was determined for 20 primer pairs that were selectively amplified, ran and scored twice. Scoring of the films was done independently.

The arithmetic mean and the SD for scoring and typing errors were 1.0 and 1.4 bands per primer pair, respectively.

The markers clustered in 22 linkage groups (Fig. 2) and 19 of them had at least three markers, while the remaining three groups consisted of only two marker loci. The number of linkage groups thus slightly exceeded the haploid chromosome number of *A. echinatior*, which is 18 (J. Gadau, unpublished data). In total, fifteen originally unlinked markers could be integrated into the existing linkage groups with the near-command (LOD 3.0 and 35 cM) and the final linkage groups that resulted from combining the preliminary groups were 1, 5–8 (Fig. 2).

The map spans 2033.8 cM. Since the number of linkage groups exceeds the haploid chromosome number, we added 35 cM (our criteria for joining the linkage groups) for each gap to cover the distance between the four additional linkage groups. This resulted in a final recombinational map size of 2174 cM. However, it is notable that this underestimates the real distance because we could not combine the existing marker groups with LOD values 3.0 and 35 cM. In addition, 33 markers remained unlinked.

To improve the quality of the linkage map we ran the error detection system of Mapmaker Version 3.0. Individuals that were marked as falsely scored by the error detection system were verified in the ordered raw data (see Materials and methods). Correcting these errors and rescoring the films reduced the map size by 4.5% (to 2076.3 cM), or 2.8% if only markers with error LOD greater than 2.0 were rescored (2112.6 cM). Using the conservative estimate of 2076.3 cM as the recombinational size of the *A. echinatior* genome, the intermarker distance in our map was about 14 cM (2076/145). The physical size of the *A. echinatior* genome was estimated to be 335 Mb so 1 cM equals approximately 161 kb (or 6.2 cM $Mb^{-1}$).

As explained above, we discarded 38 markers with strong segregation distortion. Including these markers showed that 20 of them remained unlinked whereas the remaining 18 markers joined the linkage group 1. As a result, the overall recombination rate was a little higher than reported here, so our estimate is conservative.

## DISCUSSION

### Comparison of recombination rates

Previous studies (Hunt & Page, 1995; Rueppell *et al.*, 2004; Solignac *et al.*, 2004;) found an extraordinarily high recombination rate in the honeybee, the highest recorded value in any eukaryote. A lower rate in a bumblebee led Gadau *et al.* (2001) to hypothesize that sociality alone cannot explain this high recombination rate, but that the genotypic diversity created by the high recombination rate is crucial for proper functioning of complex social insect colonies such as in the honeybee. Based on this hypothesis, they predicted (Gadau *et al.*, 2001) that the leafcutter ant *A. echinatior* should also have a high recombination rate, as its colonies resemble those of the honeybee in many respects. Our present results clearly confirm this prediction.

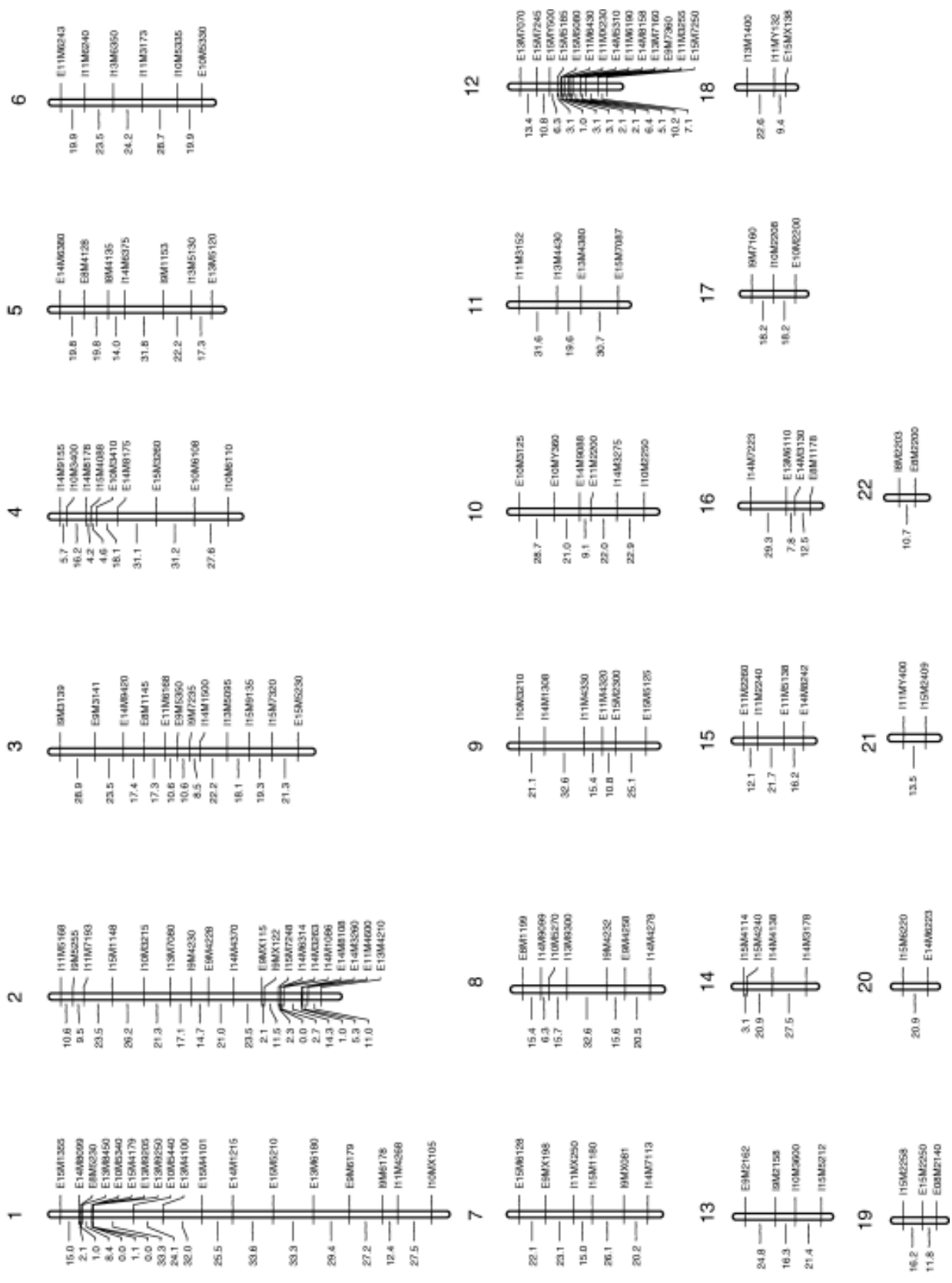

Fig. 2 Linkage groups for the ant *Acromyrmex echinatior*. On the left side are distances between markers in cM, and on the right side the names of the markers.

The linkage map of *A. echinatior* based on 145 AFLP marker loci is the first genomic map for an ant and the third for social insects in general. It shows elevated recombination rates compared to other insects and mammals (Table 1). For example, the recombination rate is four and five times higher, respectively, than in *Drosophila* and humans. Although our map is not saturated, it shows that insects with large and complex societies have a linkage map that is larger than an equivalent map of a primitively eusocial bumble bee. Furthermore, a separate project on another ant, *Pogonomyrmex rugosus*, has resulted in an estimated recombination rate of ~72 kb $cM^{-1}$ (255 Mb/3558 cM, A. Sirviö*et al.*, unpublished data) that is even higher than the one obtained for *A. echinatior* and thus adds further support to the overall pattern of increased recombination rates among highly eusocial insects. Our estimate of the physical genome size would be 10% bigger if compared against the chicken genome, but this does not alter any of the conclusions.

**Table 1 Mapping information for different species. The physical genome sizes of *Acromyrmex* and *Bombus* have been calibrated against *Apis mellifera*.**

| Species | Chromosome number (*n*) | Main method | Number of markers | Physical size of genome (Mb) | Linkage size of genome (cM) | Recombination rate (kb $cM^{-1}$) |
|---|---|---|---|---|---|---|
| Social insects | | | | | | |
| *Apis mellifera* (Hunt & Page, 1995, the physical size is from the genome release Amel_3.0) | 16 | RAPD | 395 | 238 | 3450 | 69 |
| *Acromyrmex echinatior* (this study) | 18 | AFLP | 145 | 335 | 2076 | 161 |
| *Bombus terrestris* (Gadau *et al.*, 2001) | 18 | RAPD | 79 | 367 | 1073 | 342 |
| Other insects | | | | | | |
| *Nasonia* (Gadau *et al.*, 1999) | 5 | RAPD | 91 | 312 | 765 | 410 |
| *Bracon hebetor* (Antolin *et al.*, 1996) | 10 | RAPD | 79 | 156 | 1156 | 134 |
| *Drosophila melanogaster* (Lindsley & Zimm 1992; Adams *et al.*, 2000) | 4 | Sequencing | | 180 | 280 | 643 |
| *Tribolium castaneum* (Zhong *et al.*, 2004) | 10 | AFLP | 269 | 200 | 573 | 350 |
| *Aedes aegypti* (Fulton *et al.*, 2001) | 3 | cDNA | 68 | 750–842 | 134–228 | 3693–5597 |
| *Laupala* (Parsons & Shaw, 2002) | 8 | AFLP | 231 | 1900 | 2330 | 815 |
| *Bracon* sp near | 10 | RAPD | 71 | 156 | 536 | 291 |

| | | | | | | |
|---|---|---|---|---|---|---|
| *hebetor* (Holloway *et al.*, 2000) | | | | | | |
| *Bombyx mori* (Yasukochi, 1998) | 28 | RAPD | 1018 | 495 | 2000 | 250 |
| Mammals | | | | | | |
| *Homo sapiens* (Kong *et al.*, 2002) | 23 | $\mu$sat | 5136 | 3020 | 3615 | 835 |
| *Mus musculus* (Dietrich *et al.*, 1996) | 20 | SSLP | 7377 | 2580 | 1361 | 1896 |
| *Rattus norvegicus* (Jacob *et al.*, 1995) | 21 | SSLP | 432 | 2720 | 1509 | 1803 |
| *Canis familiaris* (Neff *et al.*, 1999) | 40 | $\mu$sat | 276 | 2797 | 1510–2700 | 1852–1035 |

An exception to this general trend is provided by the parasitic wasp *Bracon hebetor* whose recombination rate is close to that estimated for the leafcutter ant (Table 1, Antolin *et al.*, 1996) and thus remarkably high for a solitary insect. However, that estimate was obtained by using different linkage criteria (LOD 3.0 and 50 cM) and the linkage size of the genome of another *Bracon* species (taxonomically near *hebetor*) is less than half of that reported for *B. hebetor* (Holloway *et al.*, 2000, Table 1). The comparison of map sizes can be problematic as different sample sizes, methods, marker numbers and software have been used. However, multiple alternative estimates for *Aedes aegypti* (Antolin *et al.*, 1996), *Tribolium castaneum* (e.g Beeman & Brown, 1999), *Bombyx mori* (Tan *et al.*, 2001), *A. mellifera* (Solignac *et al.*, 2004; Rueppell *et al.*, 2004), *Homo sapiens* (e.g Dib *et al.*, 1996) and *Canis familiaris* (e.g Lingaas *et al.*, 1997; Mellersh *et al.*, 2000) do not differ dramatically from those presented for these species in Table 1.

Another potentially interesting feature of the *Acromyrmex* genome is connected to the markers that showed clear segregation distortion. Percentage of distorted markers varies from 14.5% to 65% both in plants and animals (e.g. Voorrips *et al.*, 1997; Tan *et al.*, 2001; Zhong *et al.*, 2004). Thus, 17% in our study is in line with results obtained for other species. Biased segregation can occur mainly because of premating (meiotic drive) and postmating (viablility/fertility) selection. The occurrence of segregation distortion might also be due to methodological artefacts during the production of markers, small sample size (Hackett & Broadfoot, 2003) and breakdown of long-chain DNA during extraction from tissue samples. Solignac *et al.* (2004) detected several distorted markers in the honey bee genome that were scattered across different linkage groups, indicating that biased segregation could be due to deleterious alleles. Strong gender-specific segregation distortion has been detected in the ant *Formica aquilonia* in which the gametes produced by heterozygous queens segregate evenly in diploid daughters whereas all haploid sons receive only one type of maternal alleles (Pamilo, 1993). Such a strong segregation distortion was detected for three unlinked markers in *F. aquilonia*, whereas most distorted markers in *A. echinatior* clustered in a single linkage group. Clearly, the phenomenon needs further study.

### *Hypotheses for the high recombination rate*

The role of recombination in increasing genotypic diversity within social insect colonies can be compared to that of polyandry. Both honey bee and leafcutter ant queens are known to be highly polyandrous. Mating frequencies are up to 10 in *A. echinatior* (Sumner *et al.*, 2004) and up to 20 in the honey bee (Kraus *et al.*, 2005) and in both species division of labour among workers has been shown to include a genetic component. Workers belonging to different patrilines differ in their propensities to perform certain tasks and even in their morphology or to develop into different morphological castes (e.g. Hughes *et al.*, 2003; Jones *et al.*, 2004). Polyandry results in large allelic differences between workers, whereas recombination generates a large number of genotypes with novel and rare allelic combinations of the maternal genome. The haploid males are recombinational products or gametes produced by their mother (Laidlaw & Page, 1984). This slightly increases the diversity among the patrilines in cases where a female mates with several related males. In honey bees, multiple mating could also be relevant in ascertaining the production of heterozygous females in regard to the sex-determining locus (Beye *et al.*, 2003). Recombination and polyandry differ in the way they generate diversity. Recombination is effective in yielding variation in polygenic traits, whereas polyandry affects also single gene traits. Even though the basic consequences of recombination and polyandry are different, together they form an efficient way to generate genotypic diversity within social insect colonies.

Despite the importance of kin selection theory (Hamilton, 1964) for the origin and maintenance of social insect colonies, it has been suggested that selection acting on multiple levels and foremost on the group level would primarily promote the evolution of colonies (see Korb & Heinze, 2004 for a review). Accordingly, the hypotheses referring to the variance reducing and genotypic diversity increasing effects of recombination have been considered important in the evolution of large and socially complex insect colonies. We will discuss these hypotheses in light of our results.

The nestmate recognition system has been associated to the pattern of cuticular hydrocarbon compounds on the surface of individuals (see review by Lenoir *et al.*, 2001; Howard & Blomquist, 2005). Since multiple compounds are involved we can assume a complex, polygenic genetic basis for this pattern. Recombination could be relevant in such a system by preventing the formation of genetically distinct groups of offspring, thus avoiding the expression of nepotism among distinct groups of close kin within a colony. This could select for enhanced recombination rate in eusocial species but it is not evident why there would be a difference between small societies (bumble bee) and large and complex societies such as *A. echinatior*. The observed difference in the recombination rate between the bumble bee and species with large and complex societies therefore calls for other explanations. One of these explanations could be that enhanced recombination rates arose after the evolution of multiple queen mating, as the coexistence of full sister patrilines with other (half-sister) patrilines is particularly conducive to the expression of nepotistic tendencies when cuticular hydrocarbons would be informative about the degree of kinship (e.g. Boomsma *et al.*, 2003).

A high density of individuals within social insect nests increases inter-individual contacts and creates an ideal platform for the horizontal transmission of parasites (Schmid-Hempel, 1998). It has been suggested that well-adapted parasites can spread rapidly in genotypically less diverse colonies, resulting in an extensive pathogenic load. Thus, long-term competition between parasites and hosts could be driving the evolution of recombination (Seger & Hamilton, 1988).

Gadau *et al.* (2001) considered the parasite hypothesis as an unlikely explanation for the observed difference in the recombination rate in bees as the bumble bees suffer from pathogen loads comparable to those in the honeybee. However, it is not sure that the incidence of parasitism directly describes the genetic effects of the parasite load, as the effects can multiply over the years in feral and perennial social insect colonies. Hughes & Boomsma (2004) showed genetic variation in the disease resistance in *A. echinatior*, supporting the credibility of the parasite hypothesis. Further measures of the effects of parasites are needed also from ants in order to critically test the hypothesis.

Another aspect prominent in social insects that has been associated with the genetic diversity of colonies concerns the allocation of work. Models explaining division of labour have often targeted separate components of the differentiation process, including the stimuli for task performance, the interactions between workers, genetic, neural and hormonal factors and the effects of experience (Beshers & Fewell, 2001). The importance of genetic variation has been shown in bees, ants and waSPS (e.g. Robinson & Page, 1988; O'Donnell, 1996; Hunt *et al.*, 1998; Hughes *et al.*, 2003; Jones *et al.*, 2004). In the response threshold model for task allocation (Page & Mitchell, 1991) workers are assumed to have a genetically determined internal threshold determining how they react to stimuli and perform specific tasks. A significant proportion of the threshold variation is postulated to depend on underlying genotypic differences, creating a system that combines individual task specialization and colony task flexibility. When the level of a stimulus is low, that task is performed by specialist workers with the lowest threshold for that task. These workers respond sooner than others to the stimuli, and they also reduce the stimulus experienced by the other workers. However, when the stimuli are strong, additional workers with a high threshold are recruited to perform the task.

It has been shown that task specialization, partly based on morphological differentiation, is associated with genotypic variation in the *A. echinatior* colonies and that the benefits of diversity are likely to select for polyandry (Hughes *et al.*, 2003). This is in line with the response threshold hypothesis (Hughes *et al.*, 2003). It seems reasonable to assume that the same task specialization system can also select for increased recombination, assuming that the relevant behaviours are influenced by interacting loci relatively close to each other within a linkage group.

We should also point out some other features that may connect the recombination rate and sociality. The task specialization hypothesis discussed above relies on the benefits resulting from the increased genotypic diversity within a single cohort of individuals. Other types of selection on the recombination rate depend on long-term effects and on the product of the recombination rate ($R$) and the population size ($n$). As advanced eusocial insects, particularly those with a single queen per colony, have commonly relatively small effective population sizes in spite of the huge biomass consisting mainly of workers a high value of $R$ could be a compensation for a small $n$. Furthermore, highly eusocial species can have long generation times and the queens of *A. echinatior* species are known to live for more than 10 years (J. J. Boomsma, personal obs.). For long-term evolutionary effects, high values of $r$ could also compensate for such long generation times.

### *Future prospects*

It is difficult to discriminate between the alternative hypotheses that predict selection for increased genotypic diversity and high recombination rate, but our current results together with the earlier detected high recombination rate of the honeybee (Hunt & Page, 1995) strongly suggest that social complexity is a major factor selecting for an increased recombination frequency. To understand this phenomenon we need to exclude some of the above mentioned possibilities. One way to do that would be to determine whether recombination frequency is reduced in social parasites, which have semelparous reproduction and a very small number of workers or no workers at all. If genetic diversity of the worker force is driving the system, the social parasites should have lowered recombination frequencies. *A. echinatior* has a closely related social parasite *A. insinuator*, which has switched from polyandry to monandry, so that it would be interesting and telling to test whether this species has a reduced recombination frequency. The highly eusocial species studied so far, *Apis*, *Acromyrmex* and *Pogonomyrmex*, have also high levels of polyandry. Ants in the genus *Camponotus* are predominantly monandrous, yet they have comparable colony sizes and complex systems of division of labour similar to *Acromyrmex* or *Apis*. Determining the recombination rate for *Camponotus* would allow us to infer whether both polyandry and recombination have been selected for in order to increase the genotypic diversity within colonies, or whether they are uncoupled and differently influenced by selection. Unlike recombination, polyandry alters the levels of genetic relatedness and thus directly influences genetic conflicts among queens and workers (Crozier & Pamilo, 1996), for which reason the two traits may be under different selection pressures.

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