SOCIAL EVOLUTION

Genomic signatures of evolutionary transitions from solitary to group living

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The evolution of eusociality is one of the major transitions in evolution, but the underlying genomic changes are unknown. We compared the genomes of 10 bee species that vary in social complexity, representing multiple independent transitions in social evolution, and report three major findings. First, many important genes show evidence of neutral evolution as a consequence of relaxed selection with increasing social complexity. Second, there is no single road map to eusociality; independent evolutionary transitions in sociality have independent genetic underpinnings. Third, though clearly independent in detail, these transitions do have similar general features, including an increase in constrained protein evolution accompanied by increases in the potential for gene regulation and decreases in diversity and abundance of transposable elements. Eusociality may arise through different mechanisms each time, but would likely always involve an increase in the complexity of gene networks.

The evolution of eusociality involves changes in the unit of natural selection, from the individual to a group (*1*). Bees evolved eusociality multiple times and are extremely socially diverse (*2*) (Fig. 1), but all pollinate angiosperms, including many crops essential to the human diet (*3*). Simple eusociality may be facultative or obligate, and both forms are characterized by small colonies with a reproductive queen and one or more workers that, due to social and nutritional cues, forego reproduction to cooperatively care for their siblings (*2*). Further evolutionary elaborations have led to complex eusociality, "superorganisms" with colonies of several thousand individuals, sophisticated modes of communication, and morphological specializations for division of labor (*4*).

Theory predicts that the evolution of simple eusociality involves increased regulatory flexibility of ancestral gene networks to create specialized reproductive and nonreproductive individuals, and the evolution of complex eusociality requires genetic novelty to coordinate emergent properties of group dynamics (*5*). To test these predictions, we analyzed five de novo and five publicly available draft genome sequences of 10 bee species from three families, representing two independent origins of eusociality in Apidae and Halictidae and two independent elaborations of simple to complex eusociality in two apid tribes [Apini (honeybees) and Meliponini (stingless bees); Fig. 1]. The draft genomes were of comparable, high quality (supplementary materials).

We found that the transition from solitary to group life is associated with an increased capacity for gene regulation. We scanned the promoter regions of 5865 single-copy orthologs among the 10 species to calculate a motif score [representing the number and binding strength of experimentally characterized transcription factor binding sites (TFBSs)] for 188 *Drosophila melanogaster* TFs (*6*) with at least one ortholog in each of the 10 bees, and correlated motif score with social complexity, using phylogenetically independent contrasts (*7*). Of 2101 significantly correlated motif-gene pairs, 89% were positive and 11% negative, showing that TFs tend to have increased capacity to regulate genes in eusocial species of bees, relative to solitary species (Fig. 2A, supplementary materials).

Further evidence for increased capacity for gene regulation throughout social evolution is a positive ranked correlation between social complexity and the number of genes predicted to be methylated (*7*) (Spearman's rho = 0.76, $P = 0.01$; phylogenetically corrected Spearman's rho = 0.64, $P = 0.06$; Fig. 2B; bioinformatics predictions validated with bisulfite sequencing data for three invertebrate species; supplementary materials). DNA methylation affects gene expression in a variety of ways (*8*). Thus, this result suggests an expansion in regulatory capacity with increasingly sophisticated sociality.

The potential for increased regulatory capacity was further revealed at the protein-coding level. Increased social complexity also is associated with rapid evolution of genes involved in coordinating gene regulation. A Bayesian phylogenetic covariance analysis (*9*) of 5865 single-copy orthologs identified 162 genes with accelerated evolution in species with increased social complexity (*7*) (additional data table S3). These rapidly evolving genes were significantly enriched ($P < 0.05$) for Gene Ontology (GO) terms related to regulation of transcription, RNA splicing, ribosomal structure, and regulation of translation (supplementary text and tables S11 and S12). Similar results have been reported for bee and ant species (*10*–*13*); our findings reveal the underlying causes. Approximately two-thirds of these genes are under stronger directional selection in species with increasingly complex eusociality, but we also detected nonadaptive evolution. One-third of the rapidly evolving genes are under relaxed purifying selection in species with complex eusociality, possibly due to reduced effective population sizes (*14*).

We also found an additional 109 genes, significantly enriched ($P < 0.05$) for functions related to protein transport and neurogenesis, which evolve slower with increased social complexity (supplementary text, table S13, and additional data table S3). This includes orthologs of *derailed 2* and *frizzled*, which function as Wnt signaling receptors in *Drosophila* synaptogenesis (*15*), and *rigor mortis*, a nuclear receptor involved in hormone signaling (*16*). A similar pattern of reduced evolutionary rate has been described for genes expressed in human and honey bee brains, potentially due to increasing pleiotropic constraint in complex gene networks (*17*, *18*). Constrained protein evolution of neural and endocrine-related genes seems at odds with the evolution of complexity, but this constraint appears to be compensated for, or perhaps driven by, increased capacity for gene regulation.

We next investigated whether these molecular evolution patterns involve similar sets of genes and cis-regulatory elements among the early (facultative and obligate simple eusociality) and advanced (complex eusociality) stages of independent social transitions. We identified lineage-specific differences in coding sequences and promoter regions of 1526 "social genes" for which evolutionary rate (dN/dS) is faster or slower with increased social complexity in two independent origins and two independent elaborations of eusociality (*7*)

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(Fig. 1). Among these lineage-specific social genes, we found common patterns of cis-regulatory evolution: gains of TFBSs in the promoters of genes that evolve slower with increasing social complexity (Fig. 2C and supplementary text). This suggests that a shared feature of both independent origins and elaborations of eusociality is increasingly constrained protein evolution with increasing potential for novel gene expression patterns. The TFs responsible for this pattern were different for each social transition, even though our analysis was limited to highly conserved TFs (Table 1). Several function in neurogenesis or neural plasticity, or are prominent regulators of endocrine-mediated brain gene expression in honeybees (*19*, *20*).

We found further lineage-specific differences among the rapidly evolving "social genes" themselves. Genes undergoing accelerated evolution at the origins of eusociality were significantly enriched for GO terms related to signal transduction in both Apidae and Halictidae, but they shared only six genes (6 out of 354 and 167 genes, respectively; hypergeometric test, $P = 0.82$; Fig. 2D and additional data tables S5 and S6). Rapid evolution of signal transduction pathways may be a necessary step in all origins of eusociality to mediate intracellular responses to novel social and environmental stimuli (*10*), but selection appears to have targeted different parts of these pathways in each independent transition. Caste-specific expression and other analyses of these genes are needed to determine their function in eusociality.

Genes showing signatures of rapid evolution with the elaborations of complex eusociality were also highly disparate between honeybees and stingless bees, with only 43 shared genes and no shared enriched GO terms (43 out of 625 and 512 genes, respectively; hypergeometric test, $P = 0.70$; Fig. 2D and additional data tables S5 and S6). In addition, only 2 out of 5865 single-copy orthologs showed a signature of convergent evolution by fitting a dendrogram based on social complexity significantly better than the accepted molecular phylogeny (*7*) (supplementary text and fig. S21). Similarly, families of major royal jelly protein genes, sex-determining genes, odorant receptors, and genes involved in lipid metabolism expanded in some, but not all, lineages of complex eusocial bees (*7*) (Table 2 and supplementary text). These results suggest that gene family expansion is associated with complex eusociality as predicted (*5*), but involves different genes in each case. Despite striking convergence of social traits among the superorganisms (*4*), the final stages of transformation to this level of biological organization do not necessarily involve common molecular pathways.

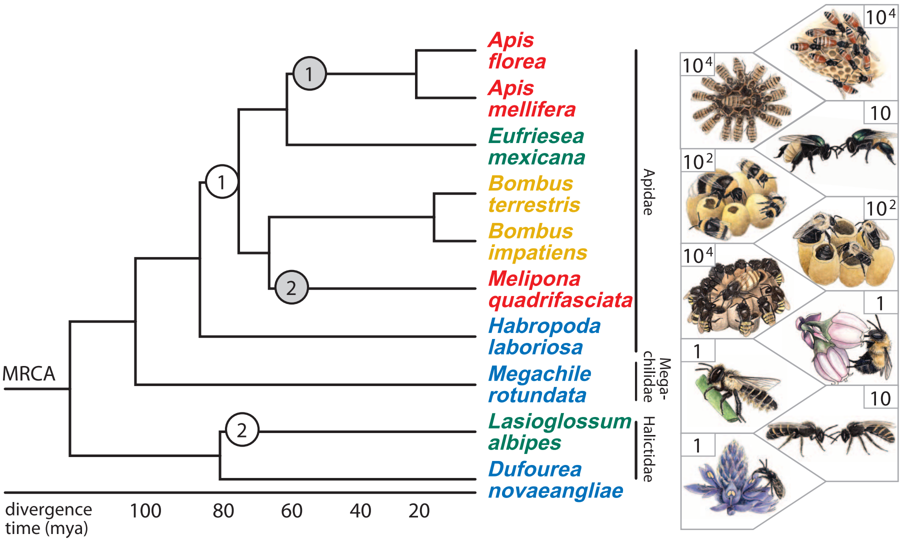

Fig. 1. Phylogeny and divergence times (*28*) of bees selected for genome analysis. We analyzed two independent origins of simple eusociality from a solitary ancestor, one each in Apidae (white circle 1) and Halictidae (white circle 2), and two independent elaborations of complex eusociality in honeybees (gray circle 1) and stingless bees (gray circle 2). Most bees mate once, but honeybees mate with multiple males. All bees eat pollen and nectar from flowering plants. Species names are colored according to degree of social complexity: blue: ancestrally solitary; green: facultative simple eusociality; orange: obligate simple eusociality; red: obligate complex eusociality. The social biology of *E. mexicana* is unknown, but is representative of the facultative simple eusocial life history (*29*). Numbers in each box are approximate colony size on a log scale. MRCA, most recent common ancestor; mya, millions of years ago.

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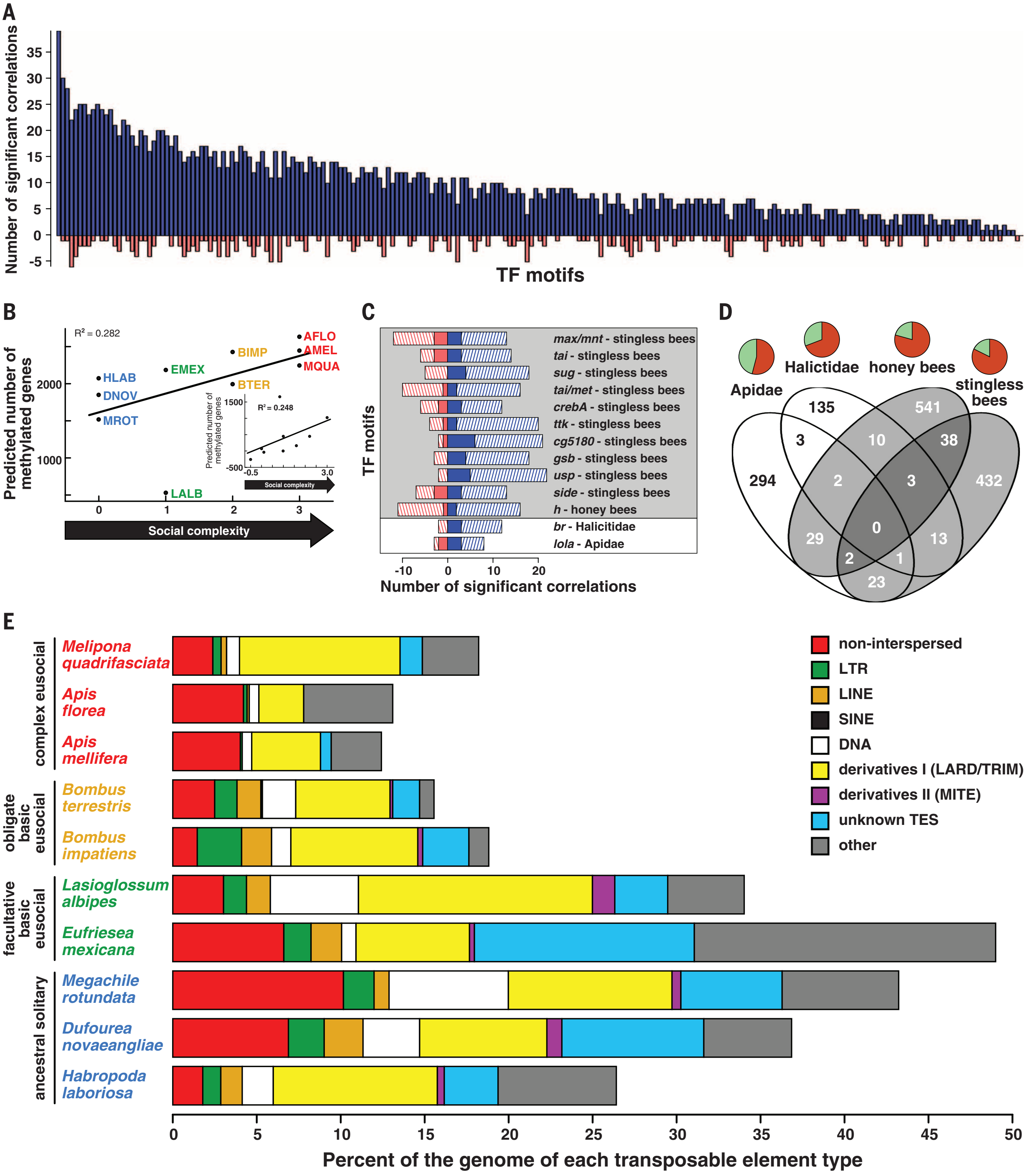

Fig. 2. Genomic signatures of evolutionary transitions from solitary to group life. (**A**) Increasing social complexity is associated with increasing presence of cis-regulatory TFBSs in promoter regions. Each bar represents a TFBS for which presence correlates significantly with social complexity (blue: positive; red: negative). (**B**) Relationship between predicted number of methylated genes and social complexity before and after (inset) phylogenetic correction (see text for statistics). (**C**) TFBS motifs showing a relationship between social complexity and evolutionary rate of coding and noncoding sequences in different lineages. Bar length indicates the number of significant correlations (blue: positive; red: negative) between each motif score and social complexity (from Table 1) among genes evolving faster (solid) or slower (hatched) in lineages with different levels of social complexity [from (D)]. Background shading follows circle shading in Fig. 1. (**D**) Number of genes for which evolutionary rate is faster or slower in lineages with higher compared to lower social complexity. Pie charts represent the proportion of genes evolving slower (light green) or faster (dark orange) with increased social complexity. Venn diagram shading follows circle shading in Fig. 1. (**E**) Complex eusocial species have a reduced proportion of repetitive DNA compared to other bees (see text for statistics). LTR, long terminal repeat; LINE, long interspersed element; SINE, short interspersed element; DNA, DNA transposon; LARD, large retrotransposon derivative; TRIM, terminal repeat retrotransposon in miniature; MITE, miniature inverted-repeat transposable element; TES, transposable elements.

The major transitions in evolution involve a reduction in conflict as the level of natural selection rises from the individual to the group (*1*). Extending this to intragenomic conflict may explain our finding of decreased diversity and abundance of transposable elements (TEs) with increasing social complexity (*7*) (regression after phylogenetic correction, $F = 8.99$, adjusted $R^2 = 0.47$, $P = 0.017$; Fig. 2E, figs. S42 to S44, and supplementary text). This may be a consequence of increased recombination rates among highly eusocial insects (*21*, *22*) or because key features of complex eusociality lead to decreased exposure to parasites and pathogens that horizontally transmit TEs (*4*, *23*). Eusociality in bees may thus provide natural immunity against certain types of intragenomic conflict.

Our results and those in (*10*–*13*) support the prediction that changes in gene regulation are key features of evolutionary transitions in biological organization (*5*). Our results further reveal the convergent adaptive and nonadaptive evolutionary processes common to both the early and advanced stages of multiple independent transitions from solitary to group living. It is now clear that there are lineage-specific genetic changes associated with independent origins of eusociality in bees, and independent elaborations of eusociality in both bees and ants. This includes different sets of genes showing caste-biased expression across species (*24*–*26*) and, as we have shown, evolutionary modifications of TEs, gene methylation, and cis-regulatory patterns associated with the suite of life-history traits that define eusociality. This suggests that if it were possible to "replay life's tape" (*27*), eusociality may arise through different mechanisms each time, but would likely always involve an increase in the complexity of gene networks.

Table 1. Transcription factors (TFs) and corresponding motifs associated with origins and elaborations of eusociality in bees. [Motif names: Fly Factor Survey (6); supplementary text.]

Motif	*D. melanogaster* TFs	Hypergeometric test *P*-value
	Solitary to simple eusociality–Apidae	
lola_PQ_SOLEXA	*Lola*	0.0047
	Solitary to simple eusociality–Halictidae	
br_PL_SOLEXA_5	*Br*	0.0016
	Simple eusociality to complex eusociality–honeybees	
h_SOLEXA_5	*dpn,h*	0.0027
	Simple eusociality to complex eusociality–stingless bees	
Side_SOLEXA_5	*E_spl, HLHm3, HLHm5, HLHm7, HLHmbeta, HLHmdelta, HLHmgamma, Side*	0.0008
usp_SOLEXA	*EcR,svp,usp*	0.0013
CrebA_SOLEXA	*CrebA*	0.0040
CG5180_SOLEXA	CG5180	0.0044
tai_Met_SOLEXA_5	*Mio_bigmax,tai_Met*	0.0045
ttk_PA_SOLEXA_5	*Ttk*	0.0078
gsb_SOLEXA	*gsb,Poxn,prd*	0.0083
tai_SOLEXA_5	*Tai*	0.0100

Table 2. Relative size of select gene families as related to social complexity in bees.

Family	Function	Eusocial bees compared to solitary bees
	Differences among bees	
Major royal jelly	Brood feeding	Expanded only in *Apis*
Sex determination pathway genes	Sex-specific development	Expanded in some eusocial lineages
Odorant receptors	Olfaction	Expanded in complex eusocial lineages
Lipid metabolism genes	Metabolic processing of lipids	Expanded in complex eusocial lineages
	Similarities across bees	
Biogenic amines receptors, neuropeptides, GPCRs*	Neural plasticity	Similar
Insulin-signaling and ecdysone pathway genes	Insect development, caste determination in honeybees, behavioral plasticity as adults	Similar
Immunity	Infectious disease protection	Similar
Cytochrome P450 monooxygenase genes	Detoxification	Similar

*GPCRs, G protein–coupled receptors.

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SUPPLEMENTARY MATERIALS

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Author Contributions

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HUMAN OOCYTES

Error-prone chromosome-mediated spindle assembly favors chromosome segregation defects in human oocytes

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Aneuploidy in human eggs is the leading cause of pregnancy loss and several genetic disorders such as Down syndrome. Most aneuploidy results from chromosome segregation errors during the meiotic divisions of an oocyte, the egg's progenitor cell. The basis for particularly error-prone chromosome segregation in human oocytes is not known. We analyzed meiosis in more than 100 live human oocytes and identified an error-prone chromosome-mediated spindle assembly mechanism as a major contributor to chromosome segregation defects. Human oocytes assembled a meiotic spindle independently of either centrosomes or other microtubule organizing centers. Instead, spindle assembly was mediated by chromosomes and the small guanosine triphosphatase Ran in a process requiring ~16 hours. This unusually long spindle assembly period was marked by intrinsic spindle instability and abnormal kinetochore-microtubule attachments, which favor chromosome segregation errors and provide a possible explanation for high rates of aneuploidy in human eggs.

Meiosis in human oocytes is more prone to chromosome segregation errors than mitosis (*1*, *2*), meiosis during spermatogenesis (*3*, *4*), and female meiosis in other organisms (*3*, *5*). Despite its importance for fertility and human development, meiosis in human eggs has hardly been studied. Human oocytes are only available in small numbers, warranting single-cell assays capable of extracting maximal information. Although high-resolution live-cell microscopy is an ideal method, oocyte development in the ovary poses challenges to direct imaging. We therefore established an experimental system (*6*) for ex vivo high-resolution fluorescence microscopy of human oocytes freshly harvested from women undergoing gonadotropin-stimulated in vitro fertilization cycles. To establish the major stages of meiosis in this system, we simultaneously monitored microtubules and chromosomes for ~24 to 48 hours (Fig. 1 and movie S1). Similar to the situation in situ (*7*), human oocytes matured into fertilizable eggs over this time course, as judged by the formation of a polar body. The morphologically identifiable stages (Fig. 1A) at characteristic times after nuclear envelope breakdown [(NEBD), set to 0 hours] provided a time-resolved framework for human oocyte meiosis (Fig. 1B). This reference timeline post-NEBD is used throughout this paper.

Before NEBD, chromosomes were highly condensed and clustered around the nucleolus. Instead of rapidly nucleating microtubules upon NEBD, human oocytes first formed a chromosome aggregate that was largely devoid of microtubules (Fig. 1A; movie S1; and fig. S1, A and B). Microtubules were first observed at ~5 hours, when they started to form a small aster within the chromosome aggregate. As the microtubule aster grew, the chromosomes became individualized and oriented on the surface of the aster with their kinetochores facing inwards. The microtubule aster then extended into an early bipolar spindle that carried the chromosomes on its surface (Fig. 1A; movie S1; and fig. S1, C to E). The chromosomes then entered the spindle but remained distributed throughout the entire spindle volume. Chromosomes first congressed in the spindle center at ~13 hours but continued to oscillate around the spindle equator. Stable chromosome alignment was typically only achieved close to anaphase onset (Fig. 1, A and B, and movie S1). Unexpectedly, the spindle volume increased over the entire course of meiosis, up until anaphase onset (Fig. 1, C and D). The barrel-shaped spindle formed in this process consisted of loosely clustered bundles of microtubules and lacked astral microtubules (movie S2 and fig. S2). At ~17 hours, the oocytes progressed into anaphase and eliminated half of the homologous chromosomes in a polar body. Nearly a day after NEBD, the oocytes had formed a bipolar metaphase II spindle and matured into a fertilizable egg. The stages and timing of meiosis were highly reproducible among oocytes (Fig. 1, A and B) and could also be observed in fixed oocytes (fig. S1, A to I). Importantly, 79.0% of imaged human oocytes extruded a polar body. This indicates that the imaging assays, as well as the methods by which the oocytes were obtained and processed, did not have a prominent effect on meiotic progression.

The surprisingly slow and gradual build-up of the spindle over 16 hours (Fig. 1, C and D) is in stark contrast to mitosis, where spindle assembly takes only ~30 min (*8*), or meiosis in mouse oocytes, where it takes 3 to 5 hours (*9*–*11*). During mitosis, two centrosomes ensure the rapid assembly of a spindle. In oocytes of many species, centrosomes are absent but functionally replaced by microtubule organizing centers (MTOCs) that lack centrioles (*9*, *12*). Human oocytes also lack centrosomes (*13*–*15*), but whether acentriolar MTOCs participate in spindle assembly is unclear (*16*–*19*). We consistently detected pericentrin- and γ-tubulin–positive MTOCs at the spindle poles of mitotic cells and metaphase I and II (MI and MII) mouse oocytes, but never at MI or MII spindles in human oocytes (Fig. 2, A and B, and fig. S3). Thus, our data suggest that meiotic spindles in human oocytes lack detectable MTOCs.

In *Xenopus* egg extracts, chromosomes can serve as sites of microtubule nucleation if centrosomes are absent (*20*). The human oocytes we imaged also initiated microtubule nucleation in the region of the chromosome aggregate (78 of 78 live human oocytes). High-resolution imaging of fixed human oocytes confirmed that microtubules were first nucleated on chromosomes, emanating primarily from kinetochores (Fig. 2C, movie S3, and fig. S4). MTOC-nucleated cytoplasmic asters, such as those seen in chromosomal proximity upon NEBD in mouse oocytes (*9*), could not be detected. Thus, chromosomes, not MTOCs, serve as major sites of microtubule nucleation in human oocytes.

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Genomic signatures of evolutionary transitions from solitary to group living

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