



# HHS Public Access

Author manuscript

*Curr Biol.* Author manuscript; available in PMC 2015 February 17.

Published in final edited form as:

*Curr Biol.* 2014 February 17; 24(4): 451–458. doi:10.1016/j.cub.2014.01.018.

## The genome of the clonal raider ant *Cerapachys biroi*

Peter R. Oxley<sup>1,\*†</sup>, Lu Ji<sup>2,†</sup>, Ingrid Fetter-Pruneda<sup>1</sup>, Sean K. McKenzie<sup>1</sup>, Cai Li<sup>2</sup>, Haofu Hu<sup>2</sup>, Guojie Zhang<sup>2,3,§</sup>, and Daniel J.C. Kronauer<sup>1,§</sup>

<sup>1</sup>Laboratory of Insect Social Evolution, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

<sup>2</sup>China National Genebank, BGI-Shenzhen, Shenzhen 518083, China

<sup>3</sup>Centre for Social Evolution, Department of Biology, University of Copenhagen, 2100 Copenhagen, Denmark

### Summary

Social insects are important models for social evolution and behavior. However, in many species experimental control over important factors that regulate division of labor, such as genotype and age, is limited [1, 2]. Furthermore, most species have fixed queen and worker castes, making it difficult to establish causality between the molecular mechanisms that underlie reproductive division of labor, the hallmark of insect societies [3]. Here we present the genome of the queenless clonal raider ant *Cerapachys biroi*, a powerful new study system that does not suffer from these constraints. Using cytology and RAD-Seq, we show that *C. biroi* reproduces via automixis with central fusion and that heterozygosity is lost extremely slowly. As a consequence, nestmates are almost clonally related ( $r=0.996$ ). Workers in *C. biroi* colonies synchronously alternate between reproduction and brood care, and young workers eclose in synchronized cohorts. We show that genes associated with division of labor in other social insects are conserved in *C. biroi* and dynamically regulated during the colony cycle. With unparalleled experimental control over an individual's genotype and age, and the ability to induce reproduction and brood care [4, 5], *C. biroi* has great potential to illuminate the molecular regulation of division of labor.

### Results and Discussion

To establish the clonal raider ant *Cerapachys biroi* (Figure 1) as a model eusocial organism, we sequenced and assembled its 214 megabase (Mb) draft genome using 33 gigabases (Gb)

© 2014 Elsevier Inc. All rights reserved.

\*corresponding author. [poxley@rockefeller.edu](mailto:poxley@rockefeller.edu).

†These authors contributed equally to the work

§These authors contributed equally to the work

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

#### Author Summary:

How division of labor in social insects evolved and is regulated are major outstanding questions. Oxley et al. present the genome of the clonal raider ant *Cerapachys biroi*. This new model system, due to its unusual biology, has great potential to provide novel insights into these fundamental topics.

of Illumina reads (119.2× coverage) and 526 Mb of Roche 454 reads (2.5× coverage). Transcriptome data (11.24 Gb) from all developmental stages and different behavioral states were generated to aid genome annotation. This assembly, and the annotation of 17,263 protein coding genes, provides the first dorylomorph genome and the first genome of an asexual ant.

The *C. biroi* genome assembly is comparable in quality and completeness to the other sequenced ant genomes (Table 1; Supplemental Experimental Procedures: Sequencing and Assembly). *Cerapachys biroi* has the smallest and most compact ant genome, with genes (including introns) accounting for 36.7% of the assembled genome. Like the other sequenced ant genomes, *C. biroi* has a complete set of DNA methylation enzymes (Supplemental Experimental Procedures: DNA Methylation and Histone Modification). However, the unimodal CpG<sub>(obs/exp)</sub> distribution (Figure S1) provides no clear evidence for germline methylation of the genome [12]. *Cerapachys biroi* has the largest set of odorant receptors of any sequenced insect (Tables 1 and S1; Supplemental Experimental Procedures: Chemoreception), suggesting that the species relies heavily on chemical cues. This is consistent with its subterranean lifestyle and lack of developed eyes. *Cerapachys biroi* also has several gene expansions in the UDP glycosyltransferase and cytochrome P450 gene superfamilies (Table 1; Figures S2 and S3; Supplemental Experimental Procedures: UDP Glycosyltransferases and Cytochrome P450 Genes), which are involved in a broad array of metabolic functions.

### Clonal Reproduction

Colonies of *C. biroi* contain no queens and consist entirely of totipotent workers, all of which reproduce asexually [10, 11]. Most forms of asexual reproduction result in genomic loss of heterozygosity (LOH) each generation. This can incur a high cost: In many eusocial Hymenoptera, development of the female sex is believed to be determined by heterozygosity at one or more sex determining loci, while haploid eggs develop into males [14]. LOH at the sex-determining loci therefore leads to the production of infertile diploid males [15]. Because less than one in 10,000 diploid offspring in *C. biroi* are male (See [8] and Experimental Procedures), we expected that the species used either a mode of reproduction with no or very little LOH (e.g. premeiotic doubling, ameiotic reproduction, or automixis with central fusion and low recombination rates), or it had evolved an alternate mechanism for sex determination.

To determine the mode of asexual reproduction in *C. biroi*, we stained ovaries and eggs to observe chromosomes during oogenesis and embryogenesis (Figures 2 and S4). Eggs undergo regular meiotic division, which initiates within the first half hour post partum (Figures 2A and 2B). Following the second meiotic division, two haploid nuclei, one from each of the reductional division daughter nuclei, fuse (Figure 2C) and migrate towards the center of the egg. This process occurs entirely within the first hour post partum. Following fusion, the diploid nucleus commences mitotic division. Meanwhile, the remaining two haploid nuclei incompletely fuse, migrate to the membrane, and eventually degenerate (Figure 2D). Therefore, rather than reproducing via premeiotic doubling or ameiotically, *C. biroi* reproduces via automixis with central fusion.

This cytological mechanism also underlies thelytoky in Cape honey bees (*Apis mellifera capensis*) [17]. It has also been inferred for several other ants [18–21], although in those cases it has not been demonstrated cytologically. Automixis with central fusion incurs LOH during meiotic recombination, because all loci distal to a chromosomal crossover event become homozygous unless a second crossover occurs to ‘rescue’ heterozygosity [22]. This LOH has been observed in other thelytokous eusocial insects: A clonal lineage of the Cape honey bee had lost 19.1% of its ancestral heterozygosity after ten years [23] (~146 generations [24], or 0.13% LOH per generation), and in the ants *Wasmannia auropunctata* and *Cataglyphis cursor*, single loci lose 0 – 2.8% and 6 – 33% (the theoretical maximum under automixis with central fusion) of heterozygosity each generation, respectively [18, 19]. As expected, recombination in these species leads to high proportions of diploid males: 15.4% of diploid eggs in the Cape honey bee [25] and 4.5% of diploid adults in *C. cursor* [26].

Using RAD-Seq, we obtained 7× coverage of ~10% of 91 individual genomes from 19 colonies from four independent clonal lineages (multi-locus lineages or MLLs) [8]. Polymorphic loci (including indels) that were scored in at least 80% of individuals were identified, providing 100,608 informative loci for analysis. These loci were used to reconstruct the ancestral genotype for each of the clonal lineages (Figure 3, *sensu* [23]), allowing us to calculate the average LOH for each clonal lineage. *Cerapachys biroi* has been established invasively for over 100 years in some locations [7], and, based on the time of first collection of a given MLL from the respective study population [8] and a generation time of 34 days [5], a minimum of 225 (MLL1 from Okinawa, Japan), 86 (MLL4 from St. Croix, USA), 43 (MLL6 from Okinawa, Japan) and 0 (MLL13 from Shenzhen, China) generations of asexual reproduction had elapsed by the time we collected samples for our analyses. Surprisingly, no more than 0.3% of ancestral heterozygosity has been lost per individual in any clonal lineage (Figure 3). This implies that the rate of LOH in *C. biroi* is as low as 0.0013% per generation: 100 times lower than in the Cape honey bee. Consequently, individuals from the same MLL are almost clonally identical across the entire genome (average within-MLL relatedness = 0.991; average within-colony relatedness = 0.996) (Figure 3).

Under automixis with central fusion, we expect to find LOH events spanning large genomic regions, and a bias of LOH towards the telomeres, as is the case for Cape honey bees [23, 27]. The exceptionally rare occurrence of haploid males in *C. biroi* [8] precluded obtaining a linkage map to assemble the scaffolds and determine their proximity to the telomeres. However, 18 of the 1,077 assembled scaffolds contained the insect telomeric repeat sequence [28], and these scaffolds were significantly enriched for LOH events (binomial probability distribution,  $P < 0.05$ ). We also found LOH events larger than 1 Mb, as expected. Consistent with a low rate of LOH, no more than 13 large LOH events were present in any single clonal lineage (Figure 3).

Although all clonal lineages show little LOH from their heterozygous lineage ancestor, there is variation in the number of ancestrally heterozygous loci between clonal lineages (Figure 3). The uniform distribution of ancestral heterozygosity across the genomes of the less heterozygous lineages (Wald-Wolfowitz Runs Test for MLL1 and MLL4,  $P = 0.19$  and  $P =$

0.34, respectively) is not consistent with localized LOH arising from recombination, but it is consistent with homozygosity arising from inbreeding in a sexual population. We therefore speculate that *C. biroi* underwent inbreeding in a (possibly facultatively) sexual population, before asexuality became fixed in MLL1 and MLL4.

*Cerapachys biroi* has the lowest LOH rate of any thelytokous eusocial insect studied, losing less heterozygosity in 21 years (MLL1) than other species lose in a single generation. It is not yet clear whether LOH in *C. biroi* arises through reduced recombination during meiosis, via selection against homozygous individuals, or both, as is the case in Cape honey bees [25]. Minimizing LOH allows asexual species with complementary sex determination to avoid the production of diploid males. Intriguingly, however, *C. biroi* lacks an ortholog of the honey bee complementary sex determiner gene *CSD* (see Supplemental Experimental Procedures: *Transformer* Genes), which, except for the fungus-growing ant *Acromyrmex echinator*, is conserved among all other sequenced eusocial Hymenoptera [29]. Further work is required to determine whether the novel mode of sex-determination in *C. biroi*, in concert with low recombination rates, helps prevent the frequent production of diploid males.

### Behavioral and Reproductive Colony Cycles

Many eusocial Hymenoptera are derived from subsocial, progressive provisioning wasps [30–32]. The lifecycle of a progressive provisioning wasp is divided into a reproductive phase, during which the wasp constructs a brood cell, has activated ovaries, and lays an egg, as well as a brood care phase, during which the wasp forages and provisions the larva while her ovaries are inactive (Figure 4A). Mary Jane West-Eberhard's "Ovarian Ground Plan Hypothesis" (OGPH) [31, 32] states that during the transition from subsocial to eusocial, the physiology and behavior expressed during the reproductive phase of the subsocial lifecycle became robustly expressed in the queen caste, while the physiology and behavior expressed during the brood care phase became expressed in the worker caste (Figure 4A). This hypothesis was later applied explicitly to the molecular regulation of division of labor in honey bee workers by Amdam *et al.* [33, 34] as the "Reproductive Ground Plan Hypothesis" (RGPH).

Colonies of *C. biroi* consistently cycle between reproductive and brood care phases that last for 18 and 16 days, respectively (Figure 4A) [5, 9]. The transitions between phases are synchronized with the development of the brood, which mature in discrete age cohorts. Larvae hatch at the end of the reproductive phase and trigger the transition to brood care by inducing foraging behavior and suppressing ovarian activity in the adults [4]. The reproductive phase, during which workers lay eggs and no foraging occurs, begins when the cohort of larvae pupates. The colony cycle of *C. biroi* and other phasic dorylomorphs, which is more recently derived from non-phasic eusocial ancestors, thereby recapitulates the phasic lifecycle of the ancient subsocial ancestor (Figure 4A). If the OGPH and RGPH are correct, we would predict that the molecular mechanisms underlying division of labor in non-phasic eusocial insects and, presumably, their subsocial ancestors, should also be involved in regulating the *C. biroi* colony cycle. In that case, *C. biroi* could become a powerful model system to establish causality between different molecular mechanisms underlying the

fundamental aspects of reproductive physiology and behavior pertinent to the evolution of eusociality. This is because reproductive and brood care behavior in *C. biroi* can be induced via simple brood swap experiments [4, 11], while the analogous experiment is not possible in most other ants, because the equivalent behaviors are fixed in queen and worker castes: Induction of caste development at the larval stage does not allow isolation and evaluation of the salient behaviors, and reversible activation of worker reproduction and queen-like behavior is usually only possible in highly contrived situations and not amenable to precisely controlled time-course analysis.

We therefore tested whether conserved genes that have previously been implicated in division of labor in other eusocial insects are dynamically regulated during the *C. biroi* colony cycle. We chose to study juvenile hormone (JH) synthesis, *Vitellogenin* (*Vg*), *Foraging* (*For*) and *Malvolio* (*Mvl*). The RGPH explicitly links JH and *Vg* to the evolution of division of labor [33]. Similarly, *For* and *Mvl* gene expression have been shown to influence worker division of labor in social insects [35–37].

Vitellogenin (*Vg*) is an egg yolk precursor protein produced for egg provisioning, while JH is a gonadotropic hormone associated with ovary activation, vitellogenesis and egg laying in solitary insects. In honey bee workers, decreasing *Vg* and increasing JH levels are associated with the transition to foraging behavior [38]. In ants, JH inhibits egg laying and increases foraging in founding queens and workers [39–44], but stimulates egg laying in established queens [44, 45].

*Vitellogenin* was duplicated in the ancestor of the Formicoid ants, creating two clades that exhibit queen/nurse-specific and forager-specific expression [46, 48] (hereafter referred to as ‘*Vgq*’ and ‘*Vgw*’ clades, respectively). To investigate the evolution and expression of *Vg* in *C. biroi*, we used the previously identified *Vg* genes from ants [46, 50–52] and *A. mellifera* to identify and re-annotate *Vg* sequences for all eight ants (See Supplemental Experimental Procedures: *Vitellogenin* Annotation and Phylogeny). Incomplete genome assembly in *Camponotus floridanus* at the *Vg* tandem duplication locus (Figure 5A) prevented identification of more than one *Vg* gene. *Cerapachys biroi* has two copies of *Vg*, one in the *Vgq* clade and one in the *Vgw* clade (Figure 5B). Unlike all other sequenced ant *Vgw* genes, *CbirVgw* does not have a 3’ truncation in the final exon relative to *Vgq*.

*CbirVgq* expression was significantly higher in workers in the reproductive phase immediately prior to egg laying (Figure 4B). *CbirVgw* expression was significantly higher in workers during the brood care phase (Figure 4B). Both *C. biroi* *Vgs* were more expressed in abdomens than in heads (Figure 4C). While in the harvester ant *Pogonomyrmex barbatus* the expression levels of *Vgq* were consistently higher than those of *Vgw* [47], the reverse was the case in *C. biroi*, where *CbirVgw* expression was higher than *CbirVgq* expression in 94% of samples.

*Cerapachys biroi* *Vg* evolution and expression is consistent with Formicoid *Vg* duplication and functional specialization [47]. The timing and abdominal expression of *CbirVgq* expression is consistent with a conserved role in egg yolk provisioning in ants [45], while the role of *CbirVgw* has yet to be determined.

3-Hydroxy-3-methylglutaryl-CoA reductase (*Hmgcr*) is the rate-controlling enzyme of the mevalonate pathway responsible for JH synthesis [54]. In *C. biroi*, *Hmgcr* was upregulated during foraging (Figure 4B) and positively correlated with *CbirVgw* ( $R = 0.39$ ,  $P < 0.05$ ), but not *CbirVgq* expression ( $R = -0.10$ ,  $P = 0.6$ ), consistent with the non-gonadotropic function of JH found in workers and foundress queens of other ant species [39–44].

*Malvolio* is a manganese transporter involved in sucrose responsiveness in *Drosophila* [55] and honey bees [35]. In honey bee workers, *Mvl* is upregulated in foragers compared to nurses, and implicated in worker division of labor [35]. *Foraging* is a conserved protein kinase that regulates feeding-related behavior in Metazoans [56, 57]. The expression of *For* is specifically associated with foraging behavior in ant and bee workers, although its mode of action is varied and expression in queens has not been studied explicitly [36, 37, 58]. We found that *Mvl* and *For* are most expressed in foraging *C. biroi* workers (Figures 4B and 4C; differences in expression based on whole bodies were only significant for *For*), with both genes significantly more expressed in heads than in abdomens during the brood care phase (Figure 4C). This expression pattern is comparable to that in honey bee workers [35, 37], but inverse to the pattern observed in harvester ants for *For* expression [36]. In honey bees, *Mvl* is upregulated in response to brood pheromone [59]. It is therefore possible that the brood-mediated regulation of *C. biroi* phasic behavior [11] is similarly influenced by *CbirMvl* expression.

These gene expression patterns in *C. biroi* are consistent with roles associated with foraging and reproductive behavior, just as in other eusocial insects. Importantly, the phase-specific expression patterns in *C. biroi* match the behaviorally and physiologically equivalent caste-specific expression patterns seen in non-phasic eusocial insects. The gene networks underlying reproduction and brood care in *C. biroi* are therefore likely to be the same conserved networks underlying caste-specific behavior in other eusocial insects. Furthermore, this raises the possibility that the phasic colony cycle in *C. biroi* and other phasic dorylomorphs represents a partial evolutionary reversal to the ancestral subsocial lifecycle.

## Conclusions

*Cerapachys biroi* maintains genomic heterozygosity, despite meiotic reproduction, at a level that is surpassed only by ameiotic species. The virtually clonal colony structure that results from this allows for experimental control and replication of individual genotypes and the genetic composition of social groups, to a degree that is unattainable in most other social Hymenoptera. The totipotency of workers and the absence of queens allows for easy colony propagation and the composition of arbitrarily sized experimental colonies, while the colony cycle allows for precise selection of age-matched workers and experimental control over colony demography. Because reproductive and brood care states can be experimentally induced in *C. biroi* [4, 11], reproductive caste-equivalent identity can be manipulated at the adult stage, facilitating the establishment of causality between different molecular mechanisms and caste differences. *Cerapachys biroi* therefore has great potential to provide novel insights into the regulation of gene networks that underlie reproductive division of labor in insect societies. Although a few other social insects share some of these favorable

traits, none of them has become a well-established model species. In conclusion, the unique biology of *C. biroi* makes the species a promising new model system to study the molecular underpinnings of social evolution and behavior.

## Experimental Procedures

### Genome sequencing, assembly, and annotation

Illumina HiSeq 2000 sequencing was performed on five paired-end libraries from individuals of MLL4. Roche 454 FLX sequencing was performed on individuals from MLL1 and MLL6. Illumina reads were assembled into scaffolds using SOAPdenovo [52], which were gap-filled using Illumina and Roche 454 data. Transcriptome data were obtained from pooled cDNA from all life stages from MLL4. Gene annotations were obtained using homology, *de novo* and transcriptome methods. We manually curated 803 genes. For more details see Supplemental Experimental Procedures: Sequencing, Assembly, Annotation and Functional Annotation.

### Estimating the rate of diploid male production in *C. biroi*

We collected five diploid males over three years from ca. 30 colonies, with each colony producing approximately 6,000 diploid workers over that period. Five diploid males among 180,000 diploid offspring corresponds to one in 36,000, well below one in 10,000 diploid offspring.

### Restriction site Associated DNA Sequencing (RAD-Seq)

Of the original 95 individuals, 91 provided a minimum genome-wide average sequencing depth of 1.3× and were included in all subsequent analyses (Figure 3). To infer the ancestral lineage genotypes (*sensu* [23]), all reads at a given locus from all individuals in a lineage were pooled, and the two most frequent alleles taken as the ancestral alleles (*sensu* [61]), as long as both had read depths greater than 20 (no locus had more than two such alleles). To account for type II errors arising from library construction and allele sequencing bias, we did not score homozygous loci with a heterozygous ancestral genotype and a single-allele sampling probability greater than 0.001 (See [62]). Additionally, homozygous loci that were present in only one individual and were unlinked to other homozygous loci were excluded as probable genotyping errors. Pairwise relatedness between individuals was calculated for each clonal lineage as in [8] using all loci that were heterozygous in the ancestral genotype of the respective lineage. See Supplemental Experimental Procedures: RAD-Seq Analysis for more details.

### RT-qPCR

For each sample, eight one-month old workers from colonies at the appropriate stage in the cycle were collected and pooled. RNA was extracted using a modified Trizol / phenol chloroform protocol. qPCR was performed on a Roche LightCycler using SYBR green. *RPS3*, *RPS6* and *RPL13α*, which showed invariable expression levels during the colony cycle, were used as reference genes. Gene expression was calculated according to [63]. MIQE [64] qPCR protocol details are given in the Supplemental Experimental Procedures: Real-Time Quantitative RT-PCR.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This work was supported by grant 1DP2GM105454-01 from the NIH and a grant from Rockefeller University to DJCK, as well as a grant from the Leon Levy Foundation for Mind, Brain and Behavior to PRO. IF was supported by a grant from the Mathers Charitable Foundation to Cori Bargmann. We would like to thank the laboratory of Hermann Steller for use of their confocal microscope.

## References

1. Sokolowski MB. Social interactions in “simple” model systems. *Neuron*. 2010; 65:780–794. [PubMed: 20346755]
2. LeBoeuf AC, Benton R, Keller L. The molecular basis of social behavior: models, methods and advances. *Curr. Opin. Neurobiol.* 2013; 23:3–10. [PubMed: 22995551]
3. Smith CR, Toth AL, Suarez AV, Robinson GE. Genetic and genomic analyses of the division of labour in insect societies. *Nat. Rev. Genet.* 2008; 9:735–748. [PubMed: 18802413]
4. Teseo S, Kronauer DJC, Jaisson P, Châline N. Enforcement of reproductive synchrony via policing in a clonal ant. *Curr. Biol.* 2013; 23:328–332. [PubMed: 23375892]
5. Ravary F, Jahyny B, Jaisson P. Brood stimulation controls the phasic reproductive cycle of the parthenogenetic ant *Cerapachys biroi*. *Insectes Soc.* 2006; 53:20–26.
6. Kronauer DJC. Recent advances in army ant biology (Hymenoptera: Formicidae). *Myrmecol. News.* 2009; 12:51–65.
7. Wetterer JK, Kronauer DJC, Borowiec ML. Worldwide spread of *Cerapachys biroi* (Hymenoptera: Formicidae: Cerapachyinae). *Myrmecol. News.* 2012; 17:1–4.
8. Kronauer DJC, Pierce N, Keller L. Asexual reproduction in introduced and native populations of the ant *Cerapachys biroi*. *Mol. Ecol.* 2012; 21:5221–5235. [PubMed: 23013522]
9. Ravary F, Jaisson P. The reproductive cycle of thelytokous colonies of *Cerapachys biroi* Forel (Formicidae, Cerapachyinae). *Insectes Soc.* 2002; 49:114–119.
10. Tsuji K, Yamauchi K. Production of females by parthenogenesis in the ant, *Cerapachys biroi*. *Insectes Soc.* 1995; 42:333–336.
11. Ravary F, Jaisson P. Absence of individual sterility in thelytokous colonies of the ant *Cerapachys biroi* Forel (Formicidae, Cerapachyinae). *Insectes Soc.* 2004; 51:67–73.
12. Elango N, Hunt B, Goodisman M, Yi S. DNA methylation is widespread and associated with differential gene expression in castes of the honeybee, *Apis mellifera*. *Proc. Natl. Acad. Sci. USA.* 2009; 106:11206–11211. [PubMed: 19556545]
13. Lorite P, Palomeque T. Karyotype evolution in ants (Hymenoptera: Formicidae), with a review of the known ant chromosome numbers. *Myrmecol. News.* 2010; 13:89–102.
14. Heimpel GE, de Boer JG. Sex determination in the Hymenoptera. *Annu. Rev. Entomol.* 2008; 53:209–230. [PubMed: 17803453]
15. van Wilgenburg E, Driessen G, Beukeboom LW. Single locus complementary sex determination in Hymenoptera: an unintelligent design? *Front. Zool.* 2006; 3:1. [PubMed: 16393347]
16. Khila A, Abouheif E. *In situ* hybridization on ant ovaries and embryos. *Cold Spring Harb. Protoc.* 2009 <http://dx.doi.org/10.1101/pdb.prot5244>.
17. Verma S, Ruttner F. Cytological analysis of the thelytokous parthenogenesis in the Cape Honeybee (*Apis mellifera capensis* ESCHOLTZ). *Apidologie.* 1983; 14:41–57.
18. Percy M, Hardy O, Aron S. Thelytokous parthenogenesis and its consequences on inbreeding in an ant. *Heredity.* 2006; 96:377–382. [PubMed: 16552429]
19. Rey O, Loiseau A, Facon B, Foucaud J, Orivel J, Cornuet J-M, Robert S, Dobigny G, Delabie J, Mariano CDSF, et al. Meiotic recombination dramatically decreased in thelytokous queens of the

- little fire ant and their sexually produced workers. *Mol. Biol. Evol.* 2011; 28:2591–2601. [PubMed: 21459760]
20. Kellner K, Heinze J. Mechanism of facultative parthenogenesis in the ant *Platythyrea punctata*. *Evol. Ecol.* 2011; 25:77–89.
  21. Rabeling C, Kronauer DJC. Thelytokous parthenogenesis in eusocial Hymenoptera. *Annu. Rev. Entomol.* 2013; 58:273–292. [PubMed: 23072461]
  22. Suomalainen, E.; Saura, A.; Lokki, J. Evolution of parthenogenetic insects in *Evolutionary Biology*. Hecht, M.; Wallace, B.; Prance, GT., editors. New York: Plenum Press; 1976. p. 209–257.
  23. Baudry E, Kryger P, Allsopp M, Koeniger N, Vautrin D, Mougél F, Cornuet J-M, Solignac M. Whole-genome scan in thelytokous-laying workers of the Cape honeybee (*Apis mellifera capensis*): central fusion, reduced recombination rates and centromere mapping using half-tetrad analysis. *Genetics*. 2004; 167:243–252. [PubMed: 15166151]
  24. Martin S, Wossler T, Kryger P. Usurpation of African *Apis mellifera scutellata* colonies by parasitic *Apis mellifera capensis* workers. *Apidologie*. 2002; 33:215–232.
  25. Goudie F, Allsopp MH, Beekman M, Oxley P, Lim J, Oldroyd BP. Maintenance and loss of heterozygosity in a thelytokous lineage of honey bees (*Apis mellifera capensis*). *Evolution*. 2012; 66:1897–1906. [PubMed: 22671554]
  26. Doums C, Ruel C, Clémencet J, Fédérici P, Cournault L, Aron S. Fertile diploid males in the ant *Cataglyphis cursor*: a potential cost of thelytoky? *Behav. Ecol. Sociobiol.* 2013 <http://dx.doi.org/10.1007/s00265-00013-01606-00266>.
  27. Goudie F, Allsopp MH, Oldroyd B. Selection on overdominant genes maintains heterozygosity along multiple chromosomes in a clonal lineage of honey bee. *Evolution*. 2013 <http://dx.doi.org/10.1111/evo.12231>.
  28. Meyne J, Hirai H, Imai HT. FISH analysis of the telomere sequences of bulldog ants (*Myrmecia*: Formicidae). *Chromosoma*. 1995; 104:14–18. [PubMed: 7587589]
  29. Schmieder S, Colinet D, Poirié M. Tracing back the nascence of a new sex-determination pathway to the ancestor of bees and ants. *Nat. Commun.* 2012; 3:895. [PubMed: 22692538]
  30. Hunt JH. Trait mapping and salience in the evolution of eusocial Vespid wasps. *Evolution*. 1999; 53:225–237.
  31. West-Eberhard, MJ. *Animal Societies: Theories and Facts*. Ito, Y.; Brown, JL.; Kikkawa, J., editors. Tokyo: Japanese Science Society Press; 1987. p. 35–51.
  32. West-Eberhard, MJ. *Natural History and Evolution of Paper-Wasps*. Turillazzi, S.; West-Eberhard, MJ., editors. New York: Oxford University Press; 1996. p. 290–317.
  33. Amdam GV, Norberg K, Fondrk MK, Page RE. Reproductive ground plan may mediate colony-level selection effects on individual foraging behavior in honey bees. *Proc. Natl. Acad. Sci. USA*. 2004; 101:11350–11355. [PubMed: 15277665]
  34. Amdam G, Csondes A, Fondrk M, Page R. Complex social behaviour derived from maternal reproductive traits. *Nature*. 2006; 439:76–78. [PubMed: 16397498]
  35. Ben-Shahar Y, Dudek N, Robinson GE. Phenotypic deconstruction reveals involvement of manganese transporter *malvolio* in honey bee division of labor. *J. Exp. Biol.* 2004; 207:3281–3288. [PubMed: 15326204]
  36. Lucas C, Sokolowski MB. Molecular basis for changes in behavioral state in ant social behaviors. *Proc. Natl. Acad. Sci. USA*. 2009; 106:6351–6356. [PubMed: 19332792]
  37. Ben-Shahar Y, Robichon A, Sokolowski MB, Robinson GE. Influence of gene action across different time scales on behavior. *Science*. 2002; 296:741–744. [PubMed: 11976457]
  38. Pinto L, Bitondi M, Simões Z. Inhibition of vitellogenin synthesis in *Apis mellifera* workers by a juvenile hormone analogue, pyriproxyfen. *J. Insect Physiol.* 2000; 46:153–160. [PubMed: 12770247]
  39. Sommer K, Hölldobler B. Colony founding by queen association and determinants of reduction in queen number in the ant *Lasius niger*. *Anim. Behav.* 1995; 50:287–294.
  40. Dolezal AG, Brent CS, Gadau J, Hölldobler B, Amdam GV. Endocrine physiology of the division of labour in *Pogonomyrmex californicus* founding queens. *Anim. Behav.* 2009; 77:1005–1010.

41. Vargo EL, Laurel M. Studies on the mode of action of a queen primer pheromone of the fire ant *Solenopsis invicta*. *J. Insect Physiol.* 1994; 40:601–610.
42. Brent C, Peeters C, Dietmann V, Crewe R, Vargo E. Hormonal correlates of reproductive status in the queenless ponerine ant, *Streblognathus peetersi*. *J. Comp. Physiol. A Neuroethol. Sens. Neural. Behav. Physiol.* 2006; 192:315–320. [PubMed: 16283330]
43. Sommer K, Hölldobler B, Rembold H. Behavioral and physiological aspects of reproductive control in a *Diacamma* species from Malaysia (Formicidae, Ponerinae). *Ethology.* 1993; 94:162–170.
44. Dolezal AG, Brent CS, Hölldobler B, Amdam GV. Worker division of labor and endocrine physiology are associated in the harvester ant, *Pogonomyrmex californicus*. *J. Exp. Biol.* 2012; 215:454–460. [PubMed: 22246254]
45. Libbrecht R, Corona M, Wende F, Azevedo DO, Serrao JE, Keller L. Interplay between insulin signaling, juvenile hormone, and vitellogenin regulates maternal effects on polyphenism in ants. *Proc. Natl. Acad. Sci. USA.* 2013 <http://dx.doi.org/10.1073/pnas.1221781110>.
46. Wurm Y, Wang J, Riba-Grognuz O, Corona M, Nygaard S, Hunt B, Ingram K, Falquet L, Nipitwattanaphon M, Gotzek D, et al. The genome of the fire ant *Solenopsis invicta*. *Proc. Natl. Acad. Sci. USA.* 2011; 108:5679–5684. [PubMed: 21282665]
47. Corona M, Libbrecht R, Wurm Y, Riba-Grognuz O, Studer RA, Keller L, Zhang J. Vitellogenin underwent subfunctionalization to acquire caste and behavioral specific expression in the harvester ant *Pogonomyrmex barbatus*. *PLoS Genet.* 2013; 9:e1003730. [PubMed: 23966882]
48. Bonasio R, Zhang G, Ye C, Mutti N, Fang X, Qin N, Donahue G, Yang P, Li Q, Li C, et al. Genomic comparison of the ants *Camponotus floridanus* and *Harpegnathos saltator*. *Science.* 2010; 329:1068–1071. [PubMed: 20798317]
49. Smith C, Zimin A, Holt C, Abouheif E, Benton R, Cash E, Croset V, Currie C, Elhaik E, Elsik C, et al. Draft genome of the globally widespread and invasive Argentine ant (*Linepithema humile*). *Proc. Natl. Acad. Sci. USA.* 2011; 108:5673–5678. [PubMed: 21282631]
50. Smith C, Smith C, Robertson H, Helmkampf M, Zimin A, Yandell M, Holt C, Hu H, Abouheif E, Benton R, et al. Draft genome of the red harvester ant *Pogonomyrmex barbatus*. *Proc. Natl. Acad. Sci. USA.* 2011; 108:5667–5672. [PubMed: 21282651]
51. Nygaard S, Zhang G, Schjøtt M, Li C, Wurm Y, Hu H, Zhou J, Ji L, Qiu F, Rasmussen M, et al. The genome of the leaf-cutting ant *Acromyrmex echinator* suggests key adaptations to advanced social life and fungus farming. *Genome Res.* 2011; 21:1339–1348. [PubMed: 21719571]
52. Suen G, Teiling C, Li L, Holt C, Abouheif E, Bornberg-Bauer E, Bouffard P, Caldera E, Cash E, Cavanaugh A, et al. The genome sequence of the leaf-cutter ant *Atta cephalotes* reveals insights into its obligate symbiotic lifestyle. *PLoS Genet.* 2011; 7:e1002007. [PubMed: 21347285]
53. Stamatakis A. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics.* 2006; 22:2688–2690. [PubMed: 16928733]
54. Bellés X, Martín D, Piulachs M-D. The mevalonate pathway and the synthesis of juvenile hormone in insects. *Annu. Rev. Entomol.* 2005; 50:181–199. [PubMed: 15355237]
55. Rodrigues V, Cheah P, Ray K, Chia W. *Malvolio*, the *Drosophila* homologue of mouse *NRAMP-1* (*Bcg*), is expressed in macrophages and in the nervous system and is required for normal taste behaviour. *EMBO J.* 1995; 14:3007–3020. [PubMed: 7621816]
56. Fitzpatrick MJ, Sokolowski MB. In search of food: exploring the evolutionary link between cGMP-dependent protein kinase (PKG) and behaviour. *Integr. Comp. Biol.* 2004; 44:28–36. [PubMed: 21680483]
57. Houte S, Ros V, Oers M. Walking with insects: molecular mechanisms behind parasitic manipulation of host behaviour. *Mol. Ecol.* 2013; 22:3458–3475. [PubMed: 23742168]
58. Ingram KK, Oefner P, Gordon DM. Task-specific expression of the *foraging* gene in harvester ants. *Mol. Ecol.* 2005; 14:813–818. [PubMed: 15723672]
59. Alaux C, Le Conte Y, Adams HA, Rodriguez-Zas S, Grozinger CM, Sinha S, Robinson GE. Regulation of brain gene expression in honey bees by brood pheromone. *Genes Brain Behav.* 2009; 8:309–319. [PubMed: 19220482]

60. Li R, Zhu H, Ruan J, Qian W, Fang X, Shi Z, Li Y, Li S, Shan G, Kristiansen K, et al. De novo assembly of human genomes with massively parallel short read sequencing. *Genome Res.* 2010; 20:265–272. [PubMed: 20019144]
61. Wang Y, Lu J, Yu J, Gibbs RA, Yu F. An integrative variant analysis pipeline for accurate genotype/haplotype inference in population NGS data. *Genome Res.* 2013; 23:833–842. [PubMed: 23296920]
62. Wheeler D, Srinivasan M, Egholm M, Shen Y, Chen L, McGuire A, He W, Chen Y-J, Makhijani V, Roth GT, et al. The complete genome of an individual by massively parallel DNA sequencing. *Nature.* 2008; 452:872–876. [PubMed: 18421352]
63. Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 2002; 3:1–12.
64. Bustin S, Benes V, Garson J, Hellems J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl M, Shipley G, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* 2009; 55:611–622. [PubMed: 19246619]

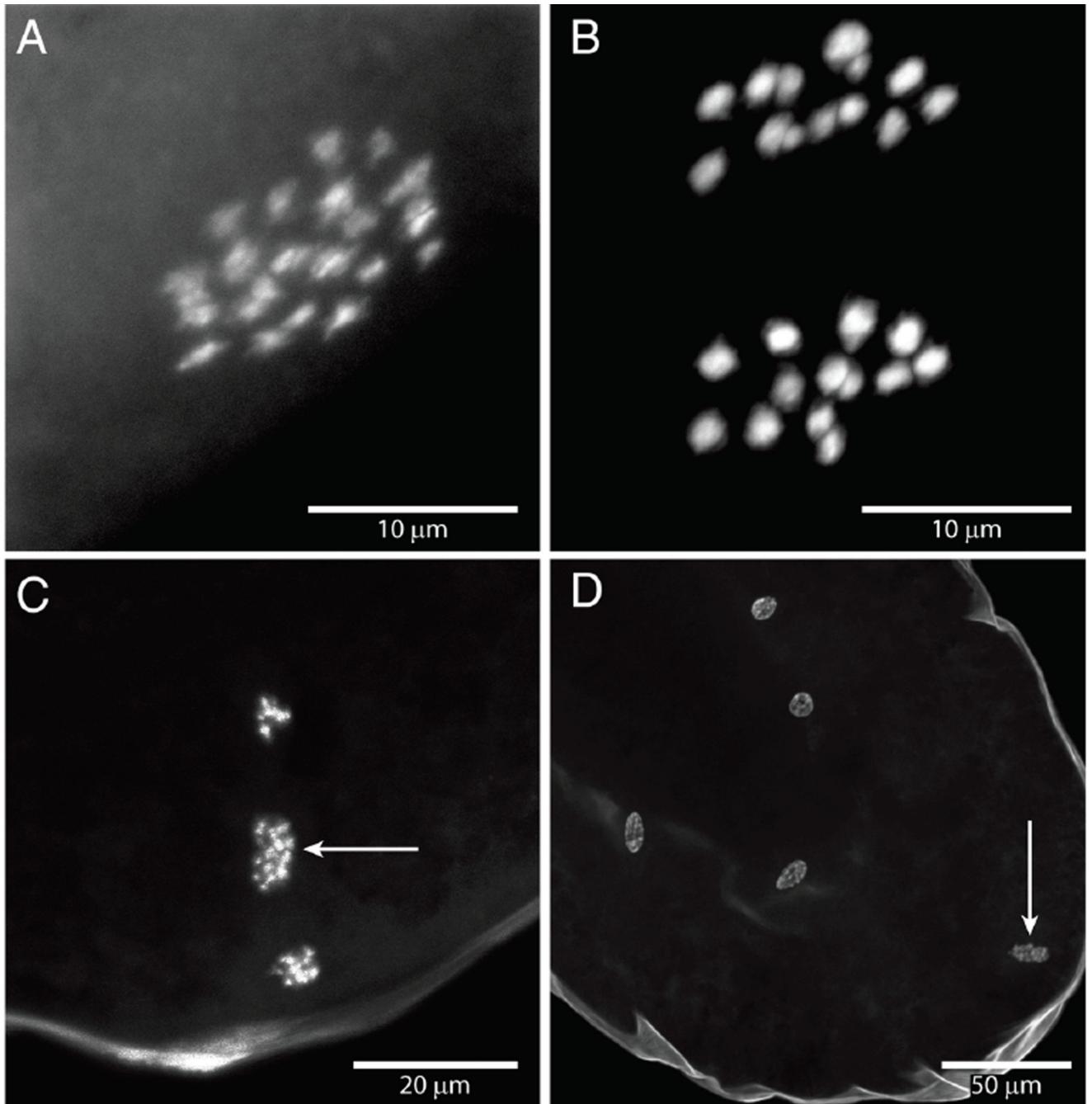
### Highlights

- The queenless clonal raider ant *Cerapachys biroi* has a small and compact genome
- Nestmates are almost clonally related and reproduce via automixis with central fusion
- Nestmates synchronously alternate between reproduction and brood care
- *C. biroi* will facilitate studies of the molecular regulation of division of labor



**Figure 1.**

A clonal raider ant (*Cerapachys biroi*) worker carrying a pupa. Ants of the genus *Cerapachys* are myrmecophagous and raid the nests of other ants [6]. The genus belongs to the dorylomorph clade of ants, which also includes the infamous army ants [6]. Since the early 1900s, introduced populations of *C. biroi* have become established on tropical and subtropical islands around the world, probably as a consequence of human traffic and trade [7, 8]. Like in many other dorylomorphs, colonies of *C. biroi* undergo stereotypical behavioral and reproductive cycles [5, 9]. Colonies of *C. biroi* lack queens and instead consist entirely of totipotent workers, all of which reproduce asexually [10, 11].



**Figure 2.**

Three-dimensional projections of DAPI-stained chromosomes in < 2hr old eggs showing that *C. biroi* reproduces through automixis with central fusion. Embryos were prepared according to [14]. The diploid chromosome number in *C. biroi* is  $2n = 28$  [13]. **A)** Prophase I immediately post partum showing a single diploid nucleus close to the posterior pole of the egg. **B)** Meiosis I (reductional division) at approximately 30 min post partum. Two nascent haploid nuclei can be seen. **C)** Fusion of central products of meiosis II (indicated by arrow) within one hour post partum. **D)** Embryo after two rounds of mitotic division with four

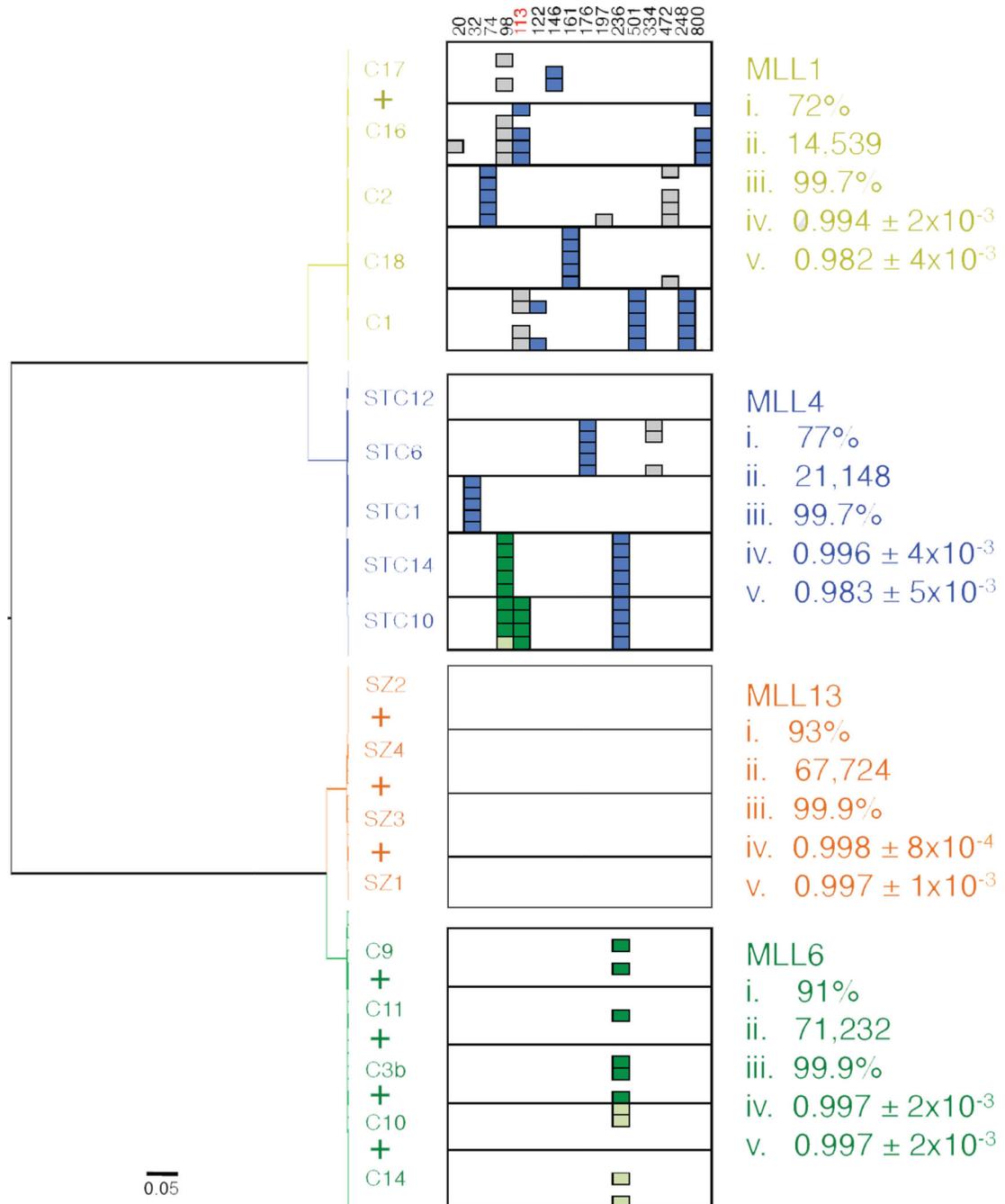
diploid nuclei, within two hours post partum. The polar bodies in panel C have fused (arrow) and migrated to the cell membrane, where they degenerate. Additional stages are shown in Figure S4.

Author Manuscript

Author Manuscript

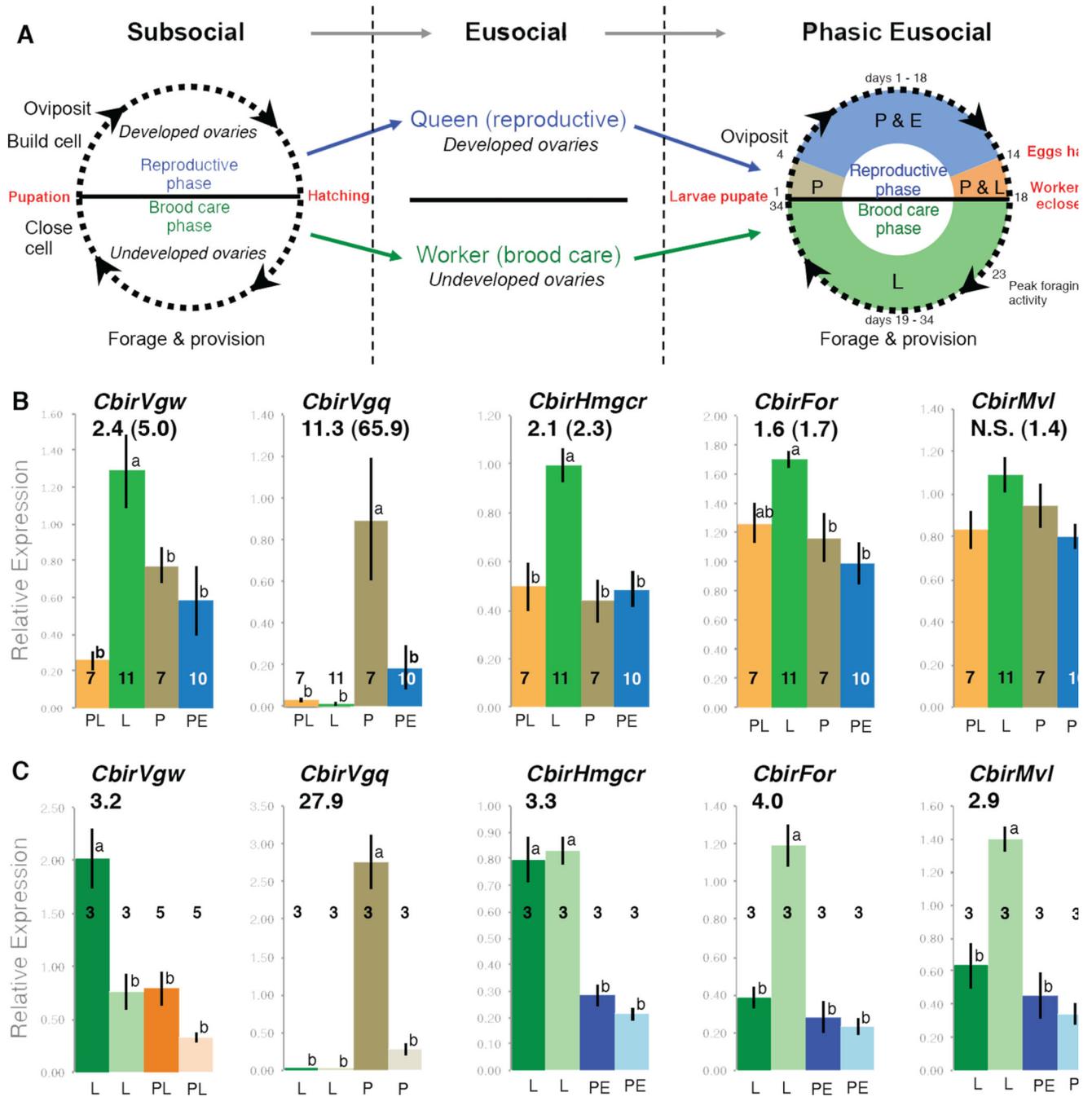
Author Manuscript

Author Manuscript

**Figure 3.**

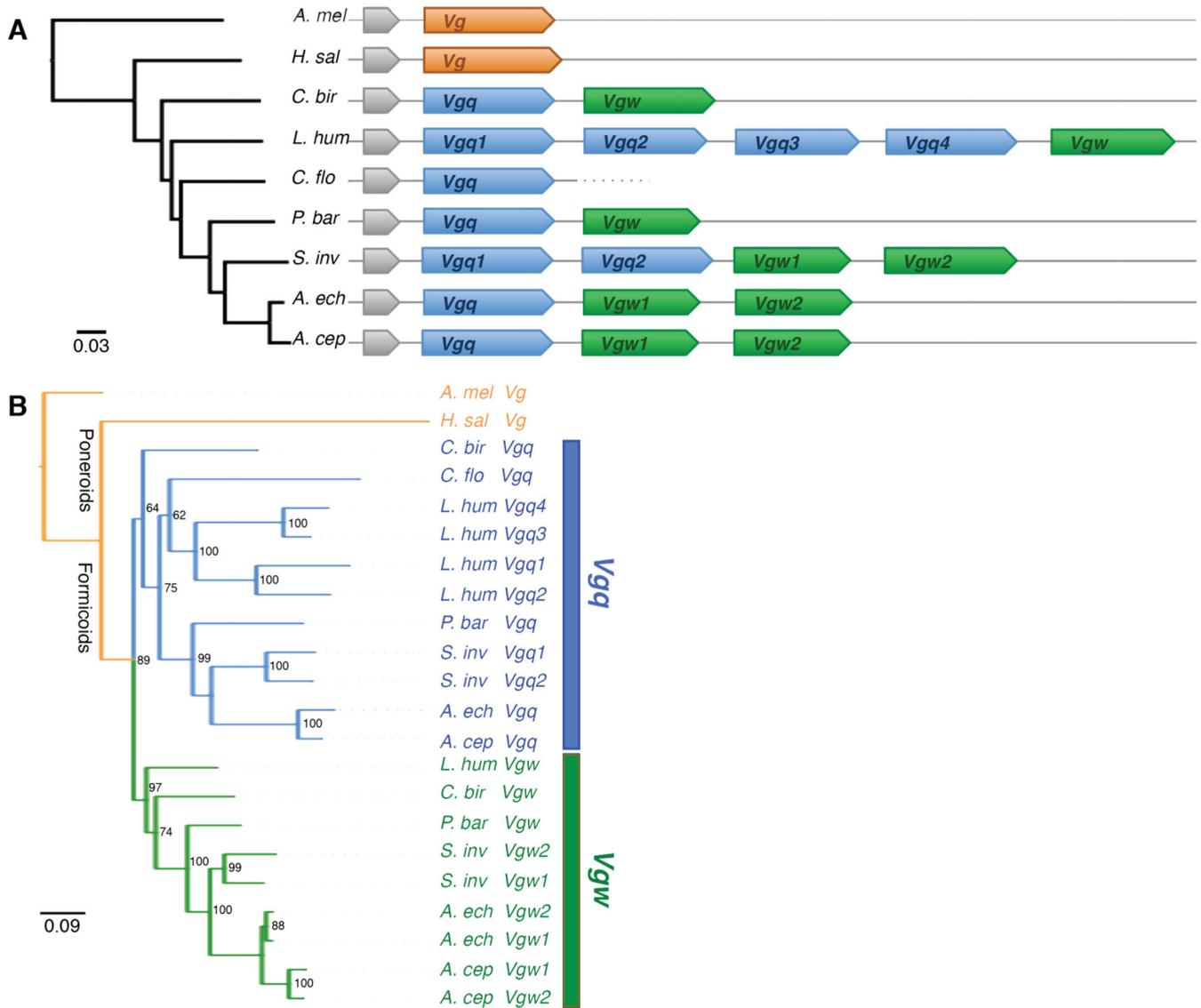
Phylogenetic and genomic relationships between *C. biroi* individuals, colonies, and clonal lineages. The UPGMA tree shows the average number of substitutions per site for 100,608 informative sites, between 91 ants from 4 clonal lineages and 19 colonies. Colonies that are not recovered as monophyletic in the phylogeny are indicated by a “+” between colony names (there is no colony-level resolution for MLL6 and MLL13). Contiguous regions of homozygous loci 1 Mb in size are shown in the map on the right: each colored square represents a putative single loss of heterozygosity (LOH) event. Columns indicate the 16

different scaffolds that contain LOH spanning 1 Mb (scaffold number given above each column). Scaffold 113 (marked in red) contains the telomeric repeat sequence. Identical block colors within a column indicate homozygous fragments with identical beginning and end positions, which most likely arose from a single ancestral LOH event. Lighter colored blocks indicate individuals that had only un-scored loci in the focal positions and are therefore consistent with either sharing the LOH of the darker colored individuals or having the ancestral heterozygous genotype. Regions marked in grey contained un-scored loci that were heterozygous in the individuals lacking the LOH fragment, and may therefore actually represent two or more smaller homozygous fragments, each < 1 Mb. Figures beneath each lineage name are (from top to bottom): i. The percentage of 1,077 scaffolds that contain some heterozygosity in the ancestral state; ii. The number of loci that are heterozygous in the ancestral genotype; iii. The average heterozygosity per individual, calculated as the percentage of heterozygous loci among the loci that were inferred to be heterozygous in the lineage ancestor (for calculations, see Supplemental Methods: RAD-Seq Analysis); iv. The average within-colony relatedness ( $\pm$  SD); v. The average between-colony relatedness ( $\pm$  SD).



**Figure 4.** Schematic of the hypothesized evolutionary transitions from subsocial to eusocial to phasic eusocial, with the phase-specific expression of candidate genes throughout the *C. biroi* colony cycle. **A)** Schematic showing the compartmentalization of subsocial behaviors into eusocial queen and worker castes, and reintegration into the phasic colony cycle of *C. biroi*. The timing of behaviors and corresponding brood stages are indicated on the subsocial and phasic eusocial cycle. The *C. biroi* reproductive phase is subdivided into three stages based on the brood present: Grey – pupae (P) only; Blue – pupae and eggs (E); Orange – pupae

and larvae (L). **B**) Whole-body gene expression for *C. biroi* *Vgw*, *Vgq*, *Hmgcr*, *For* and *Mvl* during the four stages described in panel A. Graphs show relative expression (mean  $\pm$  SEM). Colors correspond to the different stages of the colony cycle in panel A. Brood stages present are also indicated on the  $\times$  axis of each graph, and correspond to the colony cycle in A. Samples for the brood care phase (green) were collected at day 23, when foraging activity is highest. Sample size is indicated inside or above each column in bold. Letters above columns indicate significantly different groups (Bonferroni-corrected ANOVA ( $P < 0.05$ ) with Tukey's post-hoc tests ( $P < 0.05$ )). Numbers beneath gene names show average fold change in expression between significantly different groups. Maximum fold change for each gene is indicated in parentheses. **C**) Tissue-specific gene expression in some of the behavioral stages showing differences in panel B. Head and abdomen expression are indicated with light and dark colors, respectively. Graphs show mean  $\pm$  SEM gene expression as described for panel B.

**Figure 5.**

Vitellogenin sequence analysis. Previously annotated *Vg* genes were used to identify existing and novel *Vg* genes in all eight ant genomes (see Supplemental Experimental Procedures: *Vitellogenin* Annotation and Phylogeny). **A**) Phylogeny of all sequenced ant species with their corresponding *Vg* loci mapped. Maximum likelihood tree based on first and second codon positions constructed with RAxML (GTR+G model) [53] using 3,164 orthologous single-gene families present in all ants, *A. mellifera*, *N. vitripennis* (not shown) and the outgroup *D. melanogaster* (not shown) (see Supplemental Experimental Procedures: Phylogeny Reconstruction and Gene Expansions). Bootstrap support values (100 replicates) for all nodes are 100%. *A. mel* – *Apis mellifera*; *H. sal* – *Harpegnathos saltator*; *C. bir* – *Cerapachys biroi*; *L. hum* – *Linepithema humile*; *C. flo* – *Camponotus floridanus*; *P. bar* – *Pogonomyrmex barbatus*; *S. inv* – *Solenopsis invicta*; *A. ech* – *Acromyrmex echinaior*; *A. cep* – *Atta cephalotes*. Arrows indicate direction of transcription. Colors correspond to the reproduction-associated and brood care-associated *Vg* genes (*Vgq* (blue) and *Vgw* (green),

respectively). Grey genes indicate an orthologous lipid transport protein immediately upstream of all hymenopteran *Vgs*. A tandem duplication occurred at the base of the Formicoid clade, followed by several independent duplications in different Formicoid lineages. **B**) Maximum likelihood phylogram of ant *vitellogenin* (*Vg*) genes. Colors and abbreviated species names correspond to those in A. *Vgq* and *Vgw* clades are indicated with solid bars. Bootstrap values based on 1,000 replicates are given for each node. Branch lengths indicate substitutions per site.

**Table 1**

Summary statistics of the *Cerapachys biroi* draft genome (official gene set 1.8) in comparison to the eight sequenced ant genomes (including *C. biroi*). Methods are described in the Supplemental Experimental Procedures.

	<i>Cerapachys biroi</i>	Eight-ant median	Eight-ant range
Genome assembly size (Mb)	214	269	214 – 353
Diploid chromosome number <sup>a</sup>	28	32	16 – 38
Scaffold N50 (bp)	1,291,492	944,008	598,192 – 5,154,504
Contig N50 (bp)	31,934	28,034	11,606 – 62,705
Average sequencing depth	122 ×	86 ×	19 – 123 ×
Genes with EST support (%)	75.5	66.0	40.0 – 84.0
CEGMA genes (%)	99.6	99.0 <sup>b</sup>	98.0 – 99.6 <sup>b</sup>
Repeats (%) <sup>c</sup>	13.8	24.0	11.5 – 28.0
GC (%)	41.7	36.3	32.6 – 45.2
CpG <sub>(observed/expected)</sub> (CDS) <sup>d</sup>	1.20	1.42	1.17 – 1.29
CDS (% of genome)	9.76	7.52	4.87 – 9.76
Introns (% of genome)	26.87	16.62	7.67 – 26.87
miRNAs <sup>e</sup>	63	93	63 – 159
Protein coding genes (With IPR domains) (With GO terms)	17,263 (9,628) (7,835)	17,220	16,123 – 18,564
Species-specific genes	4,892	5,025	3,263 – 6,869
Odorant Receptors <sup>f</sup>	369	347 <sup>g</sup>	337 – 369 <sup>g</sup>
Gustatory Receptors <sup>f</sup>	27	63 <sup>g</sup>	21 – 117 <sup>g</sup>
Ionotropic Receptors <sup>f</sup>	26	26	23– 32
Cytochrome P450 genes <sup>h</sup>	69	60.5	28 – 84
UDP-glycosyltransferases <sup>h</sup>	21	12	9 – 21

<sup>a</sup>From [13]

<sup>b</sup>Data from *H. saltator* and *C. floridanus* not available

<sup>c</sup>See Supplemental Experimental Procedures: Repeats

<sup>d</sup>Calculated for coding sequences only; for additional CpG analyses see Figure S1 and Supplemental Experimental Procedures: DNA Methylation and Histone Modification

<sup>e</sup>See Supplemental Experimental Procedures: miRNAs

<sup>f</sup>Annotated manually (for more detailed results see Table S1)

<sup>g</sup>Data from *S. invicta*, *A. echinator* and *A. cephalotes* not available

<sup>h</sup>Annotated manually (for more detailed results see Figures S2 & S3)